A Special issue on:

International Conference on

"Current Trends in Pharmacology Research - Preclinical Trials (ICCTPR-PT)"

3rd & 4th January 2020

DOI: http://dx.doi.org/10.22376/ijpbs/ijlpr/SP09/Jan/2020.1-223

In conjunction with

BHASKAR PHARMACY COLLEGE

(Sponsored by J. B. Educational Society)

Approved by AICTE, PCI New Delhi and affiliated to the JNTU, Hyderabad

Bhaskar Nagar, Yenkaplly (V), Moinabad (M), Ranga Reddy Dist.
Hyderabad - 500 075, Telangana, India.
Phone: 08413-235027
Web site: www.bpc.edu.in
Email id: principal@bpc.edu.in

"ICCTPR-PT 2020"International Conference on Current Trends in Pharmacology Research - Preclinical Trials held on 3rd& 4th January 2020

Chief Patron	**Co-ordinator**	**Co-Convener**
Sri.J.V.Krishna Rao	Dr.A.Srinivasa Rao	Dr.A.V.Kishore Babu
Secretary	Principal	HOD, Pharmacy Practice
B.Educational Society	Bhaskar Pharmacy College	Bhaskar Pharmacy College

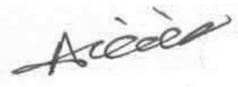
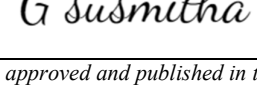
ABOUT THIS SPECIAL ISSUE

This special issue focuses on those aspects with much implication for the healthcare. The articles published in this special issue will certainly bring a positive effect for thedeveloping health care and to make use of available resources and to remove certain obsoletefactors and process which may delay or harm the existing health care system. It enhances maximum utilization of scientific knowledge to potentiate therapy and diagnosis in the healthcare system. Authors presented short review articles as well as research articles in which they focused recent developments in their subject, emphasising the aspects that, in their opinion, are most important. In addition, they provide short annotations to the papers that they consider to be most interesting from all those published in their topic over the previous year. The conference intends to focus on pre-clinical trials. The conference will be offering a unique gathering in respect to scenario of global challenges. It aims to bring together students, teachers, and researchers working in sciences. The conference is expected to be a platform for the gathering of the ideas for the development of clinical research.

Co-ordinator
Dr.A.Srinivasa Rao
Principal
Bhaskar Pharmacy College.
Hyderabad - 500 075,
Telangana, India

Peer review committee

The following Peer reviewers were appointed by the organisers to review the content of the articles and were confirmed to be of satisfactoryscholastic content after rectification by the respective authors.These reviewers solemnly take the responsibility of the scholastic and research content along with the organisers and the pblishing journal is no way responsible for the same.

Reviewer name	Designation and qualification	Signature
Dr.A.SRINIVASA RAO	M.PHARM,PH.D PRINCIPAL, BHASKAR PHARMACY COLLEGE	
Dr.A.V.KISHORE BABU	PHARM-D,[PH.D] H.O.D,PHARMACY PRACTICE	
Dr.G.SUSMITHA	PHARM-D ASSISTANT PROFESSOR	

We the above mentioned reviewers take the full responsibility of peer reviwing and assure that the contents approved and published in this issue of of good and appreciable scholastic content and would assure to cooperate any litigation arises in this regard.

CONTENT – (ABSTRACT)

CONTENT (ORIGINAL RESEARCH / REVIEW ARTICLE)

AB-01

STABLE SOLID DISPERSION INCORPORATED SUSTAINED RELEASE ORAL GEL OF 23 MG DONEPEZIL HCL FOR THE TREATMENT OF ALZHEIMER DISEASE

NEHA KANSWAMI, R.ANUDEEP REDDY, P.K. LAKSHMI*

Department of Pharmaceutics, G.Pulla Reddy College of Pharmacy, Hyderabad, Telangana, India.
Email ID: nehakanswamy95@gmail.com

ABSTRACT

The objective of the study is to formulate and evaluate stable solid dispersion incorporated sustained release oral gel of donepezil Hcl. Solid dispersions were prepared using HPMC K100M and stearic acid using various methods and incorporated into the previously prepared gel bases using sodium CMC and sodium alginate and suitable formulation was optimized and evaluated. Solid dispersions were prepared using HPMC K100 and stearic acid by using different methods like physical mixtures, co-grinding and fusion method in the ratios of 1:1, 1:2 and 1:3.From the *invitro* dissolution studies, it was found that SD made using co-grinding and fusion method showed sustained release were selected and incorporated into the gel formulations made of SCMC 4% and sodium alginate 4%. The release studies of solid dispersion incorporated gel SD-HPMC K100M showed (SDG1) 87.55% and (SDG2) 88.9% at 8th hour and SD-stearic acid showed (SDG3) 83.26% and (SDG4) 82.57% at 8th hour. It was concluded that stearic acid solid dispersions incorporated in sodium CMC gels (SDG3) was optimized using short term stability studies with no leakage of drug in comparison to SD-HPMC K100M containing gel. The optimized SD incorporated gel formulation of donepezil can be an alternative to SR tablet in improving the compliance of geriatric patients.

Keywords: Solid dispersion, HPMC K100, Stearic acid, co-grinding

AB-02

ROLE OF CARCICA PAPAYA– IN DENGUE TREATMENT

DIVYA REDDY* AND L.UMA RANI

Department of Pharmacy Practice, Bhaskar Pharmacy College, Hyderabad- 500075, India.
Email Id: dr720555@gmail.com

ABSTRACT

Dengue is one of the most important arthropod-borne diseases worldwide and is now endemic in more than 100 countries. Dengue disease creates highly complex patho-physiological, economic and ecologic conditions. About 400 million people get infected with dengue, 100 million people get sick from the infection and 22,000 die from severe dengue every year. There are no effective antiviral agents against dengue virus therefore the treatment remains supportive. Various treatments are being investigated to treat dengue. Studies have indicated that the Carica papaya plant leaf extract (CPLE) could help to increase the platelet levels in these patients. From the ancient times, the whole Papaya plant which includes (leaves, seeds, ripe and unripe fruits and its juice) due to its multiple medicinal properties it is considered as nutraceutical fruit The Carica papaya leaves therapeutic effects are presumed due to several active components such as enzymes (Papain), lycopene, carotenoids, alkaloids, monoterpenoids, flavonoids, mineral and vitamins. The abstract reviews Carica papaya leaf extract (CPLE) for the treatment of dengue with thrombocytopenia.

Keywords: Dengue, Carcica papaya, Thrombocytopenia, Nutraceutical.

AB-03

A QUALITATIVE STUDY OF ASSOCIATION BETWEEN HIGH LEVELS OF BODY TISSUES FLUORIDE IN ADOLESCENTS AND IMPAIRMENT OF PSYCHOLOGICAL, NEUROCOGNITIVE DOMAINS.

DR. SURESH A*, SHANTHI.CH, DR.TABITHA JEZREEL, DR.SATYA REVANTH.K, DR.VISHWAK REDDY.V, DR.SRUJANA.J & DR.PRAVEEN KHAIRKER

Department of Psychiatry, Dental and Pharmacology, Kamineni Institute of Medical sciences, Narketpally, Nalgonda-508254, India. Email: drsureshalladi@gmail.com.

ABSTRACT

Many researchers have discretely documented the psychological impact of high fluoride on children and adolescents particularly the slower mental functioning. In the current qualitative study, the investigators intended to find a plausible relationship between spectral levels of fluoride in drinking water and/or body tissues to qualitatively analyze psychological, clinical, neurocognitive domains in 3 different children groups exposed to varied fluoride levels from highly endemic district.3 different regions of a highly endemic district were identified based on concentrations of water fluoride levels ranging from very low to very high. Structured and standardized clinical evaluations for children aged 11 to 15 years from native schools were performed.ICD-DCR-10,CPRS, Wisconsin Card Sorting Test, Raven's Progressive Matrices and Dean's Index for teeth fluoride induced dysfunction were used and analyzed. A total of 150 students, 50 each from 3 different populations based on water fluoride were evaluated.We identified specific learning disorders, conduct disorders and motor incoordination disorder more common as well as neurocognitive dificit in children using high fluoride levels ($p<0.05$)**.**To our knowledge, this is the first comprehensive and integrated evaluation of impact of spectral levels of fluoride in 4 domains in its inherent nature for progressive understanding in neuropsychiatry.

Keywords: Spectral Fluoride levels, Neurocognitive dysfunction, Child Psychiatry,Wisconsin Card Sorting Test.

AB-04

PSYCHOPHARMACOLOGICAL EVALUATION OF ORAL KETAMINE AS ANTIDEPRESSANT IN PATIENTS WITH DEPRESSION

SHANTHI.CH*, DR.RAMA NARSIMHA REDDY.A, DR.TABITHA JEZREEL, DR.SATYA REVANTH.K DR,VAMSHI KRISHNA.I & DR. SURESH A

Department of Psychiatry and Pharmacology, kamineni Institute of Medical sciences, Narketpally,Nalgonda-508254,IndiaEmail:drsureshalladi@gmail.com.

ABSTRACT

Ketamine is a approved anaesthetic drug which is gaining ground as an intervention for patients with treatment-resistant depression (TRD) with serious suicidal ideation. In march 2019, US FDA approved use of intranasal esketamine in conjuction with an oral antidepressant for patients with TRD.10 individual patients willing to participate with written informed consents are selected and study is performed for 2months. Prospective (or) pilot study is done on every individual patients diagnosed as depression meeting ICD-10 criteria and are inpatients of the psychiatric ward between the age group of 18-50yrs. 3ml of injectable ketamine form [(i.e. 1ml=50mg strength) is diluted and mixed in 30 ml of drinking water (total 150 mg of ketamine) given orally once daily after breakfast over 15 minutes. Patients are kept under observation up to 2hrs.and adverse effects are noted. Demographic and clinical data is taken in semistructured intake proforma, baseline Hamilton depression rating scale is applied from the day of admission and scores are noted. This pilot study found oral ketamine to be safe and well tolerated, the findings also showed effectiveness of oral ketamine in treatment of depression, with 30%, 70% and 100% of patients showing response on day 3,7,10 respectively.Psychopharmacological evaluation showed effectiveness of oral ketamine in treatment of depression.

Keywords: Ketamine, depression, antidepressant, psychopharmacology.

SP-01

EFFECT OF PERMEATION ENHANCERS ON TRANSDERMAL DRUG DELIVERY OF NEBIVOLOL HYDROCHLORIDE

ARCHANA MULE*, PALLAVI KANAGALA, NASEEB BASHA SHAIK, RADHIKA KORUKONDA AND HARITHA MALLELA

Department of Pharmaceutics, G. Pulla Reddy College of Pharmacy, Osmania University, Mehdipatnam, Hyderabad-28, India. E-mail: archanamule169@gmail.com

ABSTRACT:
Transdermal drug delivery system was developed to first pass metabolism of the drug and to improve its bioavailability by resisting the drug release in acidic p^H of the stomach. Nebivolol is chosen as a model drug which is having high first pass metabolism and low oral bioavailability. Solvent casting method was adopted to prepare patches using different synthetic polymers and permeation enhancers of various concentrations. The prepared formulations were characterized for good consistency, transparency, flexibility, entrapment efficiency (%EE) drug content, *in-vitro* and *ex-vivo* studies. Further, the formulations were evaluated for physico-chemical parameters to justify their suitability for transdermal use. Permeation enhancers with high concentrations of Propylene glycol with 20% (TP2), DMSO with 10% (TP8), Tween20 with 5% (TP10), Isopropyl myristate with 20% (TP16) have shown maximum release for 8 hrs from *in-vitro* study when compared to other formulations. The ex-vivo permeation studies performed using rat skin indicated an increase in permeation of formulations through the skin compared to pure drug. Hence, these formulations can be used as transdermal patches with increased permeation and bioavailability.

Keywords: Transdermal patch, Bioavailability, NebivololHcl, Propylene glycol, DMSO, Tween20, Isopropyl myristate.

INTRODUCTION

Transdermal drug delivery systems (TDDS) are defined as self-contained discrete dosage forms designed to deliver therapeutically effective concentrations of drug/drugs through the surface of patient's skin. The most preferred route of administration is an oral route but it has disadvantages like poor bioavailability and tendency to produce rapid blood level spikes and this leads to frequent dosing, to overcome these drawbacks there is a need for the development of new drug delivery system. Transdermal drug delivery has many advantages over conventional drug delivery such as avoidance of first pass metabolism, predictable and extended or prolonged duration of activity, utility of short half-life drugs, improving physiological & pharmacological response, avoiding the fluctuation in drug levels, reducing undesirable side effects, inter and intra-patient variations low risk to digestive tract or live[1-5].

EXPERIMENTAL METHODOLOGY

MATERIALS

Nebivolol Hydrochloride was purchased from AschoNulab industries Ltd, Eudragit RL 100 was supplied by Oleomonter real SL, HPMC was purchased from Deoleo S.A. Propylene Glycol was obtained from Otto, DMSO, Tween20 and Isopropyl myristate was purchased from S.D. fine chemicals Limited. Dibutyle phthalate was purchased from Ranbaxy laboratories Ltd. Methanol was purchased from Thermo fisher scientific India Pvt. Ltd. Dichloromethane was purchased from MOLYCHEM.

METHODOLOGY

All the ingredients were weighed accurately as shown in Table 1 and dissolved in a suitable solvent with continuous stirring. Then plasticizer was added to the above solution. The resultant solution was stirred for

15 minutes to get a clear solution and was kept aside for some time to get a bubble free solution, these solutions were casted slowly on a Teflon plate with a continuous flow to avoid bubble formation and the plates were kept in hot air oven at 40^0C for 24 hrs. After 24 hrs, formed patch was taken out from hot air oven and checked for its complete dryness. The dried patch was gently separated from the Teflon plate and cut into separate patches 2cm*2cm area. The patches were preserved by wrapping in aluminium foil. These patches were used for evaluation tests further[6-11].

Table 1: Formulation Nebivolol HCL patch with four different permeation enhancer

Ingredients	TP1	TP2	TP3	TP4	TP5	TP6	TP7	TP8	TP9	TP10	TP11	TP12	TP13	TP14	TP15	TP16
Nebivolol (mg)	34	34	34	34	34	34	34	34	34	34	34	34	34	34	34	34
Eudragit RL100(mg)	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200
HPMC E15LV(mg)	300	300	-	-	300	300	-	-	300	300	-	-	300	300	-	-
HPMC E50LV(mg)	-	-	300	300	-	-	300	300	-	-	300	300	-	-	300	300
DBP(%)	15%	15%	15%	15%	15%	15%	15%	15%	15%	15%	15%	15%	15%	15%	15%	15%
Propyleneglycol(%)	15%	20%	15%	20%	-	-	-	-	-	-	-	-	-	-	-	-
DMSO	-	-	-	-	5%	10%	5%	10%	-	-	-	-	-	-	-	-
Tween 20	-	-	-	-	-	-	-	-	2%	5%	2%	5%	-	-	-	-
Isopropylemyristate	-	-	-	-	-	-	-	-	-	-	-	-	10%	20%0	10%	20%

RESULTS AND DISCUSSION

Table 2.Physico chemical parameters of films containing synthetic polymers

Code	Weight variation	Folding endurance	Thickness	Moisture content	Moisture uptake	WVTR	Drug content
TP1	72.7±0.57	230	0.23±0.02	4.1±0.01	1.20±0.48	0.042	95±0.55
TP2	66.3±0.48	255	0.22±0.01	3.8±0.12	1.01±0.5	0.010	96±0.96
TP3	77±0.50	260	0.22±0.02	5.44±0.12	1.26±0.57	0.053	92±1.73
TP4	66.7±0.58	266	0.2±0.03	2.94±0.10	1.10±0.54	0.023	97±1.62
TP5	83.0±1.00	240	0.21±0.01	6.34±0.02	3.81±0.54	0.052	94±0.48
TP6	82.7±1.12	239	0.21±0.01	3.13±0.27	1.17±0.26	0.013	96±0.94
TP7	55.3±0.58	280	0.20±0.01	3.43±0.12	3.6±0.50	0.021	97±1.86
TP8	72.2±0.43	275	0.16±0.01	2.04±0.13	1.19±0.57	0.018	98±2.18
TP9	63±0.5	190	0.26±0.01	3.19±0.12	1.20±0.48	0.059	95±2.0
TP10	75±1.2	235	0.22±0.01	2.28±0.22	1.06±0.42	0.035	94±2.25
TP11	84±2.2	230	0.21±0.02	6.74±0.02	2.16±0.57	0.033	97±2.08
TP12	67±0.57	260	0.23±0.01	5.94±0.10	2.01±0.56	0.023	98±2.23
TP13	71±1.147	246	0.26±0.02	6.84±0.02	3.83±0.57	0.052	97±1.77
TP14	66±0.59	250	0.16±0.01	4.99±0.27	3.27±0.25	0.018	93±2.05
TP15	76±2	280	0.22±0.01	3.73±0.12	2.23±0.30	0.021	97±1.60
TP16	93.3±2.3	273	0.24±0.01	2.14±0.03	2.09±0.57	0.019	96±2.8

Note: All the values are expressed as mean± SD, n=3

In-vitro drug release studies[12-13]

Table 3.Cumulative percentage drug release from TP1 to TP8 formulations.

Time	TP1	TP2	TP3	TP4	TP5	TP6	TP7	TP8
0.5	23±0.01	30±0.01	14±0.01	11±0.01	8±0.01	20±0.01	20±0.01	20±0.01
1	25±0.01	31±0.01	17±0.01	12±0.01	11±0.01	22±0.01	25±0.02	39±0.01
2	26±0.02	35±0.01	18±0.01	14±0.01	14±0.01	23±0.02	28±0.02	31±0.02
3	27±0.03	37±0.02	20±0.02	23±0.01	23±0.01	31±0.02	27±0.02	33±0.02
4	29±0.03	39±0.02	21±0.01	26±0.02	26±0.02	32±0.03	29±0.03	34±0.02
5	29±0.03	41±0.02	23±0.02	28±0.02	28±0.02	34±0.03	29±0.03	34±0.02
6	31±0.04	43±0.03	26±0.02	31±0.02	31±0.02	35±0.03	31±0.03	39±0.02
7	33±0.04	46±0.03	29±0.03	34±0.03	34±0.03	37±0.03	34±0.03	41±0.03
8	34±0.04	49±0.03	35±0.03	38±0.03	38±0.03	43±0.04	39±0.04	48±0.03

Note: All the values are expressed as mean± SD, n=3

Table 4.Cumulative percentage drug release from TP9 to TP16 formulations.

Time	TP9	TP10	TP11	TP12	TP13	TP14	TP15	TP16
0.5	18±0.01	14±0.01	12±0.01	12±0.01	8±0.01	11±0.01	8±0.01	14±0.01
1	19±0.01	20±0.01	16±0.01	17±0.01	11±0.01	14±0.01	10±0.01	17±0.01
2	22±0.01	26±0.02	18±0.01	23±0.01	13±0.01	15±0.01	11±0.01	18±0.01
3	24±0.02	29±002	20±0.01	26±0.02	16±0.01	17±0.01	14±0.01	25±0.02
4	25±0.02	31±0.02	23±0.02	29±0.02	18±0.01	21±0.01	16±0.01	30±0.02
5	26±0.02	34±0.02	26±0.02	29±0.02	20±0.01	24±0.02	20±0.01	39±0.03
6	29±0.02	41±0.03	29±0.02	31±0.02	23±0.02	26±0.02	21±0.02	46±0.03
7	34±0.03	44±0.03	33±0.02	36±0.03	26±0.02	27±0.02	23±0.02	50±0.04
8	38±0.03	49±0.04	37±0.03	39±0.03	28±0.02	29±0.02	26±0.02	54±0.05

Note: All the values are expressed as mean± SD, n=3

***Ex-vivo* diffusion studies**

From the *ex-vivo* studies formulation TP10 was optimized which showed release of 71.4% for 8 hrs.

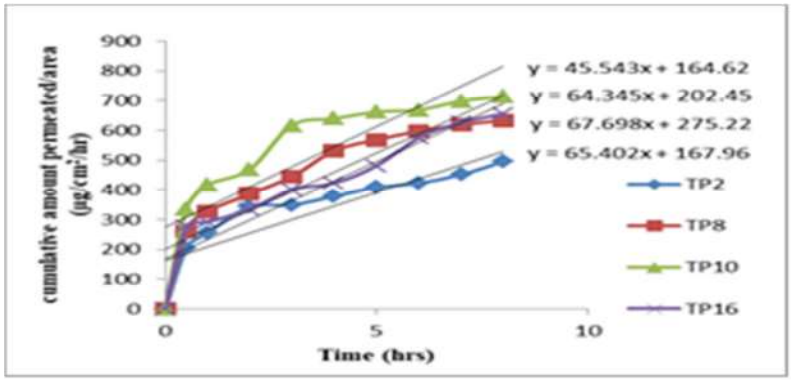


Figure 3.Flux of TP10

Table 6.Permeability parameters of optimized formulation

Formulation code	Flux(µg/cm^2/hr)	Permeability coefficient(cm/hr)	Lag time(hr)
TP10	67.698	13.84	0.5

Table 7.Model dependent kinetics of optimized formulations

Formulation code	r^2				N	Drug transport mechanism
	Zero	First	Higuchi	Peppas		
TP10	0.716	0.800	0.913	0.967	0.281	Fickian diffusion

Skin irritation studies

Skin irritation studies were conducted on depilated rabbit. Skin reaction at the site of application was assessed and scored according to Draize method and the formulations TP10 showed irritation potential of grade 0, thus proving to be non irritant. Therefore, no erythema and edema

Stability studies[14]

Stability studies were done for the optimized formulation TP10. After subjecting the optimized formulations to the stability studies, that there were no major changes in physical appearance, folding endurance and drug content. Hence the formulation was found to be stable.

CONCLUSION

NebivololHCl transdermal patches were successfully prepared by solvent casting method using different synthetic polymers and permeation enhancers of various concentrations. The prepared patches were evaluated for physico-chemical parameters to justify their suitability for transdermal use. Permeation enhancers with high concentrations of propylene glycol with 20% (TP2), DMSO with 10% (TP8), Tween20 with 5% (TP10), Isopropyl myristate with 20% (TP16) have shown maximum release for 8 hrs from *in-vitro* study when compared to other formulations. These films had good folding endurance and less moisture uptake, less moisture content and water vapour transmission rate when compared to other formulations. Formulation TP10 was optimized by conducting *ex-vivo* diffusion studies to the above four formulations. From stability studies, optimized patch formulation were found to be stable for one month at room temperature.

REFERENCES

1. Arunachalam, A., Karthikeyan, M., Kumar, D.V., Prathap, M., Sethuraman, S., Ashutoshkumar, S. and Manidipa, S. Transdermal drug delivery system: A review. Current Pharma Research 2010; 1(1): pp.70-81.
2. HimanshiTanwar, RuchikaSachdeva. Transdermal Drug Delivery System: A Review. International journal of Pharmaceutical Sciences and Research 2016;pp.2274-90.
3. Ashok Kumar J, Nikhila P, LakshmanaPrabu S, Gopal V. Transdermal drug delivery system: An overview. Int J Pharm Sci Rev Res 3 2010; pp.49-54.
4. Ashvini S. Kadam, Mukesh P. Ratnaparkhi, Shilpa P. Chaudhary. Transdermal Drug Delivery: An Overview. International Journal of Research and Development in Pharmacy and Life Sciences.June - July, 2014, Vol. 3, No.4: pp 1042-1053.
5. AudumbarDigambar Mali. An updated review on transdermal drug delivery systems. International Journal of Advances in Scientific Research 2015;1(06): pp 244-254.
6. Bhenika Sharma, BhavnaYadav, Kamal Saroha, Sanju Nanda. Formulation and Characterization of Transdermal Patch of Amlodipine Besylate. International Journal Of Pharmaceutical And Chemical Sciences 2012; ISSN: 2277-5005, Vol. 1 (3).
7. DibyalochanMohanty, VasudhaBakshi. Formulation and Characterization of Transdermal Patches of Amlodipine Besylate Using Olive Oil as the Natural Permeation Enhancer. Indo American Journalof Pharmaceutical Research. 2016:6 (06).
8. EkapolLimpongsa, KraisriUmprayn. Preparation and Evaluation of Diltiazem Hydrochloride Diffusion-Controlled Transdermal Delivery System. AAPS Pharm Sci.Tech. June 2008; Vol. 9, No. 2.
9. Jyotsana R. Madan, Nitin S. Argade and Kamal Dua. Formulation and Evaluation of Transdermal Patches of Donepezil'. Recent Patents on Drug Delivery & Formulation 2015; 9(1):95-103
10. Devi VK, Saisivam S, Maria GR, Deepti PU. Design and evaluation of matrix diffusion controlled transdermal patches of verapamil hydrochloride. Drug DevInd Pharm 2003;29:495-503.
11. EvrimAtilay. Studies on Transdermal Delivery Enhancement of Zidovudine. AAPS Pharm SciTech. March 2009; 10 (1): 88–97
12. Demou JS, Sidhom MB, Plakogiannis FM. Comparative in vitro diffusion studies for atenolol transdermal delivery systems. Pharm. ActaHelv 1994; 68:215–219.
13. Ghosh TK, Chiao CS, Gokhale RD. In-vitro permeation of some b-blockers across the hairless mouse skin. J. Pharm. Pharmacol. 1993; 45:218–219.
14. Iman, I S, Nadia A S, Ebtsam M A. Formulation and stability study of chlorpheniramine maleate transdermal patch',Asian J Pharm. 4 2014; pp.17-23

SP-02

EVALUATION OF CEREBROPROTECTIVE EFFECT OF VILDAGLIPTIN IN STZ INDUCED DIABETIC RATS BY USING BILATERAL COMMON CAROTID ARTERY OCCLUSION ISCHEMIC REPERFUSION MODEL

VKK MANDLEM[1], VAZRA SRAVANI K[2]

[1]Associate professor, Department of Pharmacology, CMR College of pharmacy, Hyderabad, India
[2]Post graduate student, Department of Pharmacology, CMR College of pharmacy, Hyderabad, India
Email-id:arunakiran4u@gmail.com

ABSTRACT

The aim of the study is to evaluate the cerebroprotective potential of Vildagliptin in bilateral common carotid artery occlusion (BCCAO) induced cerebral infarction in type-2 diabetic rats by estimating various biochemical parameters such as serum glucose, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), lactatedehydrogenase (LDH) and brain specific creatinine kinase (CK-BB), lipid peroxidation, reduced glutathione and catalase using tissue homogenate along with the study of histopathological changes in different experimental groups. Animals were grouped into 4 as normal, disease control, normal treated & disease treated. Type-2 diabetes was induced by administering 40 mg/kg STZ. On the 4th week cerebral infarction was induced in rats by BCCAO method for 30 mins with 4 hour reperfusion and then animals were sacrificed and their brains were analyzed for stroke infarct volume.Results shown that the treatment group provided a reduction in ischemic stroke infarct volume when compared with the disease control group and also lowered the elevated blood glucose levels, SGOT, SGPT, LDH, and CK-BB indicating the cerebroprotective role of vildagliptin in treating neuropathy.

Key words: Streptozotocin, cerebroprotection, DPP-4 inhibitor, bilateral common carotid artery occlusion, diabetes mellitus, ischemic stroke

INTRODUCTION

Diabetes mellitus (DM) is a chronic condition which as per WHO is a widespread metabolic disorder effecting the people all over the globe[1]. Of all the anti-diabetic drugs, studies have shown that DPP-4 inhibitors have cerebroprotective effect in type-2 diabetes[2, 3.] Incretins or incretin mimetics such as GLP-1 and GIP are GI metabolic hormones that are responsible to lower the blood glucose levels mediated by insulin release. Vildagliptin, a DPP-4 inhibitor used in treating diabetes has also found to have protective effect on brain and hence found role in treating cerebral infarction as a result of stroke which has been the second main cause of deaths worldwide[4,5].

MATERIALS AND METHODS

1. Chemicals and reagents

Thiopental was obtained from a local clinical pharmacy. Streptozotocin was purchased from Sri Sai Krishna Chemical store. Assay kits for the determination of biochemical parameters were obtained from Coral Clinic system and all other chemicals and were obtained from a local vendor.

2. Equipment

A digital balance was used to weigh the chemicals. Cold centrifuge (REMI CM 12) was used to separate serum from collected blood samples. UV-visible spectrophotometer was employed to determine the amount of constituents present in serum sample and a semi auto analyzer was used to analyze blood constituents in serum samples.

3. Animals

Animals required for the experiment were obtained from licensed animal rearing labs and were maintained and used as per CPCSEA guidelines. 12-14 weeks old albino wistar rats of either sex weighing 230-250g were used for this experiment. Animals were kept in quarantine area for about 1 week to

acclimatize with the laboratory conditions where the temperature is maintained at 20-24°C with proper air conditioning. Animals were fed on standard diet and purified drinking water. During experiment they are shifted from the quarantine area. The present study was ethically approved by CPCSEA with approval number CPCSEA/1657/IAEC/CMRCP/COL-17/56.

4. Experimental Design

Animals were checked for any diseased conditions and weighed before grouping them. They were divided into four groups each containing 6 animals.

Group 1 was treated as normal control.
Group 2 was treated as diabetic control.
Group 3 was not diseased but received the treatment vildagliptin with BCCAO.
Group 4 received treatment with vildagliptin and BCCAO in diseased rats.

4.1 Induction of type-2 diabetes

Wistar rats received 40 mg/kg intravenous STZ and diabetes was achieved within three days of the damage to beta cells. Disease treated and untreated rats were kept individually in separate cages and fed under controlled feeding conditions in diabetic rats[6].

4.2 Induction of Cerebral Infarction by using Bilateral Common Carotid Artery Occlusion (BCCAO) method

Initially rats were anaesthetized and placed on its back with the paws and tail fixed to the board. A midline incision of about 1 cm is made through the saggital plane and the salivary glands were separated to expose two common carotid arteries (CCA). A 2mm silicon tube was placed in between the CCA followed by the occlusion for 15 mins before reperfusion for 30 mins. This process is repeated for two times with the wound maintained in wet condition using saline solution. Sutures and silicon tubes were removed after the process[7,8].

4.3 Procedure for TTC staining

2,3,5-triphenyl tetrazolium chloride (TTC) stain is a common staining technique employed for the detection of cerebral necrosis. Rats in all groups were sacrified by cervical dislocation after 4 h and the brain was isolated, weighed, sliced and stored in TTC in phosphate buffer solution (1%) at 37°C for half an hour. Viable brain cells has the tendency to convert dehydrogenase to red formazan giving the tissue red color while the infarcted cells does not take in the stain and remains pale. The tissue which remained unstained is separated from the stained tissue and weighed to measure the infarct size[9].

5. Estimation of biochemical and other parameters

5.1.Estimation of stroke infarct volume

Stroke infarct volume gives a measure of cerebral necrosis which is measured staining the isolated rat brain slices with TTC stain. Viable and non-viable regions of the tissue are separated and weighed from which percentage of necrotic brain tissue was calculated by using the formula: stroke infarct volume = weight of viable tissue/weight of total brain tissue X 100[10].

5.2. Estimation of biochemical parameters

5.2.1 Glucose estimation

Measurement of serum glucose levels is a significant method for the detection of diabetes and can be estimated by GOD-POD method.The main principle involved in this method is oxidation of glucose molecules to gluconic acid and H_2O_2 catalyzed by glucose oxidase. The generated H_2O_2 further reacts with phenol and aminoantipyrine upon catalytic peroxidase reduction and forms a red color complex of quinoneimine dye which can be measured at 505nm[11].

5.2.3 Serum glutamic oxaloacetic transaminase (SGOT) estimation

The principle involved in this method is the conversion of L-aspartate &□-ketoglutarate to oxaloacetate and glutamate where the oxaloacetate further reacts with 2,4 dinitrophenyl hydrazine and yields a derivative of hydrazone which gives a brown complex in basic medium. Three solutions of blank, standard and test were prepared and stored for 10 minutes at 37°C which are then measured for absorbance at 505 nm[12].

5.2.4 Serum glutamate pyruvate transaminase (SGPT) estimation

The principle involved in this method is the conversion of L-alanine &□□-ketoglutarate to pyruvate and glutamate where the pyruvate further reacts with 2,4 dinitrophenyl hydrazine and yields a derivative of hydrazone which gives a brown complex in alkaline medium[13].

5.2.5 Lactate dehydrogenase (LDH) estimation

LDH promotes the reduction of pyruvate with NADH to form NAD. The rate of NADH oxidizing to NAD is measured by absorbance that is proportional to the concentration of LDH present in the sample. Test solution is prepared by the addition of 0.02 ml of sample to 0.8 ml of buffer reagent and incubated for 1 min at 37°C followed by the addition of 0.2 ml of starter reagent (L2) whose absorbance was measured at 340 nm[14].

5.2.6 Creatinine isoenzyme (CK-BB) estimation

0.05 ml of test sample is added to 0.8 ml of CK-BB reagent in a test tube which is incubated for 5 minutes followed by the addition of 0.2 ml of starter reagent and mixed well. Absorbance was measured at every 1, 2 and 3 minutes[15].

5.3. Estimation of *in-vivo* tissue parameters

5.3.1. Lipid peroxidation

0.5 ml of tris-hydrochloric acid buffer is mixed with 0.5 ml of PMS and incubated for 2 hours at 37°C. To this add ice cold trichloroacidic acid (1 ml) and centrifuged for 10 minutes at 1000 rpm. Supernatant mixed with 1 ml of thiobarbituric acid was boiled for 10 minutes and kept aside for the mixture to cool down followed by the measurement of absorbance[16].

5.3.2. Reduced glutathione

0.75 ml of sulfosalicylic acid (4%) was mixed with 0.7 ml of PMS and centrifuged for 5 minutes at 1200 rpm at 4°C. About 0.5 ml supernatant was mixed with 4.5 ml of Ellman's reagent (0.1mM) and the absorbance was measured at 412 nm[17].

5.3.4. Catalase

A mixture containing 0.05 ml of PMS, 1 ml of H_20_2 and 1.95 ml of phosphate buffer was prepared and the absorbance was measured at 240 nm[18].

Results and discussion:Bodyweights

The animal body weights were evaluated on Day-1 and Day-28.The animals belonging to the Group-2 shown increase in their body weights when compared to other groups as such there was the effect of diabetes which causes the animals in disease group to increase their weights and hence disease has been induced in them.They showed the significance when compared with the normal and treatment group.

Table 1: Effect of vildagliptin on body weight of animal groups

Groups	1st day (grams)	28th day (grams)
Normal	233 ± 3.21	228 ± 2.32
Disease control	226 ± 2.12	243 ± 3.24
Normal + treatment	210 ± 4.12	218 ± 5.25
Disease + treatment	225 ± 3.12	219 ± 4.23

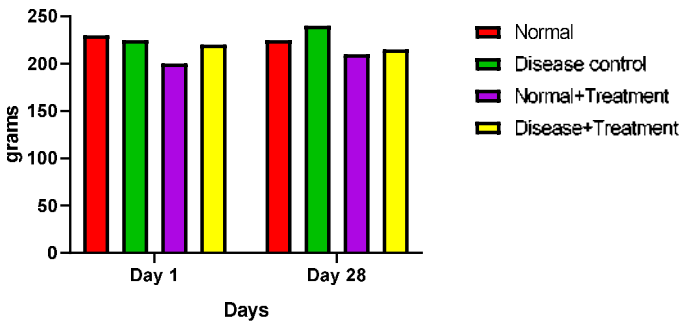


Figure 1: Graphical representation of changes in body weights of animal groups

Stroke infarct volume:

The cerebral ischemia was induced in all groups by BCCAO method and the rats belonging to normal group and disease group showed increased in the percentage of stroke infarct volume when compared to the treatments groups. The number of dead cells to live cells was calculated and compared against the diseased group. The disease group shows the value of 65.1±3.75. whereas the treatment group shown the values like 18.50±1.95 and 22.53±4.70 which indicates the protective effect of vildagliptin in treating cerebral ischemia in diabetic animals as it reduced the percentage of ischemic stroke.The values and graphs were given in table no.2 and figure no.2

Table 2: Effect of vildagliptin on stroke infarct volume

Animal groups	Percent Stroke infarct volume
Normal	36.83 ± 3.53
Disease control	65.17 ± 3.75*
Normal + treatment	18.50 ± 1.95*
Disease + treatment	22.53 ± 4.70*

Values were represented as Mean±SEM Statistical analysis performed using one way ANOVA followed by post hoc Dunnett's test, $p<0.05$ vs Disease control.

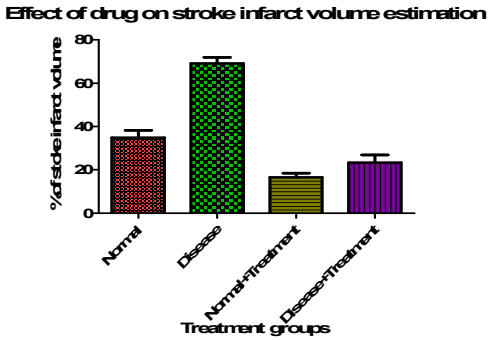


Figure 2: Graphical representation of effect of vildagliptin on stroke infarct volume

Biochemical parameters

The effect of Vildagliptin has been estimated in different groups. The diseased group showed immense rise in the blood glucose levels, SGOT, SGPT, LDH and CK-BB. The treatment with the vildagliptin showed protective effect on the blood glucose levels, SGOT, SGPT, LDH and CK-BB. In diseased group blood glucose was estimated to be 252.12±12.22 and treatment group showed decreased value like 91.26±8.26 and 117.02±6.26 which indicates the reduction of disease in animals. SGOT and SGPT value in the diseased group was estimated to be 72±20.12 and 66.6±8.22, whereas after treatment the values were decreased to be 40.7±8.26, 62.9±12.36 and 40.1±12.26, 42.1±22.12 which indicates the protective effect of drug by decreasing the damage caused by those blood parameters in disease condition. LDH was found to be 242.26±52.26 the treatment group showed decrease in the values like 142.06±32.26 and 110.22±22.62 indicating the reduction of cell destruction, in the diseased group, similarly CK-BB was found to be 636.06± 22.36 and the treatment group was showed 223.26±52.22 and 312.04±22.26 which indicates the protective effect of drug on stroke caused due to cerebral ischemia.

Table 3: Effect of vildagliptin on biochemical parameters

Animal groups	Glucose (mg/dL)	SGOT (IU/mL)	SGPT (IU/mL)	LDH (U/L)	CK-BB (IU/L)
Normal	110.22±10.22	35.1±14.62	40.6±6.26	366.12±42.26	335.28±12.26
Disease control	252.12±12.22*	72±20.12*	66.6±8.22*	242.26±52.26*	636.06±22.36*
Normal+Treatment	91.26±8.26*	40.7±8.26*	40.1±12.26*	142.06±32.26*	223.26±52.22*
Disease+Treatment	117.02±6.26*	62.9±12.36*	42.1±22.12*	110.22±22.62*	312.04±22.26*

Values were represented as Mean± SEM. Statistical analysis performed using one way ANOVA followed by post hoc Dunnett's test $p<0.05$ vs Disease control.

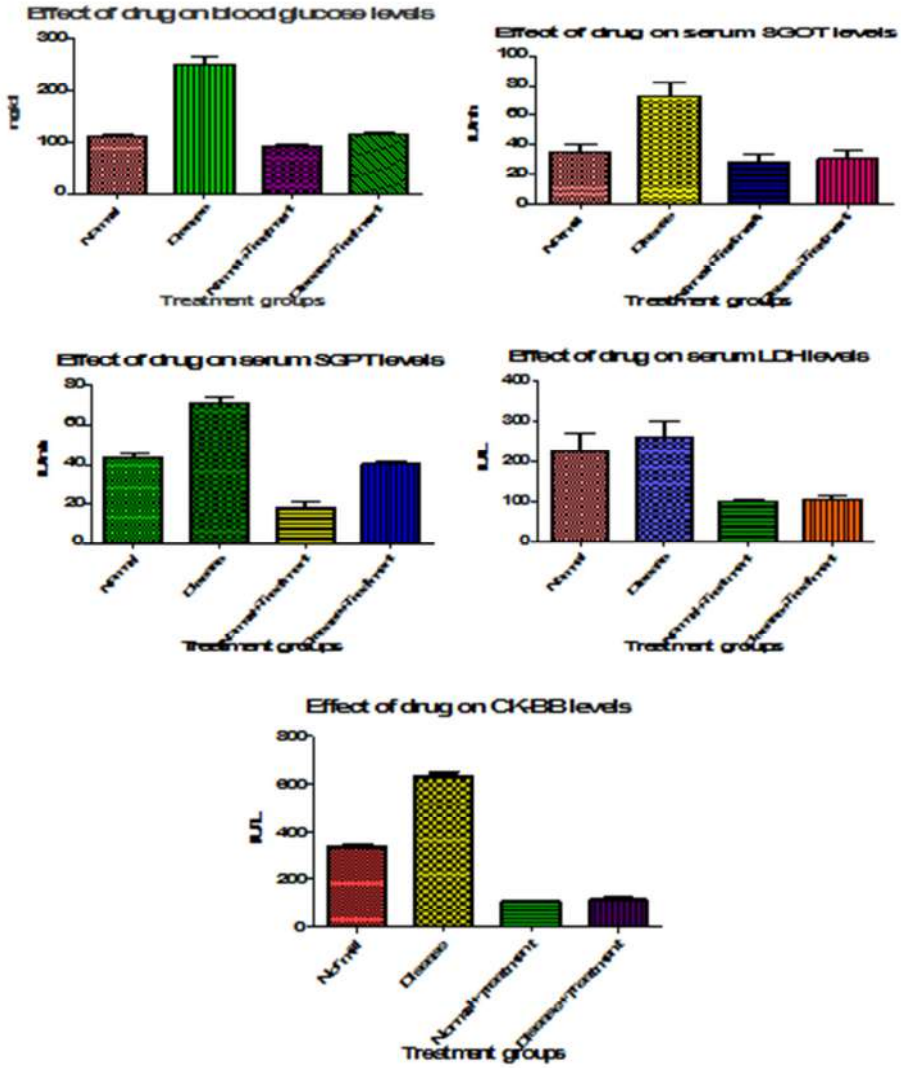


Figure 3: Graphical representation of effect of vildagliptin on biochemical parameters

Tissue anti-oxidant parameters

In case of the tissue anti-oxidant parameters the values of the diseased group raised for the lipid peroxidation was found to be 12.63±1.08 and then the treatment group showed decreased in the values like 3.60±0.53 and 2.48±0.35 indicating the reduction of damage to the cells by peroxidation, then the reduced glutathione and catalase values will be reduced in the diseased group like 2.60±0.21 and 0.04±0.04 and the treatment group showed raised in the values like 10.38±0.75,11.1±0.87 and 0.68±0.11,0.72±0.08 which are helpful in treating the disease condition. The raise in lipid peroxidation in the disease group leads to the damage of brain cells worsening the situation in diabetic people. But due to the activity of vildagliptin there was more reduction in the damaged part in the treatment group. Hence, it also showed the increased levels of glutathione and catalase in the treatment groups which shows the neuroprotection.

Table 4: Effect of vildagliptin on tissue anti-oxidant parameters

Animal groups	LPO (□mol/mg of tissue)	GSH (□mol/mg of tissue)	Catalase (□mol/mg of tissue)
Normal	2.92±0.43	6.15±0.68	0.55±0.06
Disease control	12.63±1.08*	2.60±0.21*	0.04±0.04*
Normal+Treatment	1.60±0.53*	10.38±0.75*	0.68±0.11*
Disease +Treatment	2.48±0.35*	11.71±0.87*	0.72±0.08*

Values were represented as Mean ± SEM. Statistical analysis performed using one way ANOVA followed by post hoc Dunnett's testand $p<0.05$ vs Disease control.

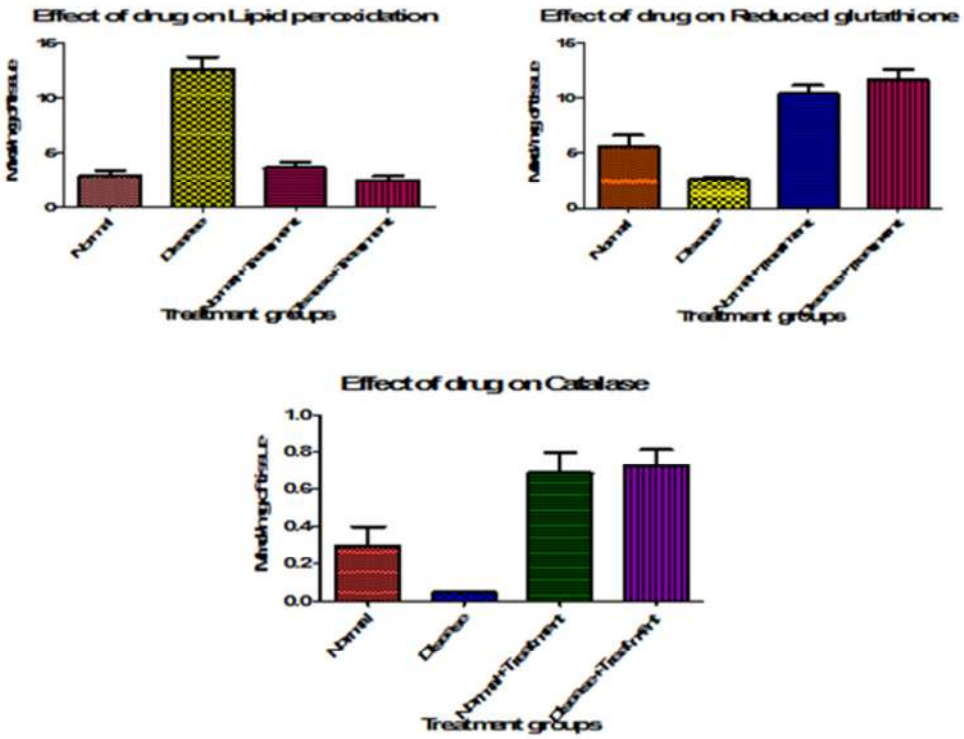


Figure 4: Graphical representation of effect of vildagliptin on tissue anti-oxidant parameters

CONCLUSION

This study was performed to evaluate the cerebroprotective effect of Vildagliptin in BCCAO induced cerebral infarction in type-2 diabetic rats. The experiment was carried out as per CPCSEA guidelines where the experimental animals were maintained in controlled environmental conditions. Animals were grouped into 4 with as normal, disease control, normal and treated and diseased and treated. Type-2 diabetes was induced by administering 40 mg/kg STZ. On the 4th week cerebral infarction was induced in rats by BCCAO method for 30 mins with 4 hour reperfusion and then animals were sacrificed and their brains were analyzed for stroke infarct volume. This is followed by the estimation of biochemical parameters such as lipid peroxidation, reduced glutathione and catalase which were compared with the diseased control group. The results obtained from this study confirmed the cerebroprotective ability of an anti-diabetic drug Vildagliptin in BCCAO induced cerebral infarction in type-2 diabetic animals.

References:

1) de la Torre JC. How do heart disease and stroke become risk factors for Alzheimer's disease? Neurological research. 2006 Sep 1;28(6):637-44.
2) Aydin S, Ozaras R, Uzun H, Belce A, Uslu E, Tahan V, Altug T, DUMEN E, Senturk H. N-acetylcysteine reduced the effect of ethanol on antioxidant system in rat plasma and brain tissue. The Tohoku journal of experimental medicine. 2002;198(2):71-7.
3) Bolay H, Dalkara T. Mechanisms of motor dysfunction after transient MCA occlusion: persistent transmission failure in cortical synapses is a major determinant. Stroke. 1998 Sep;29(9):1988-93.
4) Kalaria RN. The role of cerebral ischemia in Alzheimer's disease. Neurobiology of aging. 2000 Mar 1;21(2):321-30.
5) Caplan LR. Treatment of acute stroke: still struggling. Jama. 2004 Oct 20;292(15):1883-5.
6) Darsalia V, Ortsäter H, Olverling A, Darlöf E, Wolbert P, Nyström T, Klein T, Sjöholm Å, Patrone C. The DPP-4 inhibitor linagliptin counteracts stroke in the normal and diabetic mouse brain: a comparison with glimepiride. Diabetes. 2013 Apr 1;62(4):1289-96.
7) Garcia JH, Wagner S, Liu KF, Hu XJ. Neurological deficit and extent of neuronal necrosis attributable to middle cerebral artery occlusion in rats: statistical validation. Stroke. 1995 Apr;26(4):627-35.

8) Kalantaripour TP, Asadi-Shekaari M, Basiri M, Najar AG. Cerebroprotective effect of date seed extract (Phoenix dactylifera) on focal cerebral ischemia in male rats. J Biol Sci. 2012 Apr 1;12(3):180.
9) Gupta YK, Briyal S, Sharma U, Jagannathan NR, Gulati A. Effect of endothelin antagonist (TAK-044) on cerebral ischemic volume, oxidative stress markers and neurobehavioral parameters in the middle cerebral artery occlusion model of stroke in rats. Life sciences. 2005 May 20;77(1):15-27.
10) Gaur V, Kumar A. Protective effect of desipramine, venlafaxine and trazodone against experimental animal model of transient global ischemia: possible involvement of NO–cGMP pathway. Brain research. 2010 Sep 24;1353:204-12.
11) Gaur V, Kumar A. Behavioral, biochemical and cellular correlates in the protective effect of sertraline against transient global ischemia induced behavioral despair: possible involvement of nitric oxide-cyclic guanosine monophosphate study pathway. Brain research bulletin. 2010 Apr 29;82(1-2):57-64.
12) Gaur V, Kumar A. Effect of nonselective and selective COX-2 inhibitors on memory dysfunction, glutathione system, and tumor necrosis factor alpha level against cerebral ischemia reperfusion injury. Drug and chemical toxicology. 2012 Apr 1;35(2):218-24.
13) Majid AS, Marzieh P, Shahriar D, Zahed SK, Pari KT. Neuroprotective effects of aqueous date fruit extract on focal cerebral ischemia in rats. Pak J Med Sci. 2008 Oct 1;24(5):661-5.
14) Cuzzocrea S, Riley DP, Caputi AP, Salvemini D. Antioxidant therapy: a new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury. Pharmacological reviews. 2001 Mar 1;53(1):135-59.
15) Hussein AS, Alhadrami GA, Khalil YH. The use of dates and date pits in broiler starter and finisher diets. Bioresource Technology. 1998 Dec 1;66(3):219-23.
16) Siesjo BK. Brain Energy Metabolism John Wiley and Sons. Ltd, New York. 1978.
17) Napoli C, Palinski W. Neurodegenerative diseases: insights into pathogenic mechanisms from atherosclerosis. Neurobiology of aging. 2005 Mar 1;26(3):293-302.
18) Shohami E, Bass R, Wallach D, Yamin A, Gallily R. Inhibition of tumor necrosis factor alpha (TNFα) activity in rat brain is associated with cerebroprotection after closed head injury. Journal of Cerebral Blood Flow & Metabolism. 1996 May;16(3):378-84.

SP-03

NEUROPROTECTIVE EFFECT OF CITRULLUS LANATUS ETHANOLIC SEED EXTRACT ON CEREBRAL ISCHEMIC REPERFUSION INJURY INDUCED COGNITIVE IMPAIRMENT AND OXIDATIVE STRESS

GIRIJA PASHIKANTI[1], MAKULA AJITHA[2], GOVERDHAN PUCHCHAKAYALA[1]

[1]Centre for neurodegenerative disease and aging research department of pharmacology, Vaagdevi College of Pharmacy, Hanamkonda, Warangal 506001, India
[2]Centre for Pharmaceutical Sciences, JNTU, Kukatpally, Hyderabad-500085

ABSTRACT

Oxidative stress appears to be an early event involved in the pathogenesis of Alzheimer's disease (AD). The present study was designed to investigate the neuroprotective effects of ethanolic seed extract of *Citrullus Lanatus*on bilateral common carotid artery occlusion (BCCAO) induced cognitive impairment and oxidative stress in Wistar albino rats.Cognitive impairment and oxidative stress were induced by BCCAO for 30 minutes, followed by 7 days reperfusion of male wistar rats. Morris water maze and rectangular maze performance tests and locomotor activity were used to assess memory performance tasks. To study the activity, rats weighing 250-300g were pretreated withethanolicseed extract of *Citrullus Lanatus* (ESECL)400 mg/kg, 200 mg/kg, p.o of each for 10 days and the treatment was continued for another 7 days after cerebral ischemia. Various biochemical parameters like lipid peroxidation, Catalase, DPPH and AchE were also estimated in the brain after the treatment.There was significantly increased oxidative stress and cholinesterase activity with cognitive decline in the hippocampus in rats of BCCAO group as compared to sham operated ($p<0.05$). The animals treated with Donepezil, ESECL prevented the biochemical changes significantly ($p<0.001$) and there was significant ($p<0.01$) improvement in cognitive parameters compared to BCCAO treated rats.Thus, present study indicates neuroprotective effect of ESECLagainst BCCAO induced cognitive impairment and associative oxidative damage.

Key words: Antioxidant,bilateral common carotid artery occlusion,*Citrullus Lanatus*, cognitive impairment, oxidative stress.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerativedisease. Oxidative stress has been a major cause of neurotoxicity inAD and other neurodegenerative disorders. The *Citrullus lanatus* (Watermelon) belongs to family cucurbitaceae is one of the most popular species with high water content as high as 92% of the total weight**.** This plant has shownits pharmacological potential in different ailments. Pharmacological studies showthat *Citrullus lanatus*possesses antioxidant[4], anti-inflammatory[5], analgesic[6], anti-ulcer[7] and other activities. It is well known that plants have been a major source of natural antioxidants.

MATERIALS AND METHODS

Chemicals and drugs

Donapezil, Thiopentone sodium, Hydrogen peroxide, povidone-iodine powder, 5% w/w (Sri medical &surgicals) ethanol, ethyl acetate, n-hexane (Venkateshwara agencies) Acetylthiocholine iodide (Sigma Aldrich), Perchloric acid Formalin 10% (Finar Chemicals), DTNB (5,5-dithiobis (2- nitrobenzoic acid) reagent, DPPH (1,1-diphenyl-2- picrylhydrazyl) radical reagent (Sigma Aldrich).

Plant material and extraction method

The fresh watermelons were collected from local market of Warangal district, Telangana state, India. The seeds were separated from fruit and shade dried. The collected samples were authenticated morphologically by Dr. E. Narasimha Murthy, Department of botany,Satavahana University, Karimnagar-505002, Telangana state, India.The dried seeds of *Citrullus lanatus* were powdered in a blender. This fine powder was extracted

in soxhlet apparatus with ethanol for about 36 hours. The ethanolic extract was cooled, filtered. The filtrate was concentratedby using a rotary evaporator under reduced pressure till the concentrated mass was obtained.

Experimental Animals

Young male Wistar rats (200-250g) procured from mahaveera enterprises, medchal district-98 were used. All animals underwent surgery, they were keptin plastic cages with soft bedding under standard conditions of a 12-hour light/dark cycle with food and water *ad libitum*. The animal experiments were designed as per CPCSEA guideline and protocol of the experiments after the authorization of the Institutional Animal Ethical Committee (IAEC), Vaagdevi College of Pharmacy, Warangal (A.P) and India (1047/PO/Re/S/07/ CPCSEA, dated: 21/10/2017).

1.1. Experimental protocols

The rats were divided into five groups of 06 each. 1st group served as sham-operated control group, 2nd group served as BCCAO treated control group both groups were received vehicle i.e. distilled water. 3rd, 4th and 5th groups served as test groups i.e. drug treated BCCAO groups and received donapezil and ESECL at doses of 400 and 200 mg/kg respectively. The treatments were started once daily for 10consecutive days prior to the cerebral ischemia. On day 11, 60 minafter the last dose, all the groups received BCCAO for 30 minfollowed by reperfusion for 7 d.From the second (13) day afterinduction, the animals continued with the test substances for other7days, then the animals were assessed for behavioralparametersi.e Morris water maze, rectangular maze and locomotor activity. After behavioural study all the animals were sacrificed and their brains were isolated and subjected to biochemical analysis.[9]

1.2. Surgical procedure for bilateral common carotid artery occlusion

All surgical apparatus and surgical pad were sanitized with 70% ethanol before the surgery to keep away from any kind of infection and sepsis. Thiopentone sodium was used to anaesthetize rats at a dose of 50mg/kg, (i.p). Bilateral common carotid artery occlusion followed by 7 days reperfusion was performed according to the previous studies.[10,11]

Behavioral assessments

All animals were trained for 10 days prior to the BCCAO and drug administration.

1.2.1. Morris water maze test

The standard Morris water maze test was used to measure the capability of hippocampal-dependent spatial navigation learning and memory in rats, which was performed based on the method.[12]

1.2.2. Rectangular maze test

The rectangular maze test was used to evaluate the learning ability. This test was carried out by following method. [13,14]

1.2.3. Locomotor activity

The locomotor activity (horizontal activity) can be an evidence for the alertness of cerebral activity. The actophotometer was used to evaluate this activity, which was performed based on method.[15]

1.3. Dissection, Homogenization and biochemical examination

On day 21, after all behavioral assessments, animals were scarified by decapitation prior to deep anesthesia and brains were removed, weighed and homogenized with 10 times ice cold 0.1M phosphate buffer (pH 7.4). Then it was centrifuged at 3000 rpm at 4°C for 15 minutes and the supernatant was used for measurement of, lipid peroxidation (MDA), [16] catalase (CAT) [17], free radical scavenging activity (DPPH). [18] and acetyl cholinesterase (AchE). [19]

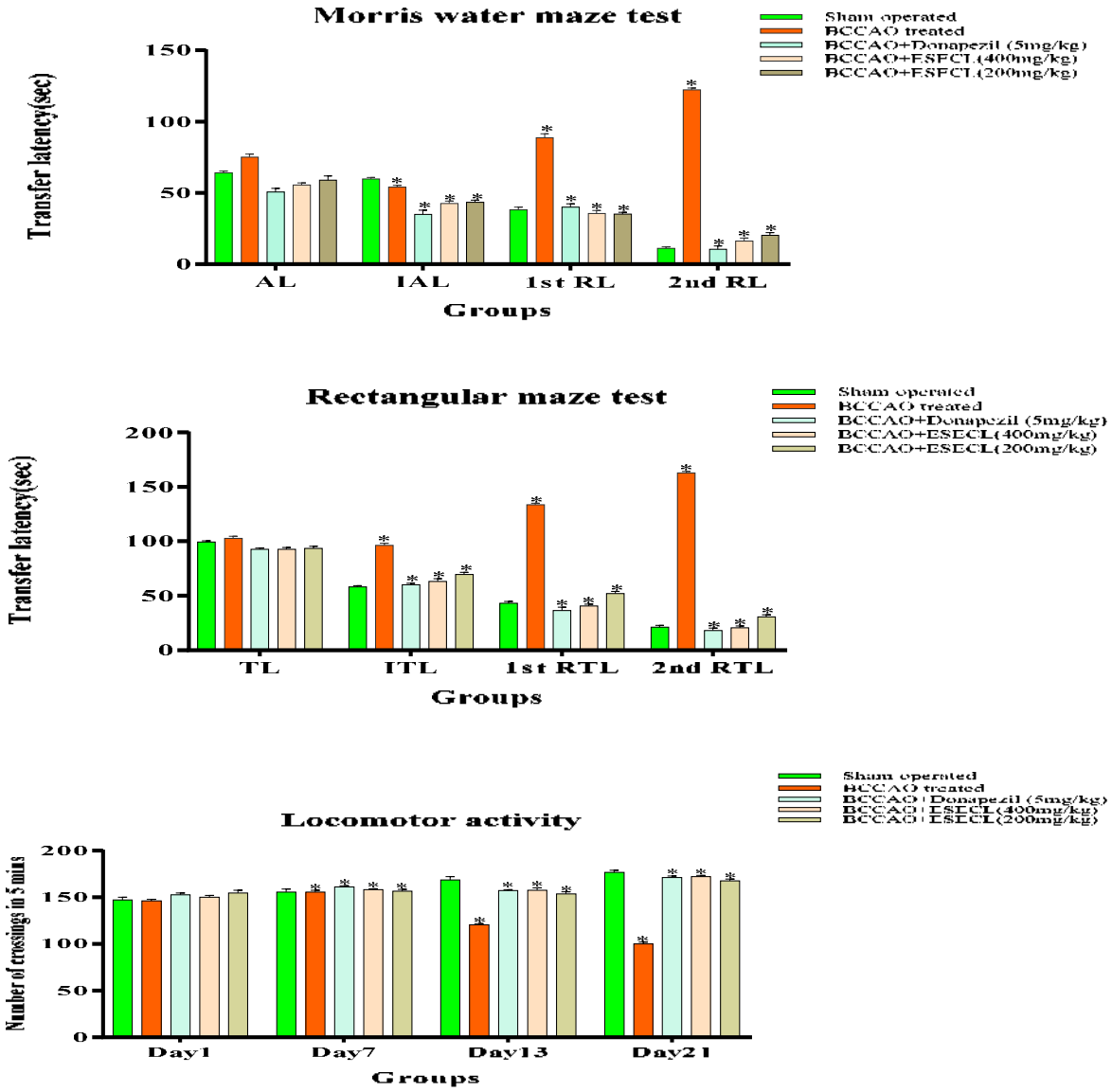
2. Results

2.1. Behavioral tests

Sham-operated, donapezil (5mg/kg, PO) and ESECL (400 and 200 mg/kg, PO) group of animals quickly learned to swim directly to the platform and passage from entry to reward chamber in the Morris water maze and rectangular maze respectively on day 12.From figure 1 there was a significant difference in

the mean IAL and ITL of BCCAO treated group compared to sham operated group on day 12 indicating BCCAO induced impaired acquisition of spatial navigation task ($P < 0.05$). In contrast, ESECL (400 and 200 mg/kg, PO) treatment after cerebral ischemia significantly decreased the IAL and ITL to reach the platform and reward chamber respectively in the pretrained rats as compared to BCCAO treated rats on day 12. Following training, the mean retention latencies (1st and 2nd RL) were significantly decreased in sham-operated, donapezil (5mg/kg, PO) and ESECL (400 and 200 mg/kg, PO) group rats on days 13 and 21, as compared to IAL on day 12 after induction. On the contrary, the performance in the BCCAO treated rats was changed after initial training in the water and rectangular maze on days 13 and 21, with significant increase in mean retention latencies compared to IAL and ITL respectively on day 12 The mean scores of locomotor activity for each rat were showed significant variation among different groups. The mean scores in sham-operated, donapezil (5mg/kg, PO) and ESECL (400 and 200 mg/kg, PO) rats cause significant increase in locomotor activity compared to BCCAO treated rats on days 13 and 21 as compared to pretraining locomotor activity on day 1 and 7. This result representing that ESECL (400 and 200 mg/kg, PO)significantly repaired the spatial cognitive andmemory deficits induced by ischemia in a dose dependent manner.

Fig. 1: Effect of Donepezil, ESECL on memory performance in a)water maze b)rectangular maze c) locomotor activitycompared to the BCCAO group.



Values are expressed as Mean ± SD (n = 6); * $p<0.01$as compared with corresponding values of BCCAOgroup.(One way ANOVA followed by dunnett's test) by using Graphpad Prism 7 statistical software.

2.2. Effect of the ESECL on anti-oxidant enzymes and AChE activity

From table 1 it was stated that cerebral ischemia followed by reperfusion caused a significant risein brain MDA levels and depletion of CAT, DPPH levels in BCCAOtreated animals as compared to shamoperated animals. However,chronic ESECL (400 and 200 mg/kg, PO)treatmentsignificantly (P<0.01) attenuated the increase in MDA levels and alsocaused a significant (P<0.01) increase in CAT and DPPH levels whencompared to the BCCAO treated rats. In order to test whether the positive effects of the ESECL on learning and memory in BCCAO treated rats were mediated by the cholinergic system, AChE activity were measured in the hippocampus of the experimental animals. BCCAO treatment caused a significant increase in AChE activity when compared to sham control. ESECL (400 and 200 mg/kg, PO) administration in BCCAO treated rats caused a significant decrease in AChE activity in the hippocampus. However,chronic ESECL (400 and 200 mg/kg, PO) treatmentsignificantly (P<0.01) attenuated enhanced AchE activity comparedto BCCAO treated rats.

Table 1.*Effect of donapezil, ESECL on BCCAO induced alteration in animals brain MDA, CAT, DPPH and AchE levels*

Treatment (mg/kg)	MDA (nmol/g tissue)	CAT(µmol of H_2O_2 min/mg tissue)	DPPH (Free radical scavenging %	AchE activity (µmol SH/g/min)
Sham operated	15.26±1.30	11.2±0.5	65.31±1.20	7.92±1.3
BCCAO treated	30.15±1.56	4.15±1.2	25.13±1.20	17.22±2.4
Donapezil- 5	16.59±3.60***	9.11±1.8***	48.9±2.6***	7.24±4.1***
ESECL – 400	20.65±4.20**	7.6±1.5***	45.31±2.50**	11.6±2.1**
ESECL – 200	21.1±3.2**	6.9±1.3**	39.18±4.10*	13.9±1.5*

Values are expressed as Mean ± SD (n=6) * p<0.05, ** p<0.01, *** p<0.001 as compared with corresponding values of disease control group (one way ANOVA followed by dunnett's test).

DISCUSSION

The present study investigated that the effect of ESECL in the prevention of cognitive impairment by using BCCAO followed by reperfusion induced rats. Significant findings of this study are that pre and post BCCAO treatment with ESECL improved cognition, decrease in brain MDA, AchE levels and increased CAT, DPPH activities. This illustrates that BCCAO followed by reperfusion characterized by progressive deterioration of learning and memory, oxidative stress and decrease in acetylcholine turnover. [20, 21]In the present study, BCCAO followed by reperfusion resulted in significant memory impairment in Morris water maze, rectangular maze and locomotory tasks which were attenuated by chronic administration with ESECL. This chronic treatment with ESECL was able to improve cognitive deficit and attenuated oxidative stress, suggesting that ESECL improves cognitive task based on its antioxidant effect as the seeds of watermelon are rich in antioxidant activity.our results also proved that the BCCAO followed by reperfusion showed the increased MDA levels which are more responsible for the oxidative damage in rats.A growing body of evidence supports the fact that free radicals are the most likely candidates responsible for producing neuronal changes mediating the behavioral deficits in AD. [24,25]

CONCLUSION

The present study suggests that chronicadministration of ESECL prevents BCCAO induced cognitiveimpairment and associated oxidative stress. It can enhance learning and memory in cognitive decline states. In addition, it also shows neuroprotective effect due to its antioxidant activity. Thus, the useof ESECL is promising for the treatment of AD and otherneurodegenerative disorders.

ACKNOWLEDGMENT

The authors would like to thank the Principal and Management of Vaagdevi College of pharmacy, Warangal for providing the necessary facilities to carry out this research work.

REFERENCES

1. Fratiglioni L, Ronchi D, Aguero-Torres H. Worldwide prevalence and incidence of dementia. Drugs & Aging. 1999;15(5):365–375.
2. Giacobini E. The cholinergic system in Alzheimer disease. Prog Brain Res. 1990; 84: 321–332.
3. CummingsJJL, MorstorfT, ZhongK. Alzheimer's disease drug-development pipeline: few candidates, frequent failures.Alzheimers Res Ther. 2014; 6(4) :37.
4. Rahman H, Manjula K, Anoosha T. In-vitro antioxidant activity of Citrullus lanatusseed extracts: Asian J Pharm Clin Res. 2013; 6 (3):152-157.
5. Madhavi P, Maruthi Rao, Kamala V, Habibur Rahman M, Eswaraiah C. Evaluation of Anti-Inflammatory Activity of Citrullus lanatus Seed Oil by In-vivo and In-vitro Models. Int Res J Pharm App Sci. 2012; 2(4): 104-10.
6. Kumari A, Rao J, Kumari J. Analgesic activity of aqueous extract of Citrullus Lanatus peels, Adv in Pharmacol and Pharma. 2013;1(3): 135-138.
7. Alok Bhardwaj, Rajeev Kumar, Vivek Dabas, Niyaz Alam. Evaluation of Anti-Ulcer Activity of Citrullus Lanatus Seed Extract in Wistar Albino Rats. International J of Pharmacy and Pharmaceutical Sciences.2012; 4(5): 135-139.
8. GuptaVK, SharmaSK. Plants as natural antioxidants. Natural Product Radiance. 2006; 5: 326–334.
9. Kalyani P, Ajith B, Sravanthi A, Goverdhan P. Neuroprotective Effect of Pyritinol and Fluvastatin in cerebral ischemic reperfusion injury and memory dysfunction: Advances in Biological Research. 2014; 8 (2): 68-78.
10. Vaishali RU, Shital SD, Swati KS, Chandrashekhar DU. Neuroprotective effect of cow colostrum and tetra methylpyrazine against global cerebral ischemia reperfusion injury, Int J of Nutrition, Pharmacology, Neurological Diseases. 2012; 2(2):111-120.
11. Rohini R, Neeraj SV, Virendra G. Evaluation of antioxidant and neuroprotective effect of date palm against bilateral common carotid artery occlusion in rats. Indian J of experimental biology. 2011; 49: 627-633.
12. Morris R. Developments of a Water- maze procedure for studying special learning in rat. J. Neurosci. Methods. 1984; 11: 47-60.
13. Agarwal A, Malini S, Bairy KL, Rao MS. Effect of Tinosporacordifolia on learning and memory in normal and memory deficit rats. Indian J pharmacol. 2002; 34:339-349.
14. Goverdhan P, Sravanthi A, Mamatha T, Neuroprotective Effects of Meloxicam and Selegiline in Scopolamine-Induced Cognitive Impairment and Oxidative Stress. Int J Alzheimer's Dis. 2012; 1-8.
15. Amites G, Jadupati M, Amitava G, Jyotirmoy D, Sanjay D, et al. The Central Nervous System Activity of *Barleriaprionitis*Linn the Locomotor Activity of swiss albino mice using Actophotometer,Int J of Pharmaceutical & Biological. 2012; 3 (2): 403-405.
16. Wills ED. Mechanisms of lipid peroxide formation in animal tissues.Biochemical Journal. 1966; 99(3): 667–76.
17. Luck H. Catalase. In:Bergmeyer HU Editor. Methods of Enzymatic Analysis. New York: Academic Press. 1971; 885–893.
18. Kaur IP, Geetha T. Screening Methods for Antioxidants A Review Mini Rev Med Chem. 2006; 48: 305-12.
19. Ellman GL, Courtney KD, Andres Jr V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity.Biochemical Pharmacology.1961; 7(2): 88–90.
20. Sorrenti V, Di Giaxomo C, Renis M, Russo A, La Della C, Perez-Polo JR. Lipid peroxidation and survival in rats following cerebral post-ischemic reperfusion: Effect of drugs with different molecular mechanisms. Drugs Exp Clin Res.1994; 20: 185–89.
21. Nakashima M, Niwa M, Iwai T, Uematsu T. Involvement of free radicals in cerebral vascular reperfusion injury evaluated in a transient focal cerebral ischemia model of rats. Free Rad Biol Med.1999; 26: 722–29.
22. Habiburrahman, Manjula K, Anoosha T, Nagaveni K, ChinnaEswaraiah M, Dipankar B.In-vitro anti-oxidant activity of citrullus lanatus seed extracts: Asian J of pharm and clin res. 2013; 6 (3): 152-57.
23. Sayre LM, Zelasko DA, Harris PLR, Perry G, Salomon RG, Smith MA. 4-hydroxynonenal-derivedadvanced lipid peroxidation end products are increased inAlzheimer's disease. J of Neurochemistry. 1997; 68(5): 2092–2097.
24. Cantuti-Castelvetri I, Shukitt-Hale B, Joseph JA. Neurobehavioralaspects of antioxidants in aging. InternationalJ of Developmental Neuroscience. 2000;18(4-5):367–381.
25. Maxwell SRJ. Prospects for the use of antioxidanttherapies. Drugs. 1995;49(3): 345–361.

SP-04

DEVELOPMENT AND CHARACTERIZATION OF ELETRIPTAN HYDROBROMIDE FASTDISSOLVING FILMS

MANNAM MOUNIKA[1] AND V LOKESWARA BABU[2]

Department of Pharmaceutics, Bhaskar Pharmacy College, Bhaskar nagar, Yenkapally (V), Moinabad (M), RR Dist., Hyderabad 500075, Telangana, India.

ABSTRACT

The aim of present study was to prepare fast dissolving films of eletriptan hydrobromide quick disintegration and faster dissolution with satisfactory taste in oral cavity. Film formulation can be taken within the pocket and patient can take it without need of water by simply putting it on the tongue without any grittiness that is frequently found during the disintegration of fast dissolving tablets. The developed formulation will disintegrate within a minute and ultimately provides good bioavailability and quick onset. The films were prepared by solvent casting method using various polymers as film former. The IR studies confirmed complete complexation of eletriptan hydrobromide with taste masking resin. The prepared formulations were evaluated for in vitro dissolution, disintegration time and physical properties. The optimized eletriptan hydrobromide film containing HPMC K15 and eudragit RS100, propylene glycol, glycerine showed better drug dissolution (more than 90% within 30 min) with satisfactory taste masking and other physicochemical properties. The development of fast dissolving film one of the alternative routes to provide quick onset of action.

Keywords: Eletriptan hydrobromide, FTIR Studies, Polymers, Solvent casting method, Fast dissolving film, Drug release studies.

INTRODUCTION

Oral route is the most preferred route of administration for systemic effect. About 60% of all the formulations are solid dosage form. Tablet is the most preferred dosage form due to ease of transportation, specially designed for the drugs which have extensive manufacturing and more patient compliance[1, 2.] Fast dissolving drug delivery system is a new generation delivery system also known as fast dissolving disintegrating film for the oral delivery of the drugs. The delivery system consists of a very thin oral strip which is simply placed on the patient's tongue or any other oral mucosal tissue and instantly gets wetted by saliva. The film rapidly hydrates onto the site of application. It then rapidly dissolves and disintegrates to release the medication for oro-mucosal absorption. It improves the efficacy of APIs by getting dissolved within few minutes in the oral cavity after coming in contact with saliva, without chewing and no need of water for administration[3.] Eletriptan hydrobromide is a selective 5-hydroxytryptamine 1B/1D (5-HT1B/1D) receptor agonist, used in the treatment of migraine attacks. The terminal elimination half-life of Eletriptan is approximately 4 hours, and is primarily metabolized by cytochrome P-450 enzyme CYP3A4 after oral administration[4, 5].

MATERIALS AND METHOD

MATERIALS

Eletriptan hydrobromide was collected as a gift sample from Hetero labs, Hyderabad, HPMCk15M, sodium alginate, eudragit RS100 and other excipients were purchased from AR chemicals.

METHODODOLOGY

FTIR Studies [6]

IR study was performed for polymer incompatibility studies with that of Eletriptan hydrobromide using Fourier transformed infrared spectrophotometry. The KBr disk technique was employed using 1:1 ratio of Eletriptan hydrobromide and various polymers. The study was repeated separately for each polymer blend with Eletriptan hydrobromide.

Formulation development

Table-1: Formulation Design of Eletriptan hydrobromide fast dissolving films

F.Code	Ingredients (mg)							
	Drug (mg)	HPMC k15M	Eudragit RS100	Sodium alginate	PEG	DMSO	Aspartame	Mannitol
HF1	50	500	-	-	1	0.1ml	1	10
F2	50		-	500	1	0.1ml	1	10
F3	50	-	500	-	1	0.1ml	1	10
1F4	50	250	-	250	1	0.1ml	1	10
F5	50	-	250	250	1	0.1ml	1	10
F6	50	250	250	-	1	0.1ml	1	10
F7	50	200	300	-	1	0.1ml	1	10
F8	50	300	200	-	1	0.1ml	1	10

Solvent casting technique [7]

The fast dissolving films composed of different ratios of HPMC, sodium alginate, Eudragit and containing Eletriptan hydrobromide were prepared using the petridish by solvent casting technique. Propylene glycol was incorporated as a plasticizer at a concentration of 10 % of dry weight of polymers. Backing membrane was casted by pouring and evaporated. Drug was transferred to the polymeric mixture containing different ratios of polymer, plasticizer and sweetener and flavormixed thoroughly with magnetic stirrer. The matrix was prepared by pouring 25 ml of the homogeneous solution on the backing membrane in a petridish and dried at 40^0C in the incubator. After 24 h the film was removed from the petridish, before removing the films was dried at 37^0 C for 1h, packed in aluminum foil and kept in desiccators until use.

Characterization of Eletriptan hydrobromide fast dissolving films

Physical appearance [8]

The films were observed visually for their physical appearance such as color, transparency and texture.

Measurement of average weight and thickness [9]

Three Fast dissolving films from each batch, as a whole (38 cm ^{2}) were weighed individually, and the average weights were calculated using digital balance. The thickness of these films was measured at six different points using thickness gauze (Mitutoyo, Japan). For each formulation, three randomly selected films were used.

Determination of drug content [10]

The drug contents of the Fast dissolving films were determined by dissolving 1 cm^2 of films in 100 ml phosphate buffer saline (pH 6.8) and shaken vigorously for 24 hours at room temperature. These solutions were filtered through Whatman filter paper (No. 42). After proper dilution, optical density was measured spectrophotometrically using a UV–VIS spectrophotometer (UV-1700 Double beam spectrophotometer, Lab India) at 220 nm against a blank.

Determination of folding endurance [11]

The folding endurance was determined manually for the prepared Fast dissolving films by repeatedly folding one films at the same place till it broke or folded upto 300 times without breaking. The number of times the Fast dissolving films folded at the same place without breaking or cracking gave the value of folding endurance.

Determination of moisture content [12]

The Eletriptan hydro bromide Fast dissolving films were weighed accurately and kept in desiccators containing anhydrous calcium chloride. After 3 days, the filmses were taken out and weighed. The moisture content (%) was determined by calculating moisture loss (%) using the formula:

Moisture content (%) =Initial weight – Final weight/Initial weightx 100

Determination of percentage moisture absorption [13]

The test was carried out to check the physical stability of the prepared Fast dissolving films at high humid conditions. The present study focuses on the moisture absorption capacity of the Fast dissolving films which was determined as follows. Three Fast dissolving films of 1 cm2 were weighed accurately and kept in a desiccator containing saturated solution of aluminium chloride, keeping 76% relative humidity inside the desiccators. After three days the films were removed from desiccator, weighed and percentage moisture absorption was calculated using following formula;

Moisture absorption (%) = Final weight - Initial weight/ Initial weightx 100

Disintegration time [14]

Disintegration time study was slightly modified to mimic the in vitro and in vivo conditions. For the study, 2 x2 cm film required for dose delivery was placed in a petri plate with 5 ml of pH 6.8 phosphate buffer. The time taken for the disintegration of the film was measured as the disintegration time.

In vitro release study [15]

The in vitro release of Eletriptan hydro bromide Fast dissolving films was carried out using Franz diffusion cell. The effective diffusion area was 1.74 cm^2. The receptor compartment (40 ml) was filled with phosphate buffer saline, pH 6.8, and its temperature was maintained at 37 ± 0.5°C. The films were applied under occlusion on the cellophane membrane fitted between the donor and receptor compartments of the diffusion cell. A 50 rpm stirring speed was applied using a magnetic stirrer. Five milliliters of the sample from receptor medium was withdrawn at regular intervals and replaced immediately with an equal volume of phosphate buffer saline, pH 6.8. The amount of Eletriptan hydro bromide released into the receptor medium was quantified by using UV–VIS spectrophotometer (UV-1700 Double beam spectrophotometer) at 220 nm against a blank.

Drug release kinetics [16]

In order to describe the Drug release kinetics from individual formulations, the corresponding dissolution data were fitted in various kinetic dissolution models.

- **Zero-order equation**

The equation for zero order release is $\mathbf{Qt = Q_0 + K_0 t}$

Q_0 = initial amount of drug,Qt = cumulative amount of drug release at time "t".(released occurs rapidly after drug dissolves.),K_0 = zero order release constant, t = time in hours

First order equation

The first order release equation is-

$$\mathbf{Log\ Qt = Log\ Q_0 + Kt\ /2.303\ OR\ Qt = Q0e\text{-}Kt}$$

Q_0 = initial amount of drug, Qt = cumulative amount of drug release at time "t".
K = first order release constant. , t = time in hours.

Stability Studies [17]

The Eletriptan hydro bromide fast dissolving films formulation having best drug content, drug release profiles both in vitro and ex vivo subjected to stability test. Formulation was stored in borosilicate glass bottles, flushed with nitrogen, and kept in stability chamber at /75% RH for a period of 3 months. A known amount of sample from the formulations subjected to stability testing was analyzed at pre determined time intervals for the drug content, in vitro release.

RESULTS AND DISCUSSION

Preformulation studies

In the present study 8 formulations with variable concentration of polymers were prepared and evaluated for physic-chemical parameters, in vitro release studies and stability studies.

Solubility

Eletriptan hydro bromide is a white to light pale colored powder that is freely soluble in water, ethanol, methanol, acetone and DMSO.

Melting point determination
The melting points were found to be in the range of 168°C-170°C
The reported melting point is 169°C.

FTIR Studies
Drug - excipient compatibility studies (FT-IR)
Drug Excipient compatibility studies shown that there is no observable changes in the peaks of Drug alone and drug with other excipients.

Physical appearance and surface texture of fast dissolving films
These parameters were checked simply with visual inspection of fast dissolving films and by feel or touch. The observation reveals that the fast dissolving films are having smooth surface and they are elegant in appearance.

Weight uniformity of fast dissolving films
The weight of the fast dissolving films was determined using digital balance and the average weight of all fast dissolving films. It was found to be in the range of 47.86±0.22 to 52.14±0.14 mg

Thickness of fast dissolving films
The thickness of the fast dissolving films was measured using screw gauge and the average thickness of all fast dissolving films. The thickness of the Fast dissolving films was found in between 0.19±0.20-0.27±0.18mm.

Folding endurance of fast dissolving films
The folding endurance gives the idea of flexible nature of fast dissolving films. The folding endurance was measured manually, fast dissolving films were folded repeatedly till it broke, and it was considered as the end point. The folding endurance was found optimum and the fast dissolving films exhibited good physical and mechanical properties and the average folding endurance of all fast dissolving films. All the films, except F6 showed good folding endurance (>76±0.12), indicated that the films have good flexibility.

Drug content uniformity of fast dissolving films
Eletriptan hydrobromide is a uniform dispersion of drug throughout the film. In each case three fast dissolving films were used and the average drug content was calculated. The F6 batch showed 97.26±0.24% drug content uniformity.

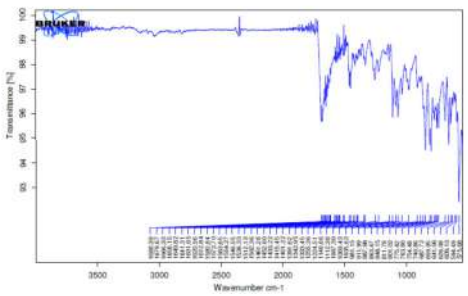
Disintegration time
The disintegration of the optimized formulation (F6) prepared with HPMC K15M and eudragit RS100 was 49.86±0.16sec respectively.

Moisture loss
The moisture loss in the patches ranged from8.75±0.27to 8.96±0.15%. The moisture loss in the Optimized formulation (F6) was found to be 8.86±0.24%.

Moisture absorption
The moisture absorption in the patches ranged from 9.92±0.40 to 10.30±0.28%. The Optimized formulation (F6) moisture uptake was found to be10.19±0.20%. The in-vitro drug release from film of all formulation was performed in triplicate using Franz diffusion cell apparatus. Dissolution study was performed in pH 6.8 phosphate buffer. F6 formulation about 99.32±0.50% of drug was released in 30 min.

Comparison studies
The comparison studies reveal that the drug release from the optimized formulation is much more as compared to marketed formulation.
Zero order & first order studies:

The drug release from the films was found to follow Zero order release based on the "r" value obtained for Zero order (0.997) and first order (0.9858) for F6 formulation.



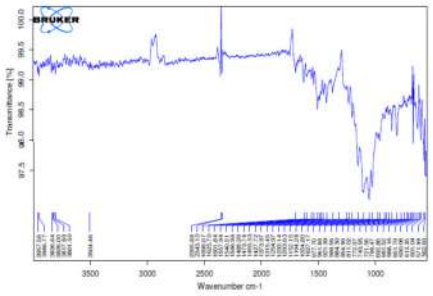


Fig-1 : FTIR graph of Pure Drug **Fig -2: FTIR graph of drug and excipient**

Table -2: Physicochemical evaluation data of Eletriptan hydrobromide Fast dissolving films

F. code	F1	F2	F3	F4	F5	F6	F7	F8
Thickness (mm)	0.26±0.23	0.20±0.15	0.27±0.18	0.22±0.30	0.25±0.18	0.23±0.32	0.20±0.28	0.19±0.20
Weight variation (mg)	49.93±0.19	48.93±0.20	52.14±0.14	50.10±0.34	51.25±0.22	47.86±0.22	51.18±0.24	46.62±0.24
Drug content Uniformity	94.41±0.25	97.16±0.26	95.84±0.32	96.82±0.36	94.50±0.33	97.26±0.24	98.82±0.14	91.25±0.28
Folding endurance	77±0.16	76±0.17	79±0.30	78±0.28	75±0.30	76±0.12	72±0.16	73±0.32
Disintegration time	48.23±0.20	51.15±0.22	49.38±0.28	50.17±0.18	53.96±0.28	49.86±0.16	50.21±0.20	45.63±0.35
Moisture content	8.96±0.15	8.78±0.18	8.90±0.23	8.75±0.27	8.91±0.30	8.86±0.24	8.93±0.32	8.91±0.20
Moisture absorption	10.26±0.42	10.52±0.38	9.92±0.40	10.23±0.32	10.24±0.26	10.19±0.20	10.21±0.23	10.30±0.28

Drug release studies

Table-3: *In vitro* release data of film F_1 to F_8

Time (min)	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F8
0	0	0	0	0	0	0	0	0
1	14.90±0.64	15.15±0.46	15.80±0.42	15.56±0.38	16.13±0.52	15.58±0.34	14.89±0.50	15.10±0.48
5	26.70±0.52	25.89±0.38	26.50±0.50	25.55±0.26	26.45±0.24	25.55±0.48	25.60±0.52	24.65±0.56
10	37.89±0.48	36.87±0.40	37.70±0.48	38.25±0.32	37.89±0.36	38.55±0.40	33.59±0.56	35.65±0.63
15	48.18±0.60	45.23±0.44	44.50±0.46	47.59±0.40	48.89±0.40	48.66±0.34	49.89±0.58	48.24±0.47
20	69.75±0.56	68.35±0.42	67.65±0.52	66.55±0.44	68.98±0.38	67.55±0.46	69.12±0.60	69.32±0.52
25	76.89±0.62	79.34±0.50	71.98±0.56	78.32±0.46	79.21±0.42	80.55±0.48	81.25±0.62	82.65±0.50
30	94.45±0.54	97.50±0.52	98.12±0.48	97.22±0.30	98.24±0.46	99.32±0.50	96.92±0.64	98.25±0.45

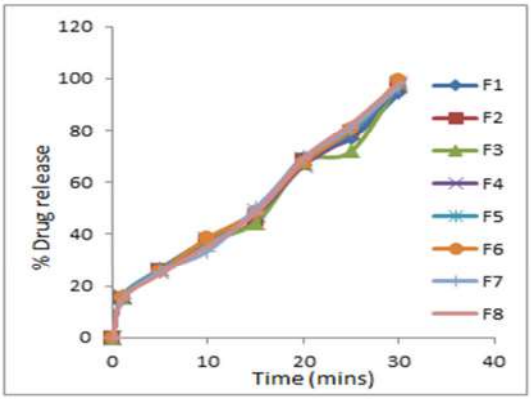


Fig-3: In vitro drug release of all formulation

The in-vitro drug release from film of all formulation was performed in triplicate using Franz diffusion cell

of drug was released in 30 min. This drug release pattern indicates that the increased concentration of polymer decreases drug release and increased concentration of plasticizer increases drug release.

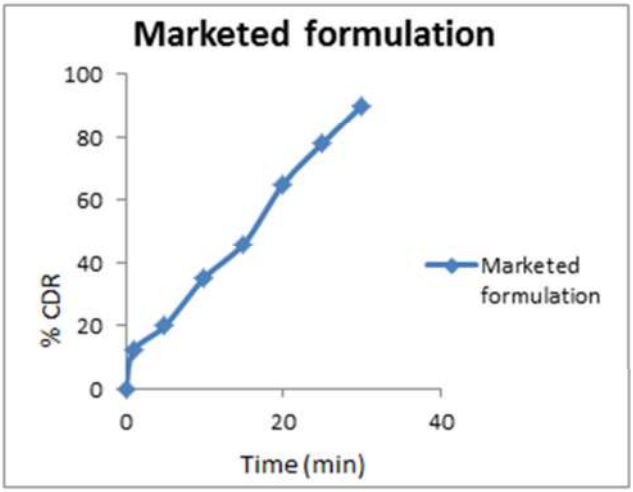


Fig-5: Drug release studies of marketed formulation

Comparison of marketed formulation

Table-6: Comparison of marketed formulation

Time	Optimized formulation (F6)	Marketed formulation
0	0	**0**
1	15.58±0.34	12.25±0.12
5	25.55±0.48	20.12±0.23
10	38.55±0.40	35.25±0.18
15	48.66±0.34	46.18±0.21
20	67.55±0.46	65.21±0.19
25	80.55±0.48	78.15±0.22
30	99.32±0.50	90.25±0.28

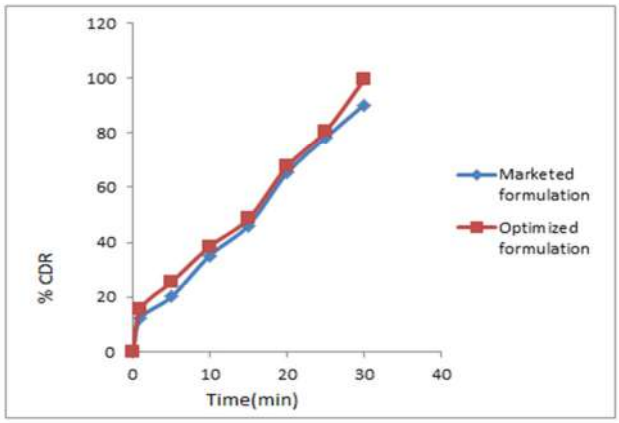


Fig-6: Drug release studies of comparison of Eletriptanhydrobromide formulation

Release order kinetics

Zero order kinetics:

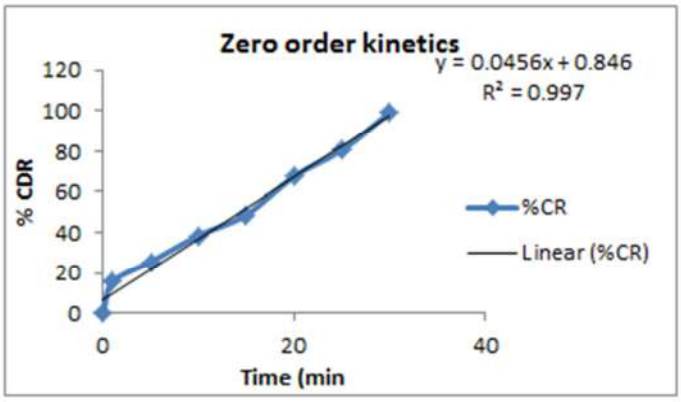


Fig-7: zero order plot for optimized formula

First order kinetics:

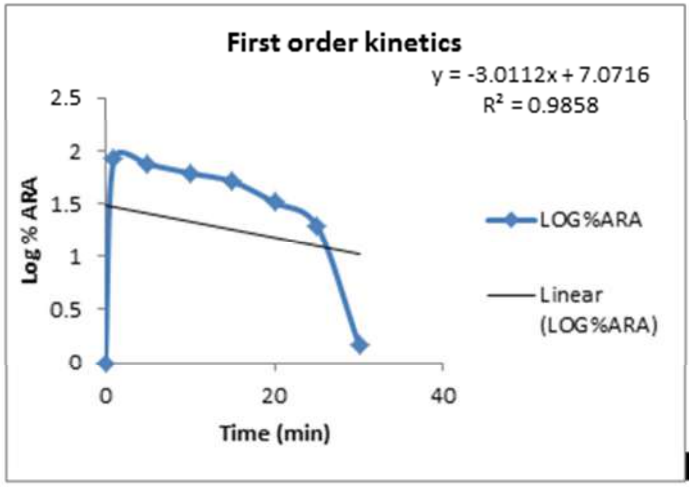


Fig-8: First order for optimized formula

Stability studies:

Optimized formulations F6 was selected for accelerated stability studies as per ICH guidelines. The fast dissolving films were observed for color, appearance and flexibility for a period of three months. The % cumulative drug release of the formulation was found to be decreasing. This decrease may be attributed to the harsh environment (40^0C) maintained during the studies.

Table-11: Stability studies of optimized formulations at 40 ± 2 ^{0}C and 75 ± 5% RH for 3 months

F. Code	Time (min)	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F6	25^{0}C/75%RH % Release	99.32±0.50	99.33±0.48	99.34±0.38	99.35±0.36	Not less than 85 %
F6	30^{0}C/75% RH % Release	99.32±0.50	99.34±0.42	99.35±0.32	99.36±0.45	Not less than 85 %
F6	40^{0}C/75% RH % Release	99.32±0.50	99.36±0.40	99.36±0.42	99.37±0.50	Not less than 85 %

Optimized formulations were intended for stability studies at 25±5 ^{0}, 30±5 ^{0} 40±5 ^{0}and 75±5% RH for three months. No significant change in physical properties and drug release of the films were observed.

CONCLUSION

From the present research work that is development and evaluation of Eletriptan hydro bromide fast films for buccal drug delivery, the following points can be concluded: The films prepared were elegant in appearance and smooth surface. The weights of films were uniform, the thicknesses of films were uniform, the films were completely dried. The films had good flexibility. There was no drug-excipients interaction between the drug and excipients used in the formulation. The drug was distributed throughout the patch uniformly. More than 90 % of the drug was released from all the formulations at the end of 30 min. In short term stability studies indicate there were no significant changes in the drug content and *in-vitro* drug release for the period of three month. Fast dissolving oral films have gained popularity because of better patient compliance; rapid onset of action, the drug is directly absorbed in systemic circulation. Eletriptan hydro bromide a second generation triptans used to treat migraine headaches. Migraine headache need fast onset of action. In this concept both are suitable candidates to prepare fast dissolving oral film technology. The developed fast dissolving film formulations of eletriptan hydro bromide will provide the alternative route for providing quick onset of action.

5. REFERENCES

1. Siddiquinehal, M.D., G. Garg and P.A. Sharma, 2011. International Research Journal of Pharmacy, Short review on A novel approach in oral fast 2: 69-74. Dissolving drug delivery system and their patents.
2. Kalyan, S. and M. Bansal, 2012. Recent Trends in Advances in Biological Research, pp: 291-303.
3. Bhyan B, Jangra S, Kaur M and Singh H. Orally fast dissolving films: innovations in formulation and technology. International Journal of Pharmaceutical Sciences Review and Research. 2011:50-57.
4. Himabindu S, Sathish D, ShaikShayeda. Formulation and Ex Vivo Evaluation of Buccal Tablets of EletriptanHydrobromide. Am. J. PharmTech Res. 2012; 2(3), 919-930.
5. Saini S, Nanda A and Hooda M. Fast dissolving films (FDF): innovative drug delivery system. 2011; 919-928.
6. Liang AC, Chen LH, 2001, Fast Dissolving Intraoral Drug Delivery Systems, Exp. Opin. Ther. Patents, 11,981.
7. Borsadia S, Halloran DO, Osborne JL, 2003. Quick Dissolving Films-A Novel Approach to Drug Delivery, Drug Delivery Technology, 3, 63.
8. Alpesh Patel R, DharmendraPrajapati S, JignyashaRaval A. Fast dissolving films (FDFs) as a newer venture in fast dissolving dosage forms. Int J Drug Dev Res 2010; 2:232-46.
9. DipikaParmar, Upendra Patel, BhavinBhimani, AditiTripathi, DhirenDaslania, Ghanshyam Patel. Orally fast dissolving films as a dominant dosage form for quick release. Int J Pharm Res Bio-sci 2012; 1:27-41.
10. Suresh B, Halloran D, James L. Quick dissolving films: a novel approach to drug delivery. Drug Dev Tech; 2006. p. 1-7.
11. SamitaGauri, Gaurav Kumar. Fast dissolving drug delivery and its technologies. Pharma Innovation 2012; 1:34-9.
12. Gowri R, Narayan N, Revathy S, Prabhavathy G, PreethyMol G, Rekha G. Melt in mouth films–an effective alternative drug delivery system. Int J Biol Pharm Res 2013; 4:645-50.
13. Kai Bin Liew, Yvonne Tze Fung Tan, Kok K KhiangPeh. Characterization of oral disintegrating film containing donepazil for the alzhemier disease. AAPS PharmSciTech 2012; 13:134-42.
14. Mona Nagar, Mayank Nagar, Vikram Chopra. Formulation and evaluation of mouth dissolving film of antipsychotic drug aripiprazole. Der Pharmacia Lettre. 2012; 4 (4):1221-1227.
15. Prabhakara P, Ravi M, Marina K, Vijaynarayana K, Ullas Souza D, Harish NM, Shastry CS. Formulation and evaluation of fast dissolving films of levocitirizinedihydrochloride. Int J Pharm Inves. 201; 1(2): 99-104. [16].
16. Nafee NA, Boraie MA, Ismail FA, Mortada LM. Design and characterization of mucoadhesivebuccal patches containing cetylpyridinium chloride. Acta Pharm. 2003; 53:199-212.
17. Mashru RC, Sutariya VB, Sankalia MG, Parikh PP. Development and evaluation of fast dissolving film of salbutamol sulphate. Drug DevInd Pharm. 2005; 31: 25-34.

SP-05

HEPATOPROTECTIVE ACTIVITY OF TABEBUIA ARGENTEA LEAF EXTRACTS AGAINST PARACETAMOL INDUCED LIVER DAMAGE INALBINO WISTAR RATS.

A SRINIVASA RAO[1], S SHOBHA RANI[2], M SRI RAMACHANDRA*[3,]

[1,3]Bhaskar Pharmacy College, Yenkapally (V), Moinabad (M), Telanagana, India.
[2] Institute of Science & Technology, JNTUH, Hyderabad, India.

ABSTRACT

The study was designed to evaluate *in vitro* antioxidant and hepatoprotective activity of *Tabebuia argentea* leaf extracts against Paracetamol induced liver damage in albino Wistar rats.The *in vitro* antioxidant activity was evaluated by measuring superoxide radical, hydrogen peroxide radical, hydroxyl radical, nitric oxide radical reducing power and estimation of phenolic content of petroleum ether, methanolic and aqueous extracts of *Tabebuia argentea*. Hepatoprotective activity of the extracts was screened against Paracetamol (3gm/kg b.w) at doses of 200 and 400 mg/kg by estimating biochemical parameters (SGPT, SGOT, SALP, TB and TP), physical parameters (liver weight, liver volume) and histopathological changes in liver with silymarin (50mg/kg, b.w.) as standard.Methanolic, aqueous and Pet.ether extracts of plant showed good antioxidant activity. Administration of plant extracts resulted in significant reduction in SGPT, SGOT, SALP and TB and increase in TP as compared to disease control group. They also reduced the liver weight and liver volume. In plant extract treated groups histological aberrations were reduced as compared to disease control. Among all the extracts the META showed a significant effect at high dose (400 mg/kg) ($p< 0.001$) compared to lower dose. This evidence showed that the plant has antioxidant and hepatoprotective activity. From the results it can be concluded thatthe*Tabebuia argentea* possesses antioxidant and hepatoprotective effect against Paracetamolinduced liver damage in rats.

Key words: ***Tabebuia argentea*, Paracetamol, Silymarin and Hepatotoxicity**

1. INTRODUCTION:

Liver is a vital organ which maintains homeostasis by performing various activities like production of bilirubin, plasma proteins, metabolism of carbohydrates, lipids, proteins and detoxification of chemicals and drugs etc[1]. *Tabebuia argentea* (*Bignoniaceae)* is a flowering trees and commonly called as 'silver trumpet tree with Silvery gray leaves, bark is corky, Leaves are palmate, opposite, 11 inches long and 4 inches wide.[3] They are rich source of many organic compounds, especially, of phenolic and polyphenolic substances. It was intended to investigate the hepatoprotective activity of *T.argentea* using paracetamol model.

2. MATERIALS AND METHODS:

2.1 Preparation of plant extracts: The leaves of *Tabebuia argentea* were collected and authenticated by Dr.*Madhava Setty, d*epartment of Botany, S.V University, tirupati. The leaves were shade dried and powdered. The powder was subjected to successive solvent extraction by using petroleum ether, methanol and water. Then the dried extract was obtained by evaporation of the solvent using a rotatory vacuum evaporator at 50 ºC and kept in dessicator and studied for phytochemical, *in vitro* antioxidant activity and examined for their hepatoprotective activity in rats.

2.2 *In Vitro* antioxidant studies: Petroleum ether, methanol and aqueous extracts were subjected for *in vitro* antioxidant studies namely viz., superoxide[4], hydrogen peroxide[5] , nitric oxide [6] hydroxyl radical scavenging activity [5] and reducing power[7]. Its total phenol content [8]were also studied.

2.3. Experimental animals:

Healthy Wistar albino rats weighing 170-180g were acclimatized for7 days and selected for study. Feed and

water were given *ad libitum* throughout the study. All the animal experiments were conducted according to the ethical norms approved by the Institutional ethical committee of CPCSEA, New Delhi (Reg.No: 1722/Ro/Ere/S/13CPCSEA).

2.4. Paracetamol induced hepatotoxic model: To evaluate the hepatoprotective potential of *T.argentea*in Paracetamol (PCM) - induced hepatic damage, rats were randomly divided into nine groups. Group I served as a control, Group-II is served as disease control(3gm/kg)[9], Group III received daily oral dose of Silymarin (50mg/kg b.w.), Group IV and V received once daily oral dose of 200, 400mg/kg b.w. of PETA respectively along, Group VI and VII received once daily oral dose of 200, 400mg/kg b.w. of META, Group VIII and IX received once daily oral dose of 200, 400mg/kg b.w. of AQTA respectively for 7 days. Except group I all were treated with single dose administration of PCM 3gr/kg oral route. Liver parameters were estimated by colleting the blood from retro orbital plexus. They includes serum enzymes SGPT, SGOT by (uv kinetic method), ALP by by p-NPP method by Schlebusch *et al.*, 1974.[10] Total Bilurubin by taylor RLS *et al*, 1996[11] and Total Protein by Lowry's method by Dunn *et al*, 1992 were estimated. Physical parameters like liver weight and liver volume were determined.

3. STATISTICAL ANALYSIS:

The data was represented as mean ± SEM. The statistical significance was analyzed by using one way analysis of variance (ANOVA) followed by Dunnett multiple comparisons test using Graph Pad Instat 3.

4. RESULTS AND DISCUSSION:

4.1. Phytochemical investigations of *T. argentea*:

Phytochemical investigations of *Tabebuia argentea* showed the presence of carbohydrates glycosides, saponins, alkaloids, phytosterols, proteins, phenolics, tannins and flavonoids in its methanolic extract whereas aqueous extract of the plant showed similar constituents except phytosterols. Petroleum ether extract showed presence of fats and fixed oils.

4.2 Estimation of total phenolic content:

The methanolic extract contains more Phenolic content (69.23 mg GAE/ g of extract) compared to petroleum ether (53.21 mg GAE/ g of extract) and aqueous extract (10.32 mg GAE/ g of extract).

4.3*In vitro* antioxidant studies:

Table: 1 Effect of T. argentea leaves extracts against superoxide, hydrogen peroxide, nitric oxide and hydroxyl radicals and its reducing power.

Free radical scavenging activity (% Inhibition)						
Compound	Conc µg/ml	superoxide	hydrogen peroxide	nitric oxide	hydroxyl	reducing power
ascorbic acid	100	45.14	44.13	34.23	41.41	41.77
	200	48.14	50.35	50.47	49.59	43.98
	300	50.06	54.57	55.07	53.93	47.99
	400	52.67	59.5	59.67	59.22	55.32
	500	55.5	62.32	64.14	61.31	60.3
Pet ether Extract	100	32.92	37.37	22.19	28.08	19.64
	200	37.31	41.46	25.3	32.1	26.69
	300	41.7	44.47	27.6	34.34	29.46
	400	43.8	48.07	32.2	39.64	32.36
	500	48.55	50.48	33.69	43.49	34.3
Methanol	100	37.99	41.78	38.29	25.04	32.78

	200	43.43	45.77	42.76	32.42	40.24
	300	46.63	49.29	44.51	37.39	41.77
	400	49.38	52.11	47.63	42.21	45.78
	500	53.08	54.81	50.6	47.51	51.31
Aqueous Extract	100	41.01	40.14	37.07	24.71	31.12
	200	44.85	45.3	41.54	29.21	34.16
	300	47.73	50.96	44.24	34.18	40.24
	400	48.97	53.48	47.63	40.44	41.77
	500	51.44	55.28	51.15	46.7	46.47

Oxidative stress is the root cause of many aliments results in production of free radicals. They are highly unstable in nature and they attack on various cellular components leads to lipid peroxidation and subsequence damage. Antioxidants are the substances which quench these free radicals and protect the tissues and organs from their invasion. The free radical scavenging activity of petroleum ether, methanolic and aqueous extracts of *T. argentea* was carried out. Three extracts of *T. argentea* showed dose dependent inhibition of hydroxyl, hydrogen peroxide, superoxide, nitric oxide and their reducing ability. The superoxide and hydroxyl radical scavenging activity of methanolic extracts were found to be relatively better than that of aqueous and petroleum ether extracts. Whereas hydrogen peroxide and nitric oxide scavenging activity and inhibition of reducing power of the free radicals of methanolic extracts was found to be prominently better than that of aqueous and petroleum ether extracts. The total phenolic content of methanolic extract of *T. argentea* which might be the reason for its better activity compared to petroleum ether and aqueous extracts.

4.3 Paracetamol induced hepatotoxicity:

Table: 2 Effect of on T.argentea various biochemical parameters in PCM Induced hepatotoxicity model in rats.

Groups	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII	Group X
AST	62.84±2.18	237.89±4.27$^{\#\#}$	78.12±2.10**	210.9±4.08**	175.7±3.31**	169.50±2.77**	**119.6±4.40****	171.8±3.38**	135.89±2.82**
ALT	86.23±1.99	290.45±4.16$^{\#\#}$	119.85±3.03**	251.3±3.90**	231..82±3.92**	201.40±2.40**	**145.8±3.51****	211.99±3.20**	161.02±5.71**
ALP	131.03±3.76	334.17±6.46$^{\#\#}$	138.02±1.27**	311.69±3.91**	281.53±4.67**	243.65±4.51**	**199.1±3.32****	249.1±7.11**	215.61±3.70**
TBL	0.525±0.05	6.23±0.12$^{\#\#}$	0.95±0.04**	5.19±0.05**	4.87±0.08**	3.78±0.14**	**2.21±0.06****	3.81±0.11**	2.56±0.09**
TP	8.07±0.21	2.96±0.06$^{\#\#}$	6.87±0.07**	3.6±0.10**	3.87±0.04**	3.89±0.07**	**4.24±0.06****	3.78±0.13**	3.98±0.14**
Liv.Wt	3.51±0.10	6.11±0.11$^{\#\#}$	4.23±0.08**	5.89±0.04^{ns}	5.97±0.05^{ns}	5.13±0.05**	**4.98±0.05****	5.33±0.05**	5.1±0.04**
liv.Vol	4.54±0.11	6.72±0.16$^{\#\#}$	5.01±0.04**	6.51±0.17^{ns}	6.3±0.05*	6.12±0.06**	**5.9±0.04****	6.22±0.07**	6.09±0.06**

Values were expressed as Mean±SEM (n=6), the statistical significance was analyzed by using one way analysis of variance (ANOVA) followed by Dunnett multiple comparisons test using GraphPadInstat3. P values: $^{\#}$P< 0.05 or *P< 0.05 (Significant), $^{\#\#}$P< 0.01 or **P< 0.01 (Highly significant) and compared to normal control group. **Values were highly significant at P< 0.01when compared to control group.^{ns}Values were Not significant at P> 0.05 when compared to toxic control group. (#-Vs Normal control; * - Vs disease control)

At higher doses, paracetamol has the potential to damage hepatocytes and cause hepatotoxicity. It undergoes metabolism/ bioactivation to release a reactive metabolite known as N-acetyl paraquinone imine (NAPQI).The levels of these three enzymes were found to increase in disease control group indicating the induction of hepatotoxicity by paracetamol. Amelioration of the levels of these enzymes after treatment with petroleum ether, methanolic and aqueous extracts of *T. argentea* indicate their protective effect against paracetamol induced hepatotoxicity. During haptopathy there will be increase in bilirubin levels due to the damage of hepatocytes. Prior treatment with various extracts of *T.argentea* significantly reduced total bilirubin level. During the hepatotoxicity the proteins of the liver tissue undergo denaturation under the catalytic influence of NAPQI. Elevation of the total protein content in treatment groups indicates antidenaturation effect of the plant. Inflammatory changes in hepatocytes leads to increase in liver weight and volume which indicate hepatotoxicity as observed in group II. Reduction in these parameters after treatment with extracts indicate their hepatoprotective activity. (Table: 2).

5. CONCLUSION:

Our investigations established that *T.argentea* showed *in vitro* antioxidant and hepatoprotective activity in PCM model in a dose-dependent manner. It should be further evaluated clinically to substantiate its use for liver related disorders.

REFERENCES:

1. Harshmohan. Textbook of pathology, Jaypee brother's medical publishers, Sixth edition, 2010,Page:593.
2. Puja Ranka, Karthik . A comparative study of *In-Vitro n*itric oxide scavenging activity of Balofloxacinvs Prulifloxacin. Asian journal of pharmaceutical and clinical research. 2017January: 1(10):380-382.
3. P. Udaya Chandrika, A. Srinivas Rao, M. Sri Rama Chandra. Antinociceptive and anti-inflammatory activity of *Tabebeuia Aurea* leaf extracts. International Journal of Ayurvedic and Herbal Medicine.2014 july-august;4(4):1520-1526.
4. Elizabeth K, Rao MNA. Oxygen radical scavenging activity of curcumin. International journal of Pharmaceutics. 58(3), February 1990; 237– 40.
5. M. Rama Prabha and K.Vasantha. Antioxidant, Cytotoxicity and Polyphenolic Content of *Calotropis procera* (Ait.) R. Br.Flowers. Journal of Applied Pharmaceutical Science, 01 (07), 2011; 136-40.
6. Puja Ranka and Karthik VP. A Comparative Study of *In-Vitro* Nitric oxide scavenging activity of Balofloxacin vs Prulifloxacin. Asian Journal of Pharmaceutical and Clinical Research,10(1), 2017; 380-82.
7. K. Himakar Reddy, P.V.G.K.Sharma& O.V.S. Reddy. A comparative *in vitro* study on antifungal and antioxidant activities of *Nervilia aragoana* and *Atlantia monophylla*. Pharmaceutical Biology, 48(5), 2010; 595-602.
8. K. Himakar Reddy, P.V.G.K. Sharma, O.V.S. Reddy. A comparative *in vitro* study on antifungal and antioxidant activities of *Nervilia aragoana* and *Atlantia monophylla*. Pharmaceutical Biology. 2010 April; 48(5): 595-602.
9. B. Hemabaratrhy, sitibalkis budin. Paracetamol hepatotoxicity in rats treated with crudeextract of *alpinia galangal*. Journal of biological sciences. 2009. 9(1): 57-62.
10. Sridhar K, Narayanan M, Goswami K. Measurement of Total Alkaline Phosphatase Activity in Human Serum using Charcoal Activated Para-Nitro Phenyl Phosphate. International Journal of Science and Research. 2016 April; 5(4): 1315-1317.
11. Garima Mishra, R. L.Khosa1, PradeepSingh, K.K.Jha. Hepatoprotective potential of ethanolic extract of Pandanus odoratissimus root against paracetamol-induced hepatotoxicity in rats. Journal of Pharmacy and Bioallied Sciences. 2015 January- march; 1(7): 45-48.

SP-06

INVITROANTI-INFLAMMATORY ACTIVITYOF ETHANOLIC PEEL EXTRACT OF HYLOCEREUS UNDATUSFRUIT

RAJESHAM V.V*, HIMA SOWMYA KANDOTI, LIKITHA KANNA, KAUSHIK M GAWALI, RAGHAVENDRA M, KIRAN KUMAR MV, ABBULU KONDE

Department of Pharmacology, CMR College of Pharmacy, Kandlakoya, Medchal Dist., Telangana-501401, India.Email-id:yvrajesham@gmail.com

ABSTRACT:
Hylocereus undatus has been proven to have various medicinal properties. The present study aims to evaluate *in vitro* anti-inflammatory activity of ethanolic peel extract of *Hylocereus undatus* fruit. The dried peels were ground into coarse powder with the help of grinder. The dry powder was subjected to cold extraction with ethanol (95%) for a week with occasional stirring. After a week, the content was filtered under vacuum. The solvent was evaporated in order to obtain the extract. At highest concentration the extract produced 73 ± 0.1% and 83.3 ± 0.5% inhibition of protein in Albumin denaturation and Mizushima-Kobayashi method respectively. On the basis of these findings, it may be inferred that *Hylocereus undatus* has significant *in vitro* anti-inflammatory activity.

Keywords : Anti-inflammatory activity, Hylocereus undatus, Albumin denaturation.

INTRODUCTION

Inflammation is a part of the body's immune response to injury. It involves a well-organized cascade of fluidic and cellular changes. It is recognizable grossly and histologically and has both beneficial and detrimental effects locally and systemically. The term inflammation is taken from the latin word "inflammare" (to burn) (de oliveira).[1,2] *Hylocereus undatus*, the white-fleshed pitahaya, is a species of Cactaceae and is the most cultivated species in the genus[3,4]. It is a sprawling or vining, terrestrial or epiphytic cactus. It is a native fruit originating from Mexico and Central and South America[5], the pitahaya or dragon fruit. Dragon fruit peel is traditionally used for various skin problems like ageing of skin, burns, etc. Earlier studies suggested that the fruit, peel and flowers of *Hylocereus undatus* has significant anti-oxidant, anti-diabetic, hepatoprotective and anti-hyperlipidaemic activity. This works aims to evaluate the invitro anti-inflammatory activity of ethanolic peel extract of *Hylocereus undatus* fruit[6].

MATERIALS AND METHODS

Drugs and Chemicals

95% ethanol, Molisch reagent, Fehling's A, Fehling's B, Benedict's solution, dil. HCl, conc. H_2SO_4, ruthenium red, aqueous KOH, acetic anhydride, chloroform, Dragendroff's reagent, Mayer's reagent, Hager's reagent, Wagner's reagent, ammonium hydroxide, sodium hydroxide, lead acetate solution, Bromine water, Dil. Iodine solution, Dil. HNO_3, Sudan Red III, egg albumin, Diclofenac Sod., phosphate buffer, 1N HCl, Dimethyl formamide obtained from SD Fine-Chem, Mumbai.

Plant Material

The plant ***Hylocereus undatus*** was selected based on its medicinal value. Healthy-looking, white-fleshed pitaya fruits which were free of bruises, rotting and odours were selected and obtained from the local market (Hyderabad, Telangana). The fruits were then cleaned of dirt and other extraneous matter. T he fruits were cut open with a clean knife and peels were separated manually. The peels were shade dried for a week at a temperature not exceeding 45^{o}C.The dried peels were ground into coarse powder by the use of grinder. The dry powder is stored in an air-tight container for use in the study.

Extraction and preparation of plant sample

The powdered plant material was subjected to cold extraction with ethanol (95%) for a week with occasional stirring. After a week, the content was filtered under vacuum. The solvent was evaporated in order to obtain the extract. The crude extract was collected and used for anti-inflammatory activity studies.

Preliminary Phytochemical screening

The ethanolic extract of dragon fruit peel was subjected to preliminary screening of phytochemical constituents. Phytochemical analysis was carried out for the presence of alkaloids, tannins, fixed oils, steroids, gums and mucilage, carbohydrates, glycosides, reduced sugars and flavonoids[7].

***In vitro* Anti-Inflammatory activity**

Anti-inflammatory activity was determined using Albumin denaturation Method and Mizushima-Kobayashi Method.

Albumin Denaturation method

Denaturation of tissue proteins is a well documented cause of inflammatory diseases. Production of auto antigens in certain inflammatory diseases may be due to denaturation of proteins *in-vivo*. The *in-vitro* anti-inflammatory effect of dragon fruit peel extract was evaluated against denaturation of egg.

The following procedure was followed for evaluating the percentage inhibition of protein denaturation:

- **Preparation of Phosphate buffer (pH 6.4)**
  Dissolve 2.5 g of disodium hydrogen phosphate, 2.5 g of sodium dihydrogen phosphate and 8.2 g of sodium chloride in 950 ml of water. Adjust the pH of the solution to 6.4 with 1 M sodium hydroxide or 1 M hydrochloric acid, if necessary. Dilute to 1000 ml with water.
- **Control solution (50ml)**
  2ml of egg albumin, 28 ml of phosphate buffer (pH 6.4) and 20ml distilled water.
- **Standard solution (50ml)**
  2ml of egg albumin 28ml of phosphate buffer and various concentrations of standard/reference drug (Diclofenac Sod.). Concentrations of 100, 200, 400, 800 and 1000 µg/ml.
- **Test solution (50ml)**
  2ml of egg albumin, 28ml of phosphate buffer and various concentrations of extract (*Hylocereus undatus* ethanolic fruit extract. Concentrations of 100,200,400,800 and 1000µg/ml.

All of the above solutions were adjusted to pH using a small amount of 1N HCl. The samples were incubated at 37^{o}C for 15mins and heated at 70^{o}C. Diclofenac Sodium was used as standard drug. The absorbance was values were measured at 276nm. After cooling the absorbance of the above solutions percentage inhibition of protein denaturation was calculated by using the given formula.

Percentage inhibition = [Vt / Vc -1] x 100

Where,
Vt = Absorbance of test sample.
Vc = Absorbance of control.
The experiment was performed in triplicate and average was taken[8].

Mizushima – Kobayashi method

The ethanolic extract of *Hylocereus undatus* fruit peel was screened for anti-inflammatory activity using inhibition of albumin denaturation technique which was studied according to Mizushima and Kobayashi with slight modification. The standard drug and extract were dissolved in minimum quantity of dimethylformamide (DMF) and diluted with phosphate buffer (0.2M, pH 7.4). Final concentration of DMF in all was less than 2.5%. Test solution (1ml) containing different concentrations of drug was mixed with 1 ml of 1mM albumin solution in phosphate buffer and incubated at 27±1 °C in BOD incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60±1 °C in water bath for 10 min. After cooling, the turbidity was measured at 276 nm. Percentage of inhibition of denaturation was calculated from

control where no drug was added. The experiment was done in triplicate and average is taken. Diclofenac Sodium was used as standard drug. The percentage inhibition of denaturation was calculated by using following formula[9].

Percentage inhibition = [Vt / Vc -1] x 100

Where,
Vt = Absorbance of test sample.
Vc = Absorbance of control.

Statistical analysis:

Data was represented as mean ±SD, which was statistically analyzed by Student's t-test and p.

2. RESULTS AND DISCUSSIONS

Preliminary Phytochemical Screenings

The preliminary phytochemical investigation revealed that the ethanolic extract of *Hylocereus undatus* peel contains carbohydrates, flavonoids, alkaloids, tannins, steroids, fats and oils.

Table 1: Preliminary phytochemical screening

Phytochemical Compounds	Results
Carbohydrates	+
Alkaloids	+
Steroids	+
Mucilage	+
Flavonoids	+
Tannins	+
Fats and Oils	+

Albumin Denaturation Method

Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti inflammation activity, ability of extract to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation (Table 2). Maximum inhibition of 73.8% was observed from leaf extract at the concentration of 10 µg/ml. Diclofenac sodium, a standard anti-inflammation drug showed the maximum inhibition 68.1% at the concentration of 10µg/ml.

Table2: Effect of ethanolic peel extract of *Hylocereus undatus* and Diclofenac sodium on protein denaturation

Concentration (µg/ml)	% Inhibition Std.	% Inhibition Test
2	17.4 ± 0.2%	35.4 ± 0.3%
4	19.6 ± 0.1%	38.05 ± 0.15%
6	42.8 ± 0.1%	52.2 ± 0.2%
8	58.2 ± 0.3%	64.3 ± 0.4%
10	68.1 ± 0.2%	73.8 ± 0.1%

Values are expressed as Mean ± Standard deviation.

Mizushima-Kobayashi Method

The ethanolic peel extract was effective in inhibiting heat induced albumin denaturation (Table 3). Maximum inhibition of 83.3% was observed from leaf extract at the concentration of 10 µg/ml. Diclofenac sodium, a standard anti-inflammation drug showed the maximum inhibition 68.5% at the concentration of 10µg/ml.

Table 3: Effect of ethanolic peel extract of *Hylocereus undatus* and Diclofenac sodium on protein denaturation

Concentration (µg/ml)	% Inhibition Std.	% Inhibition Test
2	16.6 ± 0.3%	25.9 ± 0.1%
4	44.4 ± 0.2%	35.1 ± 0.2%
6	46.2 ± 0.3%	62.9 ± 0.12%
8	57.4 ± 0.4%	68.5 ± 0.2%
10	68.5 ± 0.14%	83.3 ± 0.5%

Values are expressed as Mean ± Standard deviation

CONCLUSION

Results obtained from the study demonstrate that ethanolic peel extract of *Hylocereus undatus* fruit inhibited protein denaturation in concentration dependent manner. At highest concentration the extract produced 73 ± 0.1% and 83.3 ± 0.5% inhibition of protein in Albumin denaturationand Mizushima-Kobayashi method respectively. Therefore, form the results of the present study it can be concluded that the ethanolic peel extract possessed significant *in vitro* anti-inflammatory effect against the denaturation of protein.

ACKNOWLEDGEMENT

The authors are thankful to the Management, CMR COLLEGE OF PHARMACY, Medchal, 501401, Telangana, India for providing necessary facilities for present study.

REFERENCES

1. Chandra S, Chatterjee P, Dey P, Bhattacharya S. Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein. Asian Pacific Journal of Tropical Biomedicine. 2012 Jan 1; 2(1):S178-80.
2. Tripathi KD. Essentials of medical pharmacology. New Delhi: Jaypee Brothers Medical Publishers Ltd.; 2008, p. 189.
3. Amri O, Zekhnini A, Bouhaimi A, Tahrouch S, Hatimi A. Anti-inflammatory Activity of Methanolic Extract from Pistacia atlantica Desf. Leaves. Pharmacognosy Journal. 2018;10(1).
4. Vishal V, Sharma GN, Mukesh G, Ranjan B. A review on some plants having anti-inflammatory activity. Journal of Phytopharmacology. 2014; 2:214-21.
5. Britton NL, Rose JN. The Cactaceae: descriptions and illustrations of plants of the cactus family. Courier Corporation; 1963 Jun 1.
6. Mizrahi Y, Nerd A, Nobel PS. Cacti as crops. Hort. Rev. 1997; 18:291-319.
7. Dr. K. R. Khandelwal, Practical Pharmacognosy – Techniques and Experiments, Preliminary Phytochemical Screening, Pg. No. 149-156.
8. Musfiq Marliyah .M and Ananthi .T, In Vitro inflammation activity of seed extract of Zea mays (L.) Journal of Global Sciences, Volume 4, no.5, 2015, page no. 2168-2173.
9. Hossain MS, Chowdhury ME, Das S, Chowdhury IU. In-Vitro Thrombolytic and Anti-inflammatory Activity of Swertia Chirata Ethanolic Extract. Journal of Pharmacognosy and Phytochemistry. 2012 Nov 1; 1(4).

SP-07

STUDIES ON ANTI MICROBIAL EFFICACY OF HYDRO-METHANOLIC LEAF EXTRACT OF ERYTHROXYLUM MONOGYNUM

BHADRAMRAJU.RAMU[1], PADMAVATHI SAKINALA[2], B RAMESH [3], D.SARALAKUMARI [1*]

1.Department of Biochemistry, Sri Krishnadevaraya University, Anantapuramu-515003
2.Nirmala College of Pharmacy, Mangalagiri, Guntur-522 503
3.Department of Food Technology, Vikramasimhapuri university, Nellore-524320
1.Department of Biochemistry, Sri Krishnadevaraya University, Anantapuramu-515003*
*1*Email: skumari1@yahoo.co.in*

ABSTRACT

Erythroxylum monogynum is an one of the most important medicinal plant and it is known with different names viz: Paribhadrukamu, Devadaru, Sembulichin, Red cedar etc of variou regions in India. In Telugu it is known to be paribhadrukamu and daderaaku names. In the current work, hydro- methanolic extract of leaf of Erythroxylum monogynum is tested for antimicrobial activity (both antibacterial and antifungal activity). The antibacterial activity was conducted against the organisms Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Proteus vulgaris keeping the antibiotic ciprofloxacin as a positive control and the results falls in range of 14 mm to 18 mm zone of inhibitions was observed, the maximum zone of inhibition was 18 mm for all four organisms in 1000 ug/mL of hydro methanolic extract of leaf, where as 21 mm zone of inhibition was observed in 1000 ug/mL of ciprofloxacin for every organism. Further minimum inhibitory concentrations (MIC) were also studied for all four organisms, the results shown that 16 mm highest zone of inhibition in 100 ug/ml for S.cocci organism. And the antifungal activity was conducted against organisms aspergillus niger and candida albicans keeping the flucanozole as a positive contro and the results falls in range of 8mm to 11mm zone of inhibition was observed, the maximum zone of inhibition was in 1000ug/mL for A.niger, where as 21mm zone of inhibition was observed in 1000ug/mL of flucanazole.

Key words: Erythroxylum monogynum, Antimicrobial, E.coli, S.cocci, P.auriginosa and P.vulgaris.

INTRODUCTION

Erythroxylum monogynum is an one of the most important medicinal plant and it is known with different names viz: Paribhadrukamu, Devadaru, Sembulichin, Red cedar etc of variou regions in India [1]. In Telugu it is known to be paribhadrukamu and daderaaku names. It has widely spreaded in southern parts like Karnataka, tamilnadu, Kerala and Andhra Pradesh in India. Erythroxylum monogynum is belonging to the family Erythroxylaceae. According to Health Organization, more than 80 percentage of the world's population relies on traditional drug for their primary healthcare demands [2]. In the current study the hydro-methanolic leaf extract of erythroxylum monogynum is tested for anti bacterial and anti fungal activity, since the microbes are causatives of many human diseases.

MATERIALS AND METHODS

COLLECTION OF LEAFE:

The leaves were collected from thick forest of lankamalla in cuddappa district during October – September month, subsequently leaves were graded and fine leaves are subjected to shaded drying until the leaves get brittle in condtion. Further dried are processed for extraction.



Whole plant Collected leaves Dried leaves

PREPARATION OF LEAF EXTRACT

Erythroxylum monogynum leaves are charged to extractor along with Methanol and water. It is extracted by heating the mass, in a closed system by re pumping the extract to the herb bed. This process is repeated and filtered. The extracts are combined and concentrated under reduced pressure at low temperature. This is charged to drier unit to dry and separate the product in a powder form. This is further powdered in a multimill to a fine mesh size. It is sieved using a sifter to make uniform particle size. The product is mixed in a blender to make a uniform and homogenous lot. Finally the product is heat sterilized and sieved.

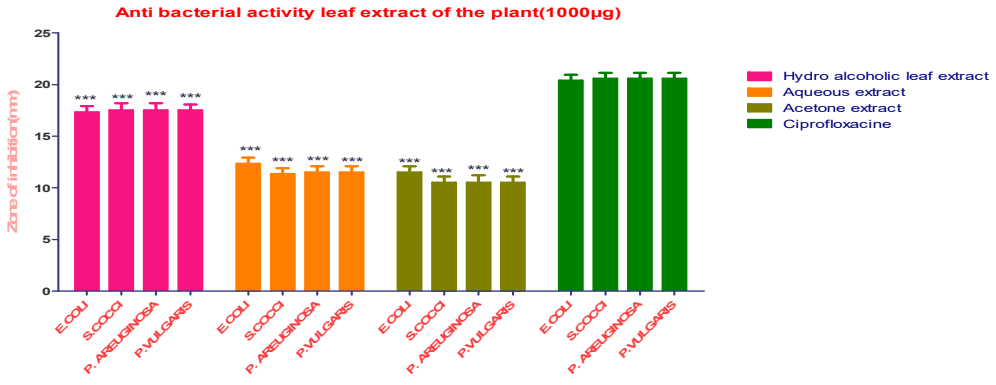
EXPERIMENTATION

Determination by Agar cup method

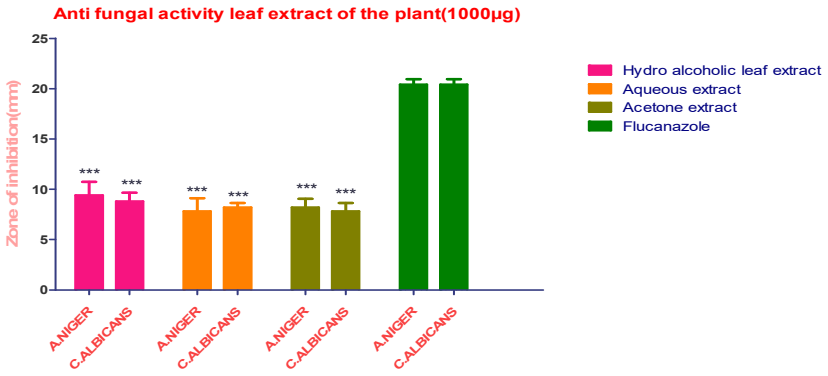
The antibacterial activity of erythroxylum monogynum hydro-methanolic extract was studied by agar cup method. To test the antimicrobial activity nutrient broth media was selected as culture media. The agar media was used for the bacterial studies and suberose dextrose was used for fungal studies. The culture media was poured onto the petridishes and later it was solidified. Before the solidification uniformly inoculate with microorganism. A borewells was made and placing the plant extract in different dilutions using the DMSO as control. Incubate the petridishes at 25°c for 24 hrs and measure the zone of inhibition.[3]

Table 1.Showing results of Zone of inhibitions (mm) Minimum inhibitory concentrations (µg) of different species of bacterial and fungal organisms

E.COLI ATCC 25922		S.COCCI ATCC 29213		P. AREUGINOSA ATCC 27953		P.VULGARIS		A.NIGER NRCC2477		C.ALBICANS	
Hydro methanolic leaf extract											
Zone of inhibition (mm)	Conc (µg)	Zone of inhibition	Conc (µg)	Zone of inhibiion	Conc (µg)	Zone of inhibition	Conc (µg)	Zone of inhibition	Conc (µg)	Zone of inhibition	Conc (µg)
18	1000	16	500	17	1000	17	1000	10	1000	9	1000
17	1000	18	1000	16	500	18	1000	8	500	8	500
16	500	16	500	16	500	18	1000	11	1000	10	1000
17	1000	16	500	18	1000	16	500	8	500	8	500
15	500	17	1000	14	500	15	500	10	1000	9	1000
15	500	18	1000	18	1000	16	500	9	500	8	500
Ciprofloxacine								Flucanazole			
20	1000	20	1000	21	1000	21	1000	20	1000	20	1000
21	1000	21	1000	20	1000	20	1000	20	1000	21	1000
20	1000	20	1000	21	1000	21	1000	21	1000	20	1000
21	1000	21	1000	20	1000	20	1000	21	1000	21	1000
20	1000	21	1000	21	1000	21	1000	20	1000	20	1000



Graphical representation of antibacterial activity of E.M



Graphical representation of antifungal activity of E.M

MIC of pseudomonas aruginosa

MIC of Aspergillus. niger

RESULTS AND DISCUSSIONS

The zone of inhibition and minimum inhibitory concentrations were measured by using agar cup plate method. The study was under taken by four bacterial species and two fungal species. The zone of inhibition and minimum inhibitory concentrations for bacterias E.Coli was 500 µg, 15-18 mm, S.Cocci 500 µg, 16-18mm, P.Areuginosa 500 µg, 14-18mm, P.vulgaris 500 µg,15-18mm, for fungi A.niger 500 µg, 8-11mm, C.albicans 500 µg, 8-10mm. comparative study was performed by using standard drugs ciprofloxacine, fluconazole. The zone of inhibitions of ciprofloxacine was 20-21mm, MIC 1000 µg, fluconazole was 20-21mm, 1000 µg. The data was subjected to the two way anova studies the results are satisfactory.

CONCLUSION

E.M leaves were collected and hydro- methanolic extract was prepared, the extract was subjected to the to the antimicrobial activity. The zone of inhibitions, minimum inhibitory concentrations were measured and compared with standard drugs ciprofloxacine, fluconazole. Basing on the experimental data the zone of inhibition and minimum inhibitory concentrations were close to the standard drugs. Thereby it can be concluded that hydromethanolic leave extract of EM was showed good antibacterial activity and can be studied for further investigations.

REFERENCES:

1.Rajagopal PL, K.Premaletha , K.R.Sreejith, Review on Erythroxylum Monogynum Roxb . Asian Journal of Medical and Health Research 2016

2.Alagesaboopathi, C. photochemical screening and antibacterial activity of Erythroxylum Monogynum Roxb International Journal of Current Research Vol. 5, Issue, 08, pp.2068-2071, August, 2013

3. Sakinala Padmavathi and Madhukar Rajaram Tajne Design, synthesis, molecular docking studies and anti-microbial activity of novel 1, 2,3,4-tetrahydrocarbazole derivatives.

4 B.Sreedharren , K. P.Jaiganesh , N.Kannappan and N. Sulochana.,Phytochemical Studies and Demonstration of Antimicrobial activity of Erythroxylum Monogynum. Int. J. Chem. Sci.: 6(1), 2008, 245-249

5.S. P. Ambasta, The Useful Plants of India, C.S.I.R. Publication, New Delhi. (1986) p.705

SP-08

FORMULATION AND EVALUATION OF ATENOLOL ORODISPERSIBLE TABLETS USING NATURAL SUPERDISINTEGRANTS AND MULTIFUNCTIONAL EXCIPIENTS

RADHIKA KORUKONDA*, PALLAVI KANAGALA, NASEEB BASHA SHAIK, ARCHANA MULE AND SRAVYA GADILI

***Department of Pharmaceutics, G. Pulla Reddy College of Pharmacy, Osmania University, Mehdipatnam, Hyderabad-28.**
E-mail: laasyaradhika@gmail.com

ABSTRACT

Orodispersible tablets are solid unit dosage forms which are composed of super disintegrants, which help them to dissolve the tablets within a matter of seconds without any difficulty of swallowing. The objective of this study was to prepare and evaluate atenolol orodispersible tablets using multifunctional excipients and natural superdisintegrants. Multifunctional excipients were used like MCC sanaq burst, starlac, Disintequik ODT, Ludipress, Pearlitol flash, F-Melt type C, SmartEx QD-100 and PROSOLV ODT G2 and few natural superdisintegrants were used like tapioca starch and Modified moringa gum. Modification of moringa gum prepared by heating the natural form of moringa gum. FTIR studies conducted and revealed that drug and excipients were compatible. Precompression parameters such as angle of repose, bulk density, tapped density, Carr's index, Hausner's ratio were performed and found to be within limits. The prepared tablets were evaluated for weight variation, thickness, hardness, disintegration time, wetting time, water absorption ratio and *invitro* dissolution studies. The results indicated that among all the formulations, optimized formulation F11 which contains tapioca starch (12%), showed disintegration time of 25 sec and % drug release 100.2 % at 14 min. The stability studies of optimized formulations revealed that formulation is stable.

Key words: Orodispersibletablets, Multifunctional excipients, natural superdisintegrants, precompression parameters, tapioca starch, Disintegration time.

INTRODUCTION

Oral route of administration is presently most acceptable route in the pharmaceutical industry where it is regarded as the safest, most economical and most convenient method of drug delivery resulting in highest patient compliance. ODT's are appreciated by a significant segment of populations particularly who have dysphagia (difficulty in swallowing) is common in among all the age groups and more specific with pediatric, geriatric population along with institutionalized patients, psychiatric patients and patients with nausea, vomiting, and motion sickness complications. European Pharmacopoeia described ODT's as uncoated tablets intended to be placed in the mouth where they disperse rapidly before being swallowed and as tablets which should disintegrate within 3 min.[1] United States Food and Drug Administration (FDA) defined ODT as "A solid dosage form containing medicinal substance or active ingredient which disintegrates rapidly usually within a matter of seconds when placed upon the tongue." The disintegration time for ODT's generally ranges from several seconds to about a minute.

EXPERIMENTAL METHODOLOGY

MATERIALS

Atenolol gift sample was obtained from APL research centre, Hyderabad, Moringa gum was purchased from Jagadish enterprises, MCC sanaq burst was purchased from Pharmatrans Sanaq AG, Starlac was supplied by Meggele Excipients & Technology, Disintequik ODT was supplied by Kerry, Ludipress was supplied by BASF India Limited, SmartEx QD-100 was supplied by Shin-Etsu, PROSOLV ODT G2 was supplied by JRS Pharma, F-Melt type C was purchased from Fuji Chemical Industries Co., Ltd. Tapioca starch was

supplied by Angel Starch & Food Pvt. Ltd, Microcrystalline cellulose was purchase from Yarrochem, Sucralose was purchased from JK Sucralose Inc, Magnesium stearate was purchased from SD fine Chemicals Limited, Talcum powder was purchased from SD fine Chemicals Limited.

METHODOLOGY

Isolation of moringa gum

The gum was collected from trees (injured site). It was dried, ground, and passed through #80. Dried gum (10 g) was stirred in distilled water (250 ml) for 6-8 h at room temperature. The supernatant was obtained by centrifugation. The residue was washed with water and the washings were added to separated supernatant. The procedure was repeated four more times.The supernatant collected was made up to 500 ml and treated with twice the volume of acetone by continuous stirring. The precipitated material was washed with distilled water and dried at 50-60°C under vacuum.[2]

Modification of Moringa gum

10g of isolated moringa gum powder was taken in china dish and subjected to heating at 120ºC for 4-5 hrs in a hot air oven. The prepared modified moringa gum was evaluated and compared with moringa gum for swelling index and viscosity.[3]

Preparation of atenolol orodispersible tablets by direct compression method

ODTs of atenolol were prepared by direct compression method using multifunctional excipients and natural superdisintegrants. Formulations F1-F8 were prepared with marketed co-processed multifunctional excipients. In formulations F9-F17 the superdisintegrants tapioca starch, modified moringa gum were used in the concentrations of 4%,8%,12%, and 2%,4%,6%8%,10%,12% respectively and diluents/ binders lactose and microcrystalline cellulose were used in the ratio 90:10 as shown in **Table 1**. Uniformly mixed blend was compressed into tablets containing 25 mg drug using 8 mm punch on a Rimek-1 rotary tablet machine by direct compression method.[4]

Table 1: Formulation of atenolol orodispersible tablets using multifunctional excipients and natural superdisintegrants

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17
Atenolol	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
Mccsanaq burst	147.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Starlac	-	147.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Disintequik ODT	-	-	147.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ludipress	-	-	-	147.8	-	-	-	-	-	-	-	-	-	-	-	-	-
Pearlitolflash	-	-	-	-	147.8	-	-	-	-	-	-	-	-	-	-	-	-
F-melt	-	-	-	-	-	147.8	-	-	-	-	-	-	-	-	-	-	-
SmartEx QD100	-	-	-	-	-	-	147.8	-	-	-	-	-	-	-	-	-	-
ProsolvODTG2	-	-	-	-	-	-	-	147.8	-	-	-	-	-	-	-	-	-
Tapioca starch	-	-	-	-	-	-	-	-	7.2	14.4	21.6	-	-	-	-	-	-
Moringa gum	-	-	-	-	-	-	-	-	-	-	-	3.6	7.2	10.8	14.4	18	21.6
Lactose									126.5	120.0	113.58	129.78	126.5	123.33	120.06	116.82	113.58
MCC									14.06	13.34	12.62	14.42	14.06	13.71	13.34	12.98	12.62

Sucralose	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Mg.st	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
Talc	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7

Note: All values are in mg, Mg.st=Magnesium stearate

RESULTS AND DISCUSSION

Table 2: Swelling index of moringa gum and modified moringa gum

Name of gum	Swelling index(%v/v)
Moringa gum	177±0.15
Modified moringa gum	26.6±0.94

Note: Values are expressed as mean± SD, n=3

Table 3: Viscosity of moringa gum and modified moringa gum

Name of the gum	RPM	Viscosity (cps)
Moringa gum	10	506
	12	494
	20	415
	30	325
	50	280
	60	250
	100	210
Modified moringa gum	10	400
	12	354
	20	314
	30	250
	50	210
	60	190
	100	180

Table 4: Data for evaluation of pre compression parameters of various powder blends

Formulation	Angle of repose(θ)	Bulk density(g/ml)	Tapped density(g/ml)	Compressibility index (%)	Hausner ratio
F1	30.80±0.01	0.742±0.02	1.021±0.23	27.37±0.01	1.377±0.21
F2	37.07±0.05	0.655±0.00	0.916±0.15	28.42±0.05	1.397±0.01
F3	27.91±0.18	0.651±0.06	0.895±0.45	27.27±0.02	1.375±0.05
F4	34.09±0.02	0.628±0.01	0.883±0.26	28.88±0.06	1.406±0.26
F5	34.08±0.03	0.627±0.15	0.829±0.01	24.35±0.02	1.322±0.28
F6	25.92±0.05	0.694±0.25	0.878±0.07	20.92±0.01	1.35±0.03
F7	20.78±0.09	0.625±0.09	0.833±0.09	25.00±0.00	1.333±0.09
F8	29.81±0.01	0.704±0.12	0.892±0.05	21.11±0.58	1.268±0.25
F9	28.83±0.01	0.585±0.00	0.796±0.02	26.51±0.25	1.361±0.18
F10	26.66±0.02	0.614±0.26	0.832±0.03	26.34±0.14	1.358±0.12
F11	24.92±0.00	0.694±0.36	0.855±0.04	18.92±0.56	1.233±0.32
F12	25.9±0.05	0.585±0.76	0.916±0.06	25.37±0.01	1.337±0.56
F13	27.8±0.09	0.625±0.90	0.883±0.21	28.42±0.06	1.357±0.00
F14	30.4±0.06	0.611±0.82	0.821±0.25	25.27±0.08	1.375±0.01
F15	28.3±0.12	0.704±0.12	1.021±0.52	28.88±0.02	1.406±0.08
F16	29.6±0.05	0.562±0.42	0.852±0.86	24.35±0.78	1.321±0.07
F17	30.2±0.06	0.581±0.58	0.895±0.98	18.92±0.05	1.231±0.08

Note: Values are expressed as mean± SD, n=3

Table 5: (a) Physical evaluation of Oro-dispersible tablets

Formulations	Weight variation (mg)*	Thickness (mm)#	Hardness (kg/cm^2)#	Friability (%)$	Drug content (%)#
F1	180.30±1.03	2.79±0.017	2.00±0.058	0.576	97.63±1.09
F2	178.80±1.74	2.60±0.015	2.07±0.153	0.290	98.10±0.95
F3	177.80±1.24	2.78±0.015	2.20±0.100	0.850	99.10±0.82
F4	178.35±1.31	2.79±0.002	2.57±0.058	0.146	97.97±1.10
F5	179.95±1.54	2.79±0.017	2.47±0.153	0.436	100.57±0.55
F6	180.00±1.72	2.76±0.015	2.70±0.100	0.434	96.59±1.54
F7	179.95±1.50	2.72±0.015	3.70±0.100	0.143	101±1.06
F8	178.50±2.12	2.62±0.002	4.77±0.252	0.433	98.03±1.02
F9	179.20±2.61	2.76±0.010	3.13±0.321	0.436	99.12±0.99
F10	177.40±2.93	2.76±0.017	3.10±0.361	0.439	99.2±1.06
F11	180.00±2.31	2.80±0.006	3.30±0.361	0.578	100.6±0.73
F12	180.30±1.03	2.79±0.017	2.30±0.058	0.576	99.41±0.46
F13	179.80±1.74	2.60±0.015	2.37±0.153	0.590	97.41±0.86
F14	178.80±1.24	2.78±0.015	2.20±0.100	0.450	99.10±0.95
F15	179.35±1.31	2.79±0.002	2.57±0.058	0.460	101.1±1.03
F16	179.95±1.54	2.79±0.017	2.47±0.153	0.436	100±0.52
F17	180.00±1.72	2.76±0.015	2.70±0.100	0.434	99.2±0.46

Note: Values are expressed as mean± SD; *n=20; #n=3; $n=10

Table 6: (b) Physical evaluation of Oro-dispersable tablets

Formulations	Disintegration time (sec)*	Wetting time(sec)	Water absorption ratio(%)#
F1	136±6.00	25±1.41	89.9±1.53
F2	90±4.24	58±0.01	88.8±1.53
F3	>180	>180	57.7±1.05
F4	>180	>180	51.3±2.12
F5	55±5.29	54±0.97	77.7±2.72
F6	>180	>180	61.6±1.39
F7	>180	>180	59.4±2.12
F8	>180	>180	57.3±3.37
F9	30±1.41	114±4.95	51.6±3.51
F10	59±3.79	60±2.45	62.9±2.45
F11	25±2.24	24±0.97	90.2±1.53
F12	170±5.00	>180	53.4±3.79
F13	161±6.00	169±7.50	62.9±4.24
F14	150±4.04	170±3.00	56.6±0.01
F15	140±3.63	180±4.95	44.4±2.12
F16	135±2.96	170±3.00	56.4±0.01
F17	130±6.78	120±7.77	57.3±0.01

Note: Values are expressed as mean± SD; *n=6; #n=3; $n=10

Table 7: %drug release profiles of F1-F6

Time (sec)	Cumulative % drug release					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
2	76.6±0.98	25.2±0.55	39.8±1.22	23.3±0.91	65.2±1.69	26.9±1.13
4	83.2±0.07	76.3±0.58	49.2±0.49	35.4±0.84	66±1.97	27.2±0.01
6	84.7±0.35	82.1±0.68	50.5±0.35	46.9±1.27	66.3±1.48	29.1±0.28
8	85.1±0.42	84.0±0.95	59.7±0.84	60.9±1.27	66.8±1.06	43.2±0.32
10	86.5±0.07	86.4±1.12	73±1.34	62.3±2.05	67.5±0.91	50.4±0.56
12	88.5±0.21	87.9±2.09	79.2±2.12	71.7±2.47	70.1±0.07	60.3±1.55
14	92.4±0.14	89.1±0.75	79.8±0.49	76.9±1.92	75.7±0.98	62.8±0.21

Note: Values are expressed as mean± S.D, n=3

Table 8: % Drug release profiles of F7-F1

Time (sec)	Cumulative % drug release					
	F7	F8	F9	F10	F11	F12
0	0	0	0	0	0	0
2	18±0.56	27.8±0.01	44.6±0.707	66.7±1.83	60.2±1.48	18.5±0.21
4	19.3±0.49	35.6±0.56	60.2±7.99	76.8±1.83	72.6±1.90	26.4±0.07
6	24.3±2.89	56.4±0.56	77.6±4.94	79.9±2.19	78.3±0.70	45.1±0.77
8	33±1.27	72.1±0.35	79.2±5.37	81.9±0.49	82.6±0.21	72.9±1.62
10	38.5±0.07	82.8±0.14	79.7±2.54	82.6±1.76	89.1±2.68	86.1±2.26
12	43.5±0.21	91.4±0.07	80.2±1.34	82.8±0.56	95.2±1.27	89.6±0.42
14	53.7±0.42	92.1±0.21	82.7±0.14	96±7.63	100.2±0.77	90.3±0.56

Note: values are expressed as mean±S.D,n=3

Table 9: % Drug release profiles of F13-F17

Time (sec)	Cumulative % drug release				
	F13	F14	F15	F16	F17
0	0	0	0	0	0
2	64.6±3.32	52.4±1.97	63.2±3.53	75.6±2.82	79.7±2.89
4	77.3±3.39	81.5±2.89	83.6±9.47	91.5±7.77	103±2.40
6	80.7±0.63	85.4±1.55	85.1±2.75	93.1±7.42	
8	84.2±0.98	88.3±1.27	85.3±3.88	94.2±3.74	
10	84.7±3.39	88.7±2.68	88.2±2.33	94.6±3.11	
12	85.4±4.87	89.0±5.16	88.6±6.58	95.0±0.42	
14	87.1±5.79	93.4±3.46	94.3±3.39	95.3±2.05	

Note: Values are expressed as mean± S.D, n

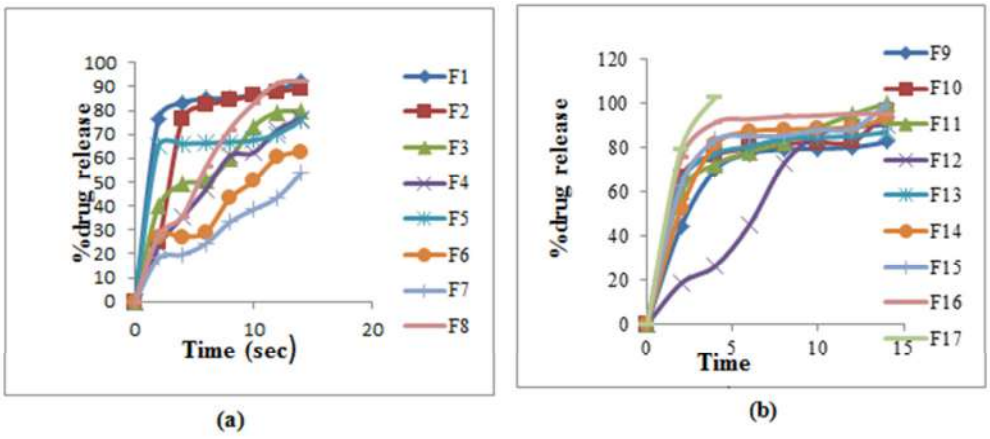


Table 10: Results of stability study of optimized formulations

S.NO	Parameter	Optimized batch	
		0 month	1 month
1	Thickness (mm)	2.80±0.006	2.79±0.01
2	Hardness(kg/cm^2)	3.30±0.361	3.20±0.23
3	Disintegration time (sec)	25±2.24	24±0.07
4	Wetting time(sec)	24±0.97	23±1.12
5	Water absorption ratio (%)	90.2±1.53	89.1±1.25
6	Drug content (%)	100.6±0.73	99±0.01

CONCLUSION

In the present research work, Orodispersible tablets of Atenolol were prepared by direct compression method using Multifunctional excipients and natural superdisintegrants. Eight formulations (F1-F8) were prepared using multifunctional excipients which act as binder, disintegrant and diluents. Nine formulations (F9-F17) were prepared using natural superdisintegrants. Formulations were prepared by using different concentrations of moringa gum have showed disintegration time more than 10 min. Hence moringa gum was modified. With modified moringa gum all the formulations showed disintegration time less than 3min.Considering all evaluation parameter results and drug release profile of all the formulations, the formulation F11 containing tapioca starch (12%) was optimized which showed disintegration time 25 sec, % drug release of 100.2% at 14min, wetting time 24 sec, water absorption ratio 90.2%. Stability studies of optimized formulation revealed that formulation was stable.

REFERENCES

1. Velmurugan S and Sundar V. Oral Disintegrating Tablets: An Overview. *International Journal of Chemical and Pharmaceutical Sciences* 2010; 1(2):1-12.
2. Githanjali Mehetre, Vishal Pande, Prakash Kendre. Isolation and Characterization of bionanofibres from moringa olifera gum as a platform for drug delivery. *Nanoscience and Nanotechnology Research* 20153(1):1-5.
3. G.V Murali mohan babu, Ch.D.S Prasad, K.V Ramana Murthy. Evaluation of Modified gum Karaya as carrier for the dissolution enhancement of poorly water-soluble drug nimodipine. *International Journal of Pharmaceutics* 2002; 234(1-2):1-17.
4. Anirudha V. Munde,Vilas P.Bharti,Vinayta R.Attal, Sanjay K. Bias. Formulation and Invitro evaluation of orodispersible tablets of Lansoprazole. *Journal of Innovations In Pharmaceutics And Biological Sciences* 2015;2(4):469-477.
5. Paramita Dey, Sabyasachi Maiti. Orodispersible Tablets: A New Trend in Drug Delivery. *Journal Of Natural Science,Biology & Medicine* 2011;1(1):1-5.
6. Kapil chauhan, Bharat Parashar,Abhishek chandel, Varun Thakur. Formulation and evaluation of fast dissolving tablets of Telmisartan. *International Journal of Pharmaceutical Sciences And Research* 2018; 0975-8232.
7. Afreen Quereshi, Salma Yasmeen, M. Suresh babu. Formulation and evaluation of oro-dispersible tablets of ivabradine by sublimation technique. *International Journal of Pharmacy And Analytical Research* 2015;4(4):2320-2831.
8. Priyanka Nagar, Kusum Singh, Iti Chauhan, Madhu Verma, Mohd Yasir, Azad Khan, Rajat Sharma, Nandini Gupta. Orally disintegrating tablets: Formulation,Preparation techniques and Evaluation. *Journal of Applied Pharmaceutical Sciences* 2011; 1(4):35-45.
9. S.P.Hiremath, Chidambar Makanapur. Formulation and evaluation of Orodispersible tablets of a model Anti-hypertensive drug. *International Journal of Pharmacy And Pharmaceutical Sciences* 2017; 9(11):0975-1491.
10. Deepak Sharma, Mankaran Singh, Dinesh Kumar, Gurmeet Singh. Formulation development and evaluation of fast disintegrating tablet of cetrizine hydrochloride: A novel drug delivery for pediatrics and geriatrics. *Journal of Pharmaceutics* 2014; 1-8.
11. Wafa Ahmed, K. Latha, V.S. Harini. Enhancement of solubility for Carbamazepine by solid dispersion using natural gums. *International Research Journal of Pharmacy* 2019; 10(9):2230-8407.
12. D.B Patel, K.J.Patel, P.D. Bharadia. Formulation and evaluation of orodispersible tablet of Ivabradine hydrochloride. *Pharmaceutical And Biological Evaluations* 2017; 4(3).

SP-09

FORMULATION AND EVALUATION OF PORTULACA OLERACEA FOR ANTI-ULCER ACTIVITY

R. SHIREESH KIRAN[1*], SAMALA SANGEETHA[1], SOWJANYA BATTU[1], KONDE ABBULU[1]

[1]Department of Pharmaceutics, CMR College of Pharmacy, Kandlakoya (V), Medchal, Hyderabad, Telangana,501401, India.Email-ID:shireeshr@gmail.com*

ABSTRACT

The herbal drugs show less toxicity when compared to synthetic drugs.In the present study the leaves of the *Portulaca Oleracea* was used for formulating and evaluating its antiulcer properties. The coarse powder of leaves were carried out for its pharmacognostic investigation. The leaves which was extracted by using different solvents such as Ethanol, methanol, water by soxhlet apparatus. Phytochemical constituents were determined and presence of tannins and Flavanoids were confirmed by phytochemical Ethanolic extract of the *Portulaca Oleracea* was used for evaluating its antiulcer activity by pyloric ligation method of albino rats.

KEYWORDS: Ulcers, *Portulaca Oleareca,* soxhlet extraction, Phytochemicaldetermination, Anti-ulcer avtivity, *Portulaca Oleracea* gel.

INTRODUCTION

An ulcer is an exposed wound of the skin on external and internal surface the body. It is caused by a breakdown in the layer of skin which fails to heal. Which range from tiny painful wounds in the mouth and serious wounds of the layer of the stomach[1]. *Portulaca Oleracea* in olden times it was used as anti-magic herbs,strewn around a bed was believed to protect averse to evil spirits and nightmares. It was used in salads and the leaves,stem juice is applied to scorpion sting.The plant approximately contains 30 and above 60 different biological activities and medicinal indications respectively,so it is considered as medicinal food to consume similar to spinach[1].

MATERIALS & METHODS

Portulaca Oleracea leaves was extraction process were Ethanol, Methanol, Distilled water and Carbopol 934, HPMC K_4M, Xanthan gum, Methyl cellulose, Triethanol amine, Propylene glycol, Methyl Paraben, Pepper mint oil

METHODOLOGY

Collection and Authentication of Plant Material:

Portulaca oleracea plants were bought from supermarket of particular vendor and authentication was given by BSI/DRC/2018-19/Tech/631. Leaves from the plant were separated manually from stem and air dried for approximately 12 days and made into coarse powder in a commercial blender.

Water soluble extractive value:

Macerated 2 gm of *Portulaca oleracea.L* coarse powder in 25 ml of chloroform mixed water in the closed stoppered flask for 1day and shaked frequently and allowed to stand for few hours then filtered it rapidly without loss of solvents.25 ml of filtrate is evaporated and dried in a tared china dish to steady weight and weighed and calculated the percent of water-soluble extract to the air dried extract[2].

Preparation of Aqueous, Ethanolic and Methanolic extracts.

Soxhlet extraction: Methods of extraction are maceration and perculation The powdered material of P.Olearacea leaves was extracted using the solvents ethanol, methanol, distilled water at temperature $<60^0$c.Extraction was carried until colourless liquid was obtained.Then the solvent was evaporated at room temperature.

Anti-ulcer activity of *Portulaca Oleracea* leavesextract in albino rats[6]

Acute toxicity study of plant:

Acute toxicity studies of the *Portulaca Oleracea* extract was studied as per OECD procedure 423 and selected doses for anti-ulcer activity of plant *Portulaca Oleracea* extract was 200mg/kg and 400mg/kg body weight.

Experimental Animals

Female albino rats (150-200g) obtained from Sri Sainath Agencies, Hyderabad.Animals were maintained at the suitable temperature of 25 ± 1^0c and were brought to lab conditions before the commencement of experiment for 12 hours light and 12 hours dark cycle.The animals had free access to pellet diet and water ad libitum. Research plan was approved from Institutional Animal Ethics Committee [IAEC NO: CPCSEA/1657/IAEC/CMRCP/COL-18/80].

Pylorus ligation

Prior to pylorus ligation rats were housed in separate cages and fasted for 2days.Then animals were anesthetised by giving diethyl ether and pyloric sphincter was ligated and kept in a cage separately to recover and after 4 hours the albino rats were sacrificed by decapitation.Later gastric content was measured and stomach was observed for ulcers under microscope.

Formulation of *Portulaca Oleracea* Gel[3,4]

Pharmaceutical gels are semi-solid or semi rigid systems containing at least two constituent consist of a condensed mass enfolded and pervaded by a liquid. The word gel is come from the gelatin which is intended for application on skin or oral cavity.

Formulation of *Portulaca Oleracea* gels using Carbopol 934, HPMC K_4M, Xanthan gum, Methyl cellulose

Accurately weighed amount of polymer was taken in a beaker and dispersed in required quantity of distilled water by using magnetic stirrer at 1200 rpm for 30min.Then the 50mg *Portulaca Oleracea* extract was made to dissolve in 2ml propylene glycol then preservative (methyl paraben) was added.After all *Portulaca Oleracea* solution was added to polymer by constant stirring then flavouring agent was added and P^H was adjusted by adding triethanol amine.

Physicochemical evaluations of *Portulaca Oleracea* gel[5]

Physical appearance: The prepared gel formulation containing *Portulaca Oleracea* were inspected visually for their colour, homogeneity,consistency and Phase separation. Spread ability, Extrudability Measurment of P^H ,Viscosity of Gel, Drug Content, Swelling Index, In-vitro drug diffusion, Drug release kinetic study of *Portulaca Oleracea* gel, Compatability studies, Selection of Optimised Formulation, Comparison of invitro drug diffusion of optimized formulation with marketed formulation (Hexigel)

RESULTS AND DISCUSSION

Pharmacognostic Investigation

Table.1: Extractive values

Extraction method	Solvent used	Wt of coarse powder	Extractive values
Soxhlet	Ethanol	28gm	44.2%
Soxhlet	Methanol	28gm	12.9%
Maceration	Water	28gm	4.2%

The percentage yield of the ethanolic extract was high when compared to methanolic and water extractive values.

Table.2: Physico-chemical Characters:

Test of *Portulaca Oleracea*	OBSERVED VALUE IN %
Total Ash	10
Acid insoluble ash	0.6
Water soluble extractive value	10

The percentage values of total ash,acid insoluble ash and water soluble extractive value were in limit.

Table 3: Solubilityof extract:

Solvent	Solubility
Water	Soluble
Ethanol	Soluble
Methanol	Soluble
Chloroform	Soluble

The extract was soluble in polar and non polar solvents such as ethanol, methanol, and chloroform.Analytical methods

Table.4: Phytochemical determination:

S.no	Identification test	Observation	Inference
1	**Flavanoids**		
	a) Shinoda test	Reddish colour appeared	+
	b)Alkaline reagent test	Yellow colour appeared	+
	c)Lead acetate test	Yellow ppt was formed	+
2	**Saponins** Froth test	Foam is developed	+
3	**Alkaloids**		
	a) Dragendroff's test	Orange red ppt was not formed	_
	b)Mayer's test	Yellow colour was formed	+
	c)Hager's test	Yellow colour was formed	+
4	**Test for Triterpenes**	Reddish brown colour was formed	+
5	**Test for Tannins**	Green black was formed	+

(+) indicates presence of respective phtochemical constituents whereas (–) indicates absence

By phytochemical determination it was confirmed that Flavanoids,saponins,alkaloids,triterpenes and tannins were present in *Portulaca Oleracea* extract[7] as show in table 4.

Table 5: Anti-ulcer activity of *Portulaca Oleracea* extract in albino rats

S.No	Name of the group	Total acid (mEq/litre)	Free acid (mEq/litre)	pH	Gastric volume(ml)	Ulcer Index
1.	Control	87.64 ± 2.35^{f}	47.5 ± 2.97^{d}	2.93 ± 0.281^{d}	5.47 ± 0.347^{d}	10.8 ± 2.66^{d}
2.	Standard Ranitidine (50 mg/kg)	54.92 ± 3.84^{ad}	16.4 ± 0.87^{a}	6.5 ± 0.157^{a}	1.9 ± 0.293^{af}	1.17 ± 0.307^{a}
3.	EEPO (200 mg/kg)	68.4 ± 3.53^{af}	15.9 ± 2.97^{a}	5.8 ± 0.129^{a}	3.38 ± 0.329^{b}	0.00 ± 0.00^{a}
4.	EEPO (400 mg/kg)	63.5 ± 2.75^{a}	15.3 ± 2.97^{a}	5.72 ± 0.281^{a}	2.98 ± 0.491^{a}	0.00 ± 0.00^{a}

Values are represented as Mean ± SEM. Statistical analysis performed using one way ANOVA followed by post hoc Dunnett's test. $^{a}p<0.001$, $^{b}p<0.01$ and $^{c}p<0.05$ Vs Toxic control; $^{d}p<0.001$, $^{e}p<0.01$ and $^{f}p<0.05$ Vs Standard Ranitidine.

Formulation of *Portulaca Oleracea* of gel (composition)

After evaluating the anti-ulcer properties of *Portulaca Oleracea* extract the human dose was calculated from animal dose by using the formula.

HED (mg/kg) = Animal dose (mg/kg) ÷ conversion factor

Where HED= Human Equivalent dose, Conversion factor =6.17

Table.6: Formulation of gel

Ingredients	F_1	F_2	F_3	F_4
PO(extract)	30mg	30mg	30mg	30mg
Carbopol 934	2gm	-	-	-
HPMC K_4M	-	2gm	-	-
Xanthan gum	-	-	2gm	-
Methyl cellulose	-	-	-	2gm
Triethanol amine	q.s	q.s	q.s	q.s
Propylene glycol 600	2ml	2ml	2ml	2ml
Methyl paraben	0.02mg	0.02mg	0.02mg	0.02mg
Pepper mint oil	q.s	q.s	q.s	q.s
Distilled water	q.s	q.s	q.s	q.s

Physical appearance of *Portulaca Oleracea* gels:

All formulation batches were found to be green in colour.There was slight phase seperation in f2 and f3 formulation when compared to f1 and f4.

Table.7: Physical appearance of *Portulaca Oleracea* gels:

Formulations	Spreadability (gm.sm/sec)	Extrudability	P^H	Viscosity	Drug Content %	Swelling index %
F1	38.57	Good	6.18±0.02	2975±0.23	94.03±0.03	77.2±0.25
F2	30.01	Satisfactory	6.18±0.03	2106±0.25	90.06±0.02	22.6±0.45
F3	33.33	Good	6.01±0.05	1979±0.24	91.34±0.03	44.2±0.58
F4	28.01	Poor	6.09±0.02	2505±0.23	93.23±0.03	59.4±025

The spreadability of F1 formulation was high when compared to other formulations it may be due to the polymer property variations. F1 formulation showed good extrudability. The P^H of all formulations was similar but F1 and F2 was found to be slight variation with F3 and F4. The viscosity of F1 formulation was found to be high when compared to other formulations because F1 formulation consists of carbopol 934 which shows high viscosity property. The percentage of drug content was higher in formulation F1 it may be due to the polymeric variations. Swelling index of F1 formulation was high when compared to other formulations it may be due to high swelling property of carbopol 934.

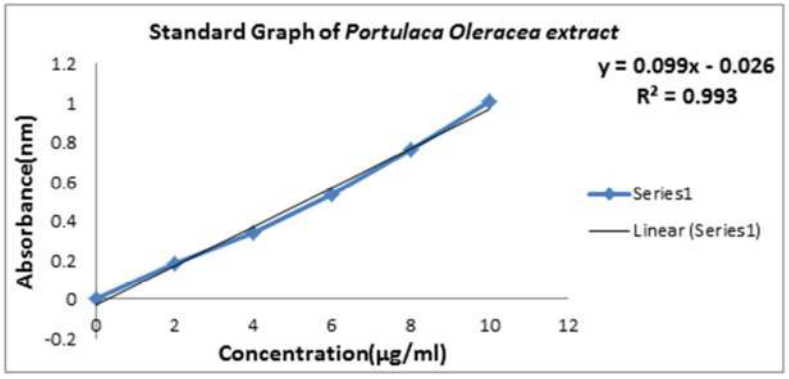


Table.8: Cumulative amount of drug release of F1, F2, F3, F4 and Market formultion:

Time in min	%CDR				
	F1	F2	F3	F4	Marketed formulation
30	5.92±0.25	5.05±0.24	5.92±0.88	4.49±0.58	10±0.21
60	11.5±0.35	9.93±0.24	6.95±0.21	7.56±0.75	12±0.34
90	16.6±0.34	14.61±0.65	14.98±0.64	12.38±0.24	15.9±0.38
120	27.1±0.25	23.9±0.66	23.6±0.66	19.16±0.36	17.2±0.21
150	34.85±0.24	31.12±0.48	26.7±0.47	22.6±0.24	18.6±0.34
180	40.20±0.02	36.26±0.65	31.1±0.72	29.4±0.24	20.5±0.04
210	48.44±0.25	43.71±0.65	35.27±0.48	34.6±0.67	21.98±0.34
240	51.82±0.84	48.08±0.48	45.42±0.48	46.3±0.32	23.5±0.56
270	62.34±0.48	61.65±0.25	59.34±0.48	57.66±0.25	48.44±0.25
300	73.54±0.25	74.86±0.48	68.76±0.25	68.34±0.4	68.56±0.24
330	83.75±0.02	84.56±0.48	80.87±0.02	81.64±0.4	78.98±0.27
360	96.76±0.4	94.78±0.02	93.86±0.02	94.34±0.02	87.67±0.24

***n=3**

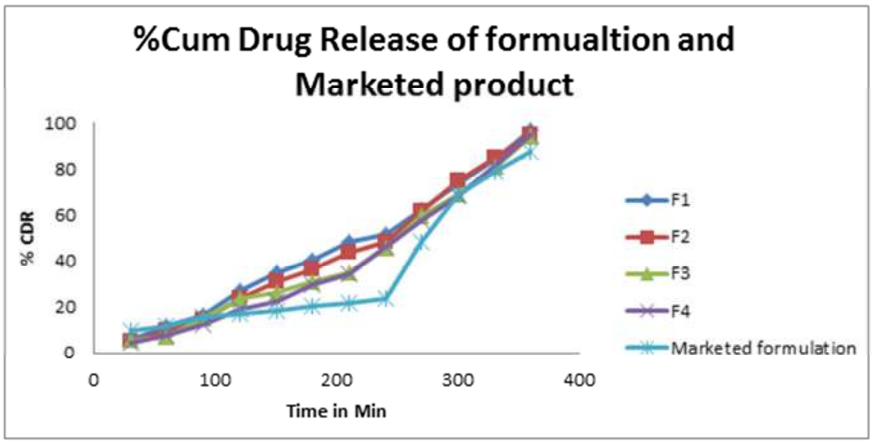


Fig 2: %CDR OF FORMULATIONS

Cumulative amount of drug release of F2 and F4 was similar but when compared to these two formulations F1 formulation showed high amount of drug release properties it may be due to swelling property of carbopol 934.

Table.9: In-vitro Drug Release Kinetic study:

Formulations	Zero-order		First-order		Higuchi-plot		Korsmeyer-peppas
	(r^2)	(K_0)	(r^2)	(K_1)	(r^2)	(K_H)	(r^2)
F1	**0.992**	3.38	0.993	0.514	0.89	3.87	0.72
F2	0.991	3.105	0.985	0.495	0.88	3.6	0.73
F3	0.982	2.905	0.984	0.481	0.87	3.406	0.76
F4	0.97 1	2.97	0.978	0.486	0.83	3.47	0.75

Optimised formulation:

F1 was determined as optimised formulation after evaluating all *Portulaca Oleracea* formulations for physical appearance, drug content, P^H,extrudability, spreadability,swelling index and invitro diffusion studies. F1 consist of polymer carbopol 934 which shows higher compatability with extract there was no

formation of cakes or physical changes after storing the polymer-extract for 1 month. Optimised formulation F1 which consist of the polymer carbopol 934 showed high amount of drug release when compared to the marketed gel (Hexigel) as show in table 9.

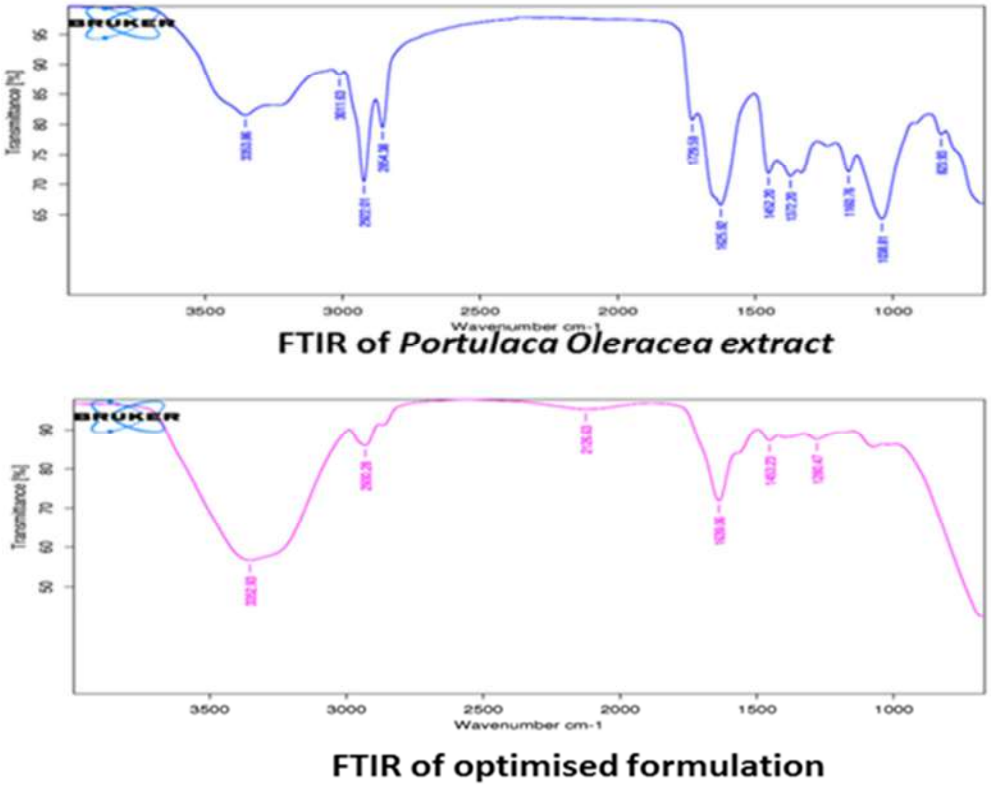


Fig 4: FTIR studies:

Table10: Description of FTIR

Extract(cm^{-1})	Optimised formulation(cm^{-1})	Type of vibrations	Intensity	Functional group
3353.86	3352.93	Stretching	strong	O-H
2922.01,2854.38	2930.28	Stretching	strong	CH_3 ,CH_2 ,CH
1625.92	1639.06	Bending	Medium-strong	NH_2 (primary amines
1452.20	1453.23	Bending	Medium	CH_3 ,CH_2

From the compatibility studies (FTIR) it was concluded that the functional groups that were present in the *Portulaca Oleracea* extract was present in the optimized formulation with very minute changes, from this we can concluded that the extract and excipients have no interactions[8] as show in table 10.

The present research work on *Portulaca Oleracea* leaves can be concluded that the ethanolic extraction of *Portulaca Oleracea* leaves by soxhlet method showed the high practical yield and presence of Flavanoids and tannins in *PO* leaves which plays major role in anti-ulcer activity confirmed by the phytochemical screening and presence of quercetin in leaves was confirmed by TLC method. Protective effect of *Portulaca Oleracea* leaves in treating ulcers was proved by animal model by pyloric ligation method. *PO* extract showed the better compatibility with polymers like carbopol 934,HPMCK_4M,xanthan gum, methyl cellulose which was studied by FTIR. All four prepared formulations are evaluated for their properties in which was found that all parameters like P^H, extrudability, swelling index, physical appearance, viscosity, spreadability, drug content, drug diffusion was affected by four polymers. F1 formulation which was formulated by using

high viscosity of 2975±0.23,higher drug content i.e.,94.03±0.03 and swelling index. The drug diffusion of F1 formulation was high comparable to other gels it showed 96.76%.On evaluating invitro drug release kinetics it found to be follow first order kinetics that the gel shows the immediate release of drug. On comparing the F1 formulation with marketed gel (Hexigel) and it was found that the marketed gel showed less drug diffusion and F1 showed good stability after storing the gel for 3 months which can be confirmed by stability studies performed.

CONCLUSION

Overall it can be concluded that formulated herbal gel can be used for treating the mouth ulcers.

REFERENCES

1. Devansh Mehta: "Ulcer-Review on Types,Anti-Ulcer drugs ,Anti-ulcer medicinal plants,Anti-ulcer drug market, Diagnostics and Current global Clinical Trials Status",(2016) 2.
2. Kaushik Avinash *et al,* "Peptic Ulcer: A Review with Emphasis on plants from Cucurbetaceae family with Anti-ulcer Potential''International Journal of Research in Ayurveda and Pharmacy 2(6):1714-1716 ·
3. Sivapotha Sundharam B *et al,* "Oral Ulcer-A Review", Journal of Dentistry &Oral disorder (2018), 4(4):1098.
4. Anjali Teresa *et al,* "Herbal Remedies for mouth ulcer:A Review (2017) J.Bio.Innov 6(4).
5. Arshiya Sultana,Khaleequr Rahman *et al*."*Portulaca oleracea* Linn: A Global Panacea with Ethnomedicinal and Pharmacological Potential", Int J Pharmacy and Pharmaceutical Sciences,(2013) 5(2).
6. Mubashir H.Masoodi *et al*,"*Portulaca oleracea* linn.A Review", Journal of Pharmacy Research(2011) 4.(9).
7. Okofor Izuchkwu Azuka *et al*. "A review on *Portulaca oleracea* (Purslane) plant-Its nature and biomedical benefits",International Journal of Biomedical Research (2014)05(02).

SP-10

STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF DAUNORUBICIN AND CYTARABINE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY

MOHAN GOUD V[1], ROJA Y*[2], JVC SHARMA[3], AVSSS GUPTA[4]

[1,2]Department of Pharmaceutical Analysis, [3]Department of Pharmacognosy, [4]Department of Pharmacology, Joginpally B.R. Pharmacy College, Hyderabad-500075, Telangana, India.

ABSTRACT

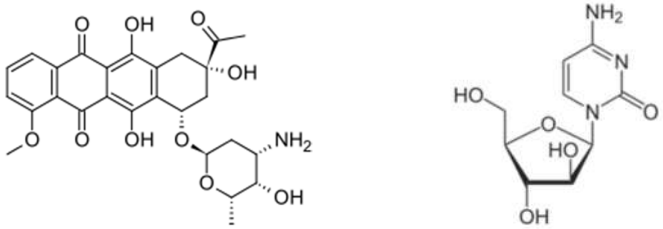
An economical UPLC method was developed for the simultaneous estimation of the Daunorubicin and Cytarabine in vial dosage form. In present study, the Chromatogram was run through X-Bridge C18 (100 x 2.1 mm, 1.6µm) column with mobile phase containing Buffer 0.01N KH_2PO_4: Acetonitrile taken in the ratio 50:50, was pumped at a flow rate of 0.3 ml/min. Buffer used in this method was 0.01N KH_2PO_4 (3.5 pH) and buffer pH was adjusted with 0.1% OPA. Optimized wavelength selected was 240.0 nm. Retention time of Daunorubicin and Cytarabine were found to be 0.958 min and 1.510 min. % RSD of the Daunorubicin and Cytarabine were found to be 0.8 and 0.6 respectively. % Recovery was obtained as 99.37 % and 100.10 % for Daunorubicin and Cytarabine respectively. LOD, LOQ values obtained from regression equations of Daunorubicin and Cytarabine were 0.14, 0.41 and 0.33, 1.00 respectively. Linearity equations were obtained for Daunorubicin was y = 2177.2x + 718.38 and Cytarabine was y = 4896.8x + 2531.1 respectively. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Key Words: ***Daunorubicin, Cytarabine, UPLC, Validation.***

1. INTRODUCTION

Cytarabine IUPAC name is 4-amino-1-[(2R, 3S, 4S, 5R)-3, 4-dihydroxy-5-(hydroxymethyl) oxolan2-yl] pyrimidin-2-one (Figure 1). It is a chemotherapy drug used to treat acute myeloid leukemia, acute lymphocytic leukemia, chronic myelogenous leukemia, and non-Hodgkin's lymphoma. Daunorubicin IUPAC name is (1S,3S)-3-Acetyl-3,5,12-trihydroxy-10methoxy-6,11-dioxo-1, 2, 3, 4, 6, 11-hexahydro-1-tetracenyl 3-amino2,3,6-trideoxy-α-L-lyxo-hexopyranoside (Figure 1). It is used to treat acute leukemia, lymphocytic leukemia, chronic myelogenous leukemia and Kaposi's sarcoma.[1-5]

Figure 1: Structures for (i) Daunorubicin and (ii) Cytarabine



(i) (ii)

A detailed Literature review [6-10] reveals that different methods RP-HPLC, UV, LCMS for its analysis in formulations, but there is no UPLC method was reported. Hence our present plan is to develop a new, sensitive, robust& accurate method for its analysis in formulation, after a detailed study, a new UPLC method was decided to be developed and validated as per ICH norms. [11-12]

2. MATERIALS AND METHODS

2.1. Instruments and ReagentsUsed

Electronics Balance-Denver, p^H meter -BVK enterprises, India, Ultra sonicator-BVK enterprises, WATERS UPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software. UV-VIS spectrophotometer integrated with UV win 6 Software was used for measuring absorbance. Distilled water, Acetonitrile, Phosphate buffer, Methanol, Ortho-phosphoric acid [All are HPLC grade], potassium dihydrogen phosphate buffer [AR].

2.2. Analytical methodology

2.2.1. Preparation of Standard and Sample stock solutions

Accurately weighed 11 mg of Daunorubicin, 25 mg of Cytarabine and transferred to 25ml volumetric flasks and 3/4th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (440 µg/ml of Daunorubicin and 1000 µg/ml Cytarabine)

2.2.2. Preparation of Standard and Sample working solutions (100 % solution)

1 ml from each stock solution was pipetted out and taken into a 10 ml volumetric flask and made up with diluent. (44 µg/ml of Daunorubicin and 100 µg/ml of Cytarabine)

3. METHOD DEVELOPMENT

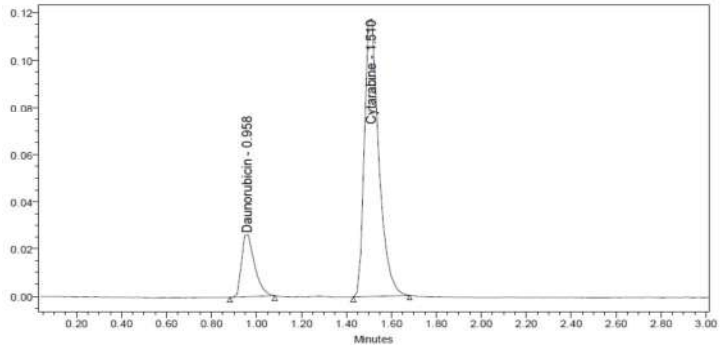
3.1. Optimized method

Trials were performed for the method development and the best peak with least fronting factor was found to be with RT = 0.958 min., for Daunorubicin and 1.510 min., for Cytarabinerespectively (Figure 2; Table 1).

Table 1: Optimized Chromatographic conditions

Parameter	Content
Column	X-Bridge C18 (100 x 2.1 mm, 1.6 µm)
Mobile Phase	50% 0.01N KH_2PO_4: 50% Acetonitrile
Flow Rate	0.3 ml/min.
Temperature	30^0C
Injection Volume	0.30 µl
Wavelength	240 nm

Figure 2: Optimized chromatogram



4. METHOD VALIDATION

4.1. Accuracy:

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.37% and 100.31 % for Daunorubicin and Cytarabine respectively (Table 2).

Table 2: Recovery studies for Daunorubicin and Cytarabine

% Concentration	Daunorubicin			Cytarabine		
	50 %	100 %	150 %	50 %	100 %	150 %
Trail-I	98.55	99.92	99.60	100.71	99.08	100.50
Trail-II	99.12	99.04	100.21	99.95	100.01	100.31
Trail-III	98.90	99.80	99.18	100.53	99.95	99.85
AVG (% Recovery)	98.9	99.59	99.66	100.39	99.68	100.22
SD	0.29	0.48	0.52	0.396	0.5178	0.3363
% RSD	0.29	0.48	0.52	0.39	0.52	0.34
Mean % Recovery	99.37±0.54			100.31±0.49		

4.2. Precision:

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned in table 3. Precision % RSD values obtained as 0.3 % and 0.9 % and Intermediate precision values obtained as 0.6 % and 0.6 % respectively for Daunorubicin and Cytarabine. As the limit of Precision was less than "2" the system precision was passed in this method (Table 3).

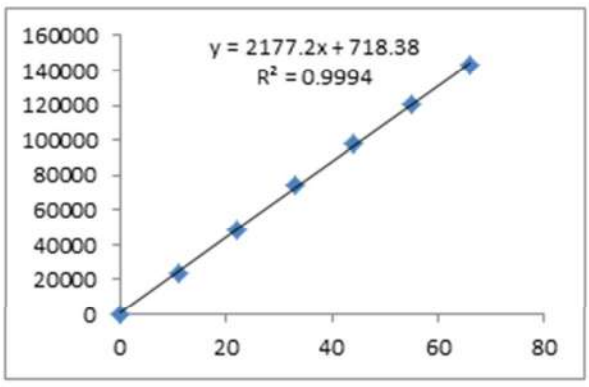
Table 3: System precision table of Daunorubicin and Cytarabine

S. No	Peak area of Daunorubicin		Peak area of Cytarabine	
	Precision	Day_ Day Precision	Precision	Day_ Day Precision
1.	103838	103398	492723	451949
2.	103225	105155	500146	459018
3.	103409	102473	496550	457349
4.	104038	103028	495758	456419
5.	103249	103268	495001	453657
6.	103294	102673	499675	453511
Mean	103509	103333	496642	455317
S.D	344.3	958.8	2839.7	2698.2
% RSD	0.3	0.9	0.6	0.6

4.3. Linearity:

Six linear concentrations of Daunorubicin (11-66 µg/ml) and Cytarabine (25-150 µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Daunorubicin was y = 2177.2x + 718.38 and of Cytarabine was y = 4896.8x + 2531.1. Correlation coefficient obtained was 0.999 for the two drugs (Figure 3&4; Table 4).

Figure 3: CalibrationcurveofDaunorubicinFigure 4: CalibrationcurveofCytarabine



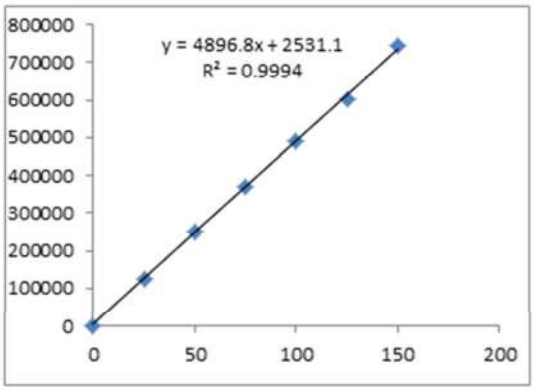


Table 4: Linearity table for Daunorubicin and Cytarabine

Daunorubicin		Cytarabine	
Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area
0	0	0	0
11	23739	25	126910
22	48836	50	251164
33	74414	75	371185
44	97759	100	491169
55	120501	125	602795
66	142708	150	745339

4.4. Robustness:

Robustness conditions like Flow minus (0.20 ml/min), Flow plus (0.40 ml/min), mobile phase minus (45B:55A), mobile phase plus (55B:45A), temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. % RSD was within the limit (Table 5).

Table 5: Robustness data for Daunorubicin and Cytarabine

S. No	Condition	% RSD of Daunorubicin	% RSD of Cytarabine
1	Flow rate (-) 0.2 ml/min	0.9	0.9
2	Flow rate (+) 0.4 ml/min	0.4	0.8
3	Mobile phase (-) 45B:55A	0.3	0.3
4	Mobile phase (+) 55B:45A	0.4	0.5
5	Temperature (-) 25°C	0.6	0.4
6	Temperature (+) 35°C	0.7	0.8

4.5. DEGRADATION STUDIES:

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation (Table 6).

Table 6: Degradation Data of Daunorubicin and Cytarabine

S. No	Degradation Condition	% Drug Degraded	
		Daunorubicin	Cytarabine
1	Acid	9.36	3.41
2	Alkali	8.78	3.07
3	Oxidation	8.38	2.52
4	Thermal	2.08	2.43
5	UV	1.23	1.81
6	Water	1.23	0.44

5. CONCLUSION

A simple, precise method was developed for the simultaneous estimation of the Daunorubicin and Cytarabine in injection form. Retention time of Daunorubicin and Cytarabine were found to be 0.958 min., and 1.510min. % RSD of the Daunorubicin and Cytarabine were found to be 0.8 and 0.6 and % Recovery was obtained as 99.33 % and 99.70 % respectively. LOD, LOQ values obtained from regression equations of Daunorubicin and Cytarabine were 0.14, 0.44 and 0.33, 1.00 respectively. Regression equation of Daunorubicin is y = 2177.x + 718.3, and y = 4896.x + 2531.1, of Cytarabine.Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

6. ACKNOWLEDGEMENT

The authors would like to acknowledge the Department of Pharmaceutical Analysis, Joginpally B.R. Pharmacy Campus for providing an institutional research platform and necessary facilities.

7. REFERENCES

1. Jazz Pharmaceuticals, Inc. Vyxeos (daunorubicin and cytarabine liposome) for injection prescribing information. Palo Alto, CA; Aug 03, 2017.
2. Shelton J, Lu X, Hollenbaugh JA, Cho JH, Amblard F, Schinazi RF. Metabolism, Biochemical Actions, and Chemical Synthesis of Anticancer Nucleosides, Nucleotides, and Base Analogs. Chem Rev. 2016; 116 (23): 14379–14455.
3. Murry DJ, Blaney SM. Clinical pharmacology of encapsulated sustained-release cytarabine. Ann Pharmacother. 2000; 34: 1173-8.
4. Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. N Engl J Med. 2006; 354:166-78.
5. Kirthi C, Azra A, Mounika R, Aamir AS, Aparna Y, Sanjeev S. A study on the adverse effects of anticancer drugs in an oncology center of a tertiary care hospital. Int J Pharm Pharm Sci 2014; 6:580-3.
6. Murthy Varanasi, Rohini A, Pravallika KE, Prameela R A and Rahaman SA: Development and validation of RP-HPLC method for estimation of Cytarabine in bulk and pharmaceutical dosage forms. International Journal of Pharmaceutical Sciences and Research 2013; 4573-4576.
7. Bhatnagar A, Loura S and Chaudhary M: A stability indicating RP-HPLC method for determination of anticancer agents Cytarabine in the lyophilized dosage form. Eurasian Journal of Analytical Chemistry 2012; 160-167.
8. Lukawska M, Oszczapowicz L, Jelinska A, Piontek JC, Zajac M, Dobrowolski, L. Development and validation of RP-HPLC method for determination of novel derivatives of daunorubicin. Chem Anal 2009; 54:907-17.
9. Suman P, Sivarao T and Reddy VSK: Development and validation of a stability-indicating RP-HPLC method for estimation of Daunorubicin - a chemotherapic drug in bulk and pharmaceutical formulations. World Journal of Pharmaceutical Research 2017; 1158-1174.
10. Rajvanshi Kushagra. Method development and validation of RP- HPLC method for simultaneous estimation of Daunorubicin and Cytarabine in the synthetic World Journal of Pharmacy and Pharmaceutical Sciences 2018; 1301-1310.
11. ICH Harmonised Tripartite Guideline. Validation of analytical procedures, Text and methodology, Q1 R2. International Conference on Harmonization, 2005, 1-13.
12. ICH Harmonised Tripartite Guideline, Stability Testing of New Drug Substances and Products, Q1A (R2). International Conference on Harmonization, 2003, 1-18.

SP-11

INVITRO ANTIDIABETIC ACTIVITY OF METHANOL EXTRACT OF RED MARINE ALGAE GRACILARIA FOLIFERA

P.PANDIAN* AND K.KATHIRESAN
Department of Pharmacy, Annamalai University, Chidambaram-608002, Tamil Nadu.
Email-id:pandian0071@rediffmail.com

ABSTRACT Diabetes mellitus include the group of metabolic disease which increase in glucose level, by increase in hyperglycemia it affects the metabolism of carbohydrates, fat and proteins due to pancrease does not produce the required amount of insulin. In the present study the methanol extracts of *Gracilariafolifera*was studied for alpha (α) - amylase and alpha (α)-glycosidase inhibition using an in vitro model. α-amylase and α-glycosidase are two methods used for the invitro antidiabetic activity with methanol extract. The IC50 values is calculated and compared with acarbose standard drug. The presence of anti-diabetic activity with two methods was concluded with the standard one and the plant extract is used to reduce the hyperglycemia and the coexisting disease.

KEYWORDS:Methanol extract, α-amylase inhibitory activity, α-glycosidase inhibitory activity.

INTRODUCTION

Diabetes mellitus is an important chronic metabolic disorder that affects the metabolism of carbohydrate, fat and protein[1]. There are three forms of diabetes. The three main types of diabetes are type 1, type 2, and gestational diabetes. Both women and men can develop diabetes at any age.

MATERIAL AND METHODS

Collection of marine algae: Fresh algae of *Gracilariafolifera* were collected from different locations of Mandapam area, Tamilnadu, South East Coast of India. In the earlymorning 5 am to 11.30am during which the tidal height was from 0.77 meter to 0.08 meter (lat 9° 15' N; long 79°E). Then the algae were washed thoroughly with sea water to remove extraneous materials and brought to the laboratory in plastic bag containing water to prevent evaporation. Samples were then shade dried until constant weight obtained and ground in an electric mixer. The powdered samples subsequently stored in refrigerator.[2]

Preparation of Extracts: The shade dried material was extracted with analytical grade petroleum ether for defatting and Methanol for 8 hours by continuous hot percolation in Soxhlet apparatus. The Extracts were dried under vacuum the dry drug extract were dissolved in dimethyl formamide (DMF) theMethanol extractis used for further studies

In Vitroα -amylase inhibitory assay

A starch solution (1% w/v) was prepared by stirring 1g starch in 100 ml of 20 mM of phosphate buffer (pH 6.9) containing 6.7mM of sodium chloride. The enzyme solution was prepared by mixing 27.5 mg of porcine pancreatic amylase (PPA) in 100 ml of 20 mM of phosphate buffer (PBS, pH 6.9) containing 6.7mM of sodium chloride. To 100 µl of (2,4,8,10,15 µg/ml) plant extracts, 200 µl porcine pancreatic amylase was added and the mixture was incubated at 37 ºc for 20 min. To the reaction mixture 100 µl (1%) starch solution was added and incubated at 37 ºC for 10 min. The reaction was stopped by adding 200 µl DNSA (1g of 3,5 di nitro salicylic acid, 30g of sodium potassium tartrate and 20 ml of 2N sodium hydroxide was added and made up to a final volume of 100 ml with distilled water) and kept it in a boiling water bath for 5 minutes. The reaction mixture diluted with 2.2 ml of water and absorbance was read at 540 nm. For each concentration, blank tubes were prepared by replacing the enzyme solution with 200 µL in distilled water. Control, representing 100% enzyme activity was prepared in a similar manner, without extract. The experiments were repeated thrice using the same protocol.[3]

In Vitro α-Glycosidase Inhibition Assay

The inhibition of α -glycosidase activity was determined using the modified published method [4]. One mg of α - glycosidase was dissolved in 100 ml of phosphate buffer (pH 6.8). To 100 µl of (2, 4, 8, 10, 15 µg/ml) plant extracts, 200 µlα - glycosidase were added and the mixture was incubated at 37ºC for 20 min. To the

reaction mixture 100 µl 3mM -nitrophenyl –Dglucopyranoside (p-NPG) was added and incubated at 37 ºC for 10 min. The reaction was terminated by the addition of 2ml Na2CO3 0.1M and theα-glucosidase activity was determined spectrophotometrically at 405 nm on spectrophotometer UV-VIS (Shimadzu UV-1800) by measuring the quantity of -nitrophenol released from p-NPG. Acarbose was used as positive control of α-amylase and α-glycosidase inhibitor. The concentration of the extract required inhibiting 50% ofα -amylase and α-glycosidase activity under the assay conditions was defined as the IC50 value.

Result and discussion

In Vitro -amylase inhibitory assay:

In this study the in vitro –amylase inhibitory activities of the methanol extract of *Gracilariafolifera*was investigated. The result of experiment showed that, there was a dose-dependent increase in percentage inhibitory activity against -amylase enzyme. The methanol extract (2-15µg/ml) of the plant exhibited potent - amylase inhibitory activity in a dose dependent manner. The extract showed inhibitory activity from 3.48±0.04 to 19±0.02% with an IC50 value of 0.95 µg dry extract (Table 1). Acarbose is a standard drug for -amylase inhibitor. Acarbose at a concentration of (2-15 µg/ml) showed -amylase inhibitory activity from 5.89±0.07 to 46.28±0.05% with an IC50 value 0.42 µg dry extract. So, the plant extract might be used as starch blockers since it prevents or slows the absorption of starch in to the body mainly by blocking the hydrolysis of 1,4-glycosidic linkages of starch and other oligosaccharides into maltoseand other simple sugars.In our study, the methanol extract of the plant showed maximum α- amylase inhibitory activity (IC50 = 0.95 µg dry extract) Our results are in accordance with the previous study wherein, there is a positive relationship between the total polyphenol and flavonoid content and the ability to inhibit intestinal pancreatic α-amylase.[5,6,7]

In Vitroα-glucosidase inhibitory assay

The results of antidiabetic activity using α-glucosidase inhibitory assay of the methanol extracts of *Gracilariafolifera*are shown in Table 2. The extract revealed a significant inhibitory action of -glucosidase enzyme. The percentage inhibition at 2- 15 µg/ ml concentrations of *Gracilariafolifera*extract showed a dose dependent increase in percentage inhibition. The percentage inhibition varied from 25.54% - 5.65% for highest concentration to the lowest concentration. Thus, the inhibition of the activity of α-glycosidase by *Gracilariafolifera*would delay the degradation of carbohydrate, which would in turn cause a decrease in the absorption of glucose, as a result the reduction of postprandial blood glucose level elevation. In this study acarbose was also used as a standard drug for α-glycosidase inhibitor. Acarbose at a concentration of (2-15 µg/ml) showed α-glycosidase inhibitory activity from 28.67 ±0.14 to 50.82±0.08 % with an IC50 value 0.36 µg dry extract. This indicates that the methanolic extract of *Gracilariafolifera*is very potent α-amylase and α-glycosidase inhibitor in comparison with acarbose. This could be justified that the nature of some extract constituents (phenols, flavonoids saponins, steroids, alkaloids, terpenoids) present in the extract could be responsible as being effective inhibitors of α-amylase and α-glycosidase.

Table 1: *In vitro* antidiabetic activity of the methanol extract of *Gracilariafolifera*using alpha amylase method and comparison with standard drug acarbose

Sl No	Plant extract/ standard drug	Concµg/ml	% of Inhibition	IC 50 µg dry extract
1	Methanol extract of *Gracilariafolifera*	2	3.48±0.04	0.95
		4	5.74±0.06	
		8	9.81±0.03	
		10	13.62±0.05	
		15	19.00±0.02	
2	Acarbose	2	5.89±0.07	0.42
		4	10.52±0.05	
		8	21.25±0.02	
		10	35.54±0.06	
		15	46.28±0.05	

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM.

Table 2: In vitro antidiabetic activity of the methanol extract of Gracilariafoliferausing alpha glucosidase method and comparison with standard drug acarbose.

Sl No	Plant extract/ standard drug	Concµg/ml	% of Inhibition	IC 50 µg dry extract
1	Methanol extract of *Gracilariafolifera*	2 4 8 10 15	5.65±0.09 9.23±0.05 15.82±0.08 19.74±0.10 25.54±0.11	0.88
2	Acarbose	2 4 8 10 15	28.67± 0.14 35.28±0.17 41.93±0.12 45.28±0.09 50.82±0.08	0.36

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM.

To investigate the biological activities of *Gracilariafolifera*forantidiabetic activities of the methanol extract of the plant has been analysed. As a result, we found that the extract of *Gracilariafolifera*haveinhibitory activity against α-amylase and α-glycosidase and this therapeutic potentiality could be exploited in the management of post prandial hyperglycemia in the treatment of type 2 diabetes mellitus. Although the effects of *Gracilariafolifera*extract have been established *in vitro*, these results indicate that *Gracilariafolifera*has potential as a crude drug and a dietary health supplement. The plant showed significant enzyme inhibitory activity, so the compound isolation, purification and characterization which are responsible for inhibiting activity, have to be done for the usage of antidiabetic agent.

Conculsion

Further studies are also required to elucidate whether the plant have antidiabetic potential by *in vivo* for corroborating the traditional claim of the plant.

References

1.Keerthana G, Kalaivani MK, Sumathy A: In-vitro alpha amylase inhibitory and anti-oxidant activities of ethanolic leaf extract of Croton bonplandianum. Asian J Pharm Clin Res, 2013; 6(4): 32-36.

2.Sellappa Sudha, PalanisamySenthilkumar, and Subramanian Prakash. Antidiabetic activity of aqueous extract of *Padina boergesenii*in Streptozotocin-induced diabetic rats. International Journal of Pharmacy and Pharmaceutical Sciences.2014. 6(5), P 418-422.

3. Ali H, Houghton PJ, Soumyanath A: α- Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to phyllanthusamarus. J Ethnopharmacol, 2006; 107: 449- 55.

4. Kim JS, Hyun TK, Kim MJ: The inhibitory effects of ethanol extracts from sorghum, foxtail millet and proso millet on -glucosidase and -amylase activities. Food Chem., 2011; 124: 1647–51

5. Mai TT, Thu NN, Tien PG, Van Chuyen N: Alpha-glucosidase inhibitory and antioxidant activities of Vietnamese edible plants and their relationships with polyphenol contents. J Nutr Sci Vitaminol (Tokyo), 2007, 53: 267–76.

6. Ramkumar KM., Thayumanavan B, Palvannan T, Rajaguru P. Inhibitory effect of GymnemaMontanum leaves on α-glucosidase activity and α-amylase activity and their relationship with polyphenolic content. Medicinal Chemistry Research, 2010; 19(8): 948-961.

7. Manikandan R, Vijaya A, Muthumani GD: Phytochemical and in vitro anti-diabetic activity of methanolic extract of Psidium guajava leaves. International Journal of Current Microbiology and Applied Sciences, 2013; 2(2):15-19.

SP-12

LENS LIPID PEROXIDATION, PROTEIN OXIDATION AND PROTEIN CONTENT IN NONDIABETIC AND DIABETIC CATARACT PATIENTS

HARITHA KETHAM[1],RAMESH BELLAMKONDA[2*], BHADRAMRAJU.RAMU[1], SARALAKUMARI DESIREDDY[1*]

[1]Department of Biochemistry, Sri Krishnadevaraya University, Anantapur - 515003,Andhra Pradesh, India
[2]Department of Food Technology, Vikrama Smhapuri University, Nellore - 524320, Andhra Pradesh, India
**Department of Biochemistry, Sri Krishnadevaraya University, Anantapur-515003*
***Email** id-rammygp@gmail.com

ABSTRACT

Diabetic cataract is the leading cause of blindness worldwide**.** More than 17 million people are blind because of cataract and 28000 new cases are reported daily worldwide. In the US, the economic impact of cataract surgery alone is estimated to be over $34 billion per year. Therefore, it is imperative to look for means and ways to effectively prevent or delay development of diabetic cataract. We performed lipid peroxidation, protein oxidation and protein content assays on 150 nondiabetic and 100 diabetic patients who had undergone cataract surgery. The present studies revealed that lipid peroxidation, protein oxidation are enhanced in diabetic cataractous lenses compared to nondiabeticcataractous lenses whereas protein content was decreased in diabetic lenses compared to nondiabetic lenses. The results indicate that the increased lipid peroxidation and protein oxidation may be due to increased oxidative stress or decreased antioxidant capacity. Further, these results imply that ingredients in the study's dietary sources may be explored for anticataractogenic agents that prevent or delay the development of cataract.

Keywords: Cataract, Protein Oxidation, Lipid Per oxidation, Protein Content

INTRODUCTION

Cataract, the opacification of the lens of the eye, is the leading cause of blindness worldwide, it accounts for approximately 42% of all blindness[1]. It is known that cataract may also form as a consequence of certain inborn metabolic derangements including diabetes mellitus. Diabetes has been considered to be one of the major risk factors of cataract[2, 3]. Diabetes is characterized by an excessive increase in blood glucose caused by a relative or absolute lack of insulin in the blood.

METHODS AND MATERIALS

The present study was performed on cataractous lenses of nondiabetic and diabetic subjects. Cataractous lenses were surgically extracted from Department of Ophthalmology, Government General Hospital, Kurnool, St.Werberghs Hospital, Nandyal and SSSIHMS, Prashanthipuram, Puttaparthy by extracapsular extraction from 150 nondiabetics (40% females, 60% males) and 100 diabetic subjects (44% females, 56% males). The average age of the nondiabetic and diabetic subjects was 65.8±1.5 and 61.4±1.4 years respectively. After surgery lens were immediately stored at -20ºC.

Preparation of sample

Immediately after separation of lens 10% homogenate was prepared in 50mM potassium phosphate buffer pH 6.2 using Potter-Elvehjem homogenizer at 0ºC. The whole homogenate was used for lipid peroxidation. For protein oxidation and protein content, the homogenate was centrifuged at 16,000rpm for 45min in cooling centrifuge and the supernatant was used for assays.

Lipid peroxidation

The extent of LPO was determined by assaying malandialdehyde (MDA) formation according to the method of Utley et al (1967)[4]. To 0.1ml of lens of 10% homogenate 4ml of 0.67% thiobarbutyric acid and 2ml of

10% TCA were added, heated in a water bath for 30min. After cooling and centrifugation, the absorbance of the supernatant was read at 535nm. Appropriate blank was employed for correction. The extent of LPO was measured as nmoles of MDA formed/10mg lens using a molar extinction coefficient of MDA as $1.56x10^5$ M^{-1} CM^{-1}.

Protein oxidation

The content of free carbonyls in the lens was determined by the method of Levine et al (1990)[5]. 0.1ml of sample was precipitated with 0.5ml of 20% TCA and centrifuged at 1200g for 15min at 4ºC. After centrifugation, the pellets were treated with 0.5ml of 10mM dinitrophenyl hydrazine (DNPH) in 2N HCl and kept at room temperature for 1hr with vortexing for every 10-15min. After 1hr protein was reprecipitated with 0.5ml of 20% TCA and then washed with ethanol:ethylacetate (1:1) to remove free DNPH and lipid carbonyl DNPH derivatives. Proteins were then dissolved in 0.6ml of 6M guanidine HCl in 2mM potassium phosphate buffer pH 6.5. The protein carbonyl products were quantified measuring the absorbance 370nm against the HCl treated blanks using UV/VIS spectrophotometer. The activity was calculated using a molar absorption coefficient of 2200 mol/L^{-1} cm^{-1}.

Protein

Protein content in the lens was determined by Lowry method (Lowry et al., 1951)[6] using bovine serum albumin as a standard.

SATISTICAL ANALYSIS

Data was analyzed using Student's t-test, $P < 0.05$ was considered to be statistically significant. P values were calculated to assess the significance of the changes observed.

RESULTS

Oxidative stress

The increased LPO levels in diabetic cataractous lenses compared to nondiabeticcataractous lenses indicate increase LPO in the lens due to hyperglycemia (Table 1). Protein carbonyl content, a measure of oxidative damage to proteins was found to be increased in diabetic lens compared to nondiabetic lens, suggesting enhanced protein oxidation under hyperglycemia (Table 1).

Table 1 Lens Lipid peroxidation, Protein oxidation and Protein content of nondiabetic and diabetic cataract patients

Parameter		***Nondiabeticcataractous lenses***	***Diabetic cataractous lenses***
Lipid peroxidation (nmoles of MDA formed /10mg lens)		145.80±2.99	208.00* ±8.37
Proteinoxidation (µmoles/mg protein)		35.25±3.44	150.72* ±21.16
Protein content (mg/g lens)	**Soluble proteins**	25.66±0.99	7.59* ±0.61
	Total proteins	169.17±7.30	54.51 *±6.88

Values are Mean ± SE (*n=20*). Significantly different from Nondiabetic

Protein aggregation

Alteration in protein profile and insolubilization of soluble protein has been considered to be the ultimate change that results in lens opacification. Therefore, we analyzed soluble and total protein content. There was a significant decrease in both total and soluble proteins in diabetic cataractous lens compared with nondiabeticcataractous lens (Table 1)

DISCUSSION

Cataract is an important complication of diabetes mellitus (DM) responsible for blindness worldwide, epidemiological studies have reported positive association between cataract and DM. At present, the only

treatment. It has been estimated that a delay in cataract onset 10 years could reduce the need for cataract surgery by as much as half [7]. Any strategy that prevents or slows the progression of cataract has a significant health impact. Human studies, as well as in vitro and in vivo animal experiments strongly suggested that there was an association between increased oxidative stress and the development of cataract [8, 9, 10, 11.]

Lipid peroxidation appears to play a key role in human cataractogenesis. The polyunsaturated fatty acid side chains of membrane lipids are susceptible to oxidizing radicals with the formation of lipid peroxide. Later it is eventually decomposed to a variety of end products including (MDA) [12]. The highly membranous photoreceptor cells are extremely rich in polyunsaturated lipid. Degeneration of these cells, which is the primary pathology in such diseases, would lead to peroxidation with the generation of toxic products with in the eye. Such products produce secondary damage to other ocular structures including the lens. The increase in LPO and protein carbonyls in the present study suggest increased oxidative stressing diabetic conditions. The accumulation of carbonyl groups in proteins is generally attributed to oxidative damage and is thought to contribute to general protein dysfunction. It is believed that OH the chemically most reactive species of 'activated oxygen' is responsible for peptide bond cleavage during protein oxidation [13]. Gallagher et al., reported that cleavage site could be anywhere around the peptide and results in various products. Many of these oxidative products have newly formed carbonyl groups [14].

The decrease in total and soluble protein content in diabetic cataractous lenses compared to nondiabeticcatarctous lenses. In this study could be due to partly leakage of proteins and insolubilization. The perfect physiochemical balance of the lens proteins gives its transparency. Since the lens proteins are long-lived, they are susceptible to post-translational modifications such as glycation which leads to change in physiochemical properties and function of lens proteins [15].

CONCLUSIONS

Improvement in the medical management of DM during the last 50 years has increase in life expectancy of a large number of diabetic patients. This increase has resulted in a significant rise in diabetic complications, including diabetic cataract and diabetic retinopathy. In view of the wide spread prevalence of diabetes in developing countries such as India [16] diabetic cataract may pose a major problem in the management of blindness. The present leads to the conclusion of increased oxidative stress as the cataract advances with decreased antioxidant capacity. In order to overcome the oxidative stress, the exogenous compounds taken concurrently are beneficial for the prevention of cataract; endogenous antioxidants also reduce the adverse effects of cataract.

REFERENCES

1. Thylefors B. Prevention of blindness. WHO's mission for vision. *World Health Forum;* 1998:19:53-9.
2. Harding J. Cataract: Biochemistry, Epidemiology and Pharmacology. London: Chapman and Hall; 1991.
3. Lee A Y W and Chung SS M. contributions of polyol pathway to oxidative Stress in diabetic cataract. *Faseb Journal* 1999:13:23-30.
4. Utley H G, Bernheim F, Hochstein P. Effect of sulfhydryl reagents on peroxidation in microsomes. *Arch BiochemBiophys* 1967:118:29-32.
5. Levine, R.L., Garland, D., Oliver, C.N., Amici, A., Climent. I., Lenz, A., Ahn BW, Shaltier S, Stadtman ER, 1990. Determination of carbonyl content in oxidatively modified proteins. Method Enzymol 186, 464–478.
6. Lowry O H, Rosenbrough N J, Farr A L, Randall R J. Protein measurement with the Folin phenol reagent. *Biol. Chem* 1951:193:265.
7. Kupfer C. Bowman lecture: the conquestof cataract-a global challenge. *Trans OphthalmolSoc* 1985:104:1-10.
8. Bhuyan K C, Bhuyan D K. Molecular mechanism of cataractogenesis. III. Toxic metabolites of oxy initiators of lipid peroxidation and cataract.*Curr EyeRes* 1948:3:67-81.

9. Altomare E, Vendemiale G, Grattagliano I, Angelini P, Micelli-Ferrari T, Cardia L. "Human diabetic cataract: role of lipid peroxidation". *Diabetic Meta* 1995:21(3):173-179.
10. Kubo E, Miyoshi N, Fukuda M, Akagi Y. Cataract formation through thePolyol pathway is associated with free radical production. *Exp Eye Res* 1999:68:1665-1676.
11. Boscia F, Grattagliano I, Vendemiale G, Micelli-Ferrari T, Altomare E. Protein oxidation and lens opacity in humans.*Invest Ophthalmol Vis Sci* 2000:41:2461-2465.
12. Trotta R J, Sullivan S G, stern A. Lipid peroxidation: effects of HMP shunt as mediated by GSH and Ascorbate. *Biochem J* 1982:204:405-415.
13. Berlett B S, Stadtman E R. Protein oxidation in aging, disease and oxidative stress. *J BiolChem* 1997:272:20313-30316.
14. Gallagher J, zelenko O, Walts A D, Sigman D S. Protease activity of 1, 10- phenanthrollne-copper 1. Targeteds scission of the catalytic site of carbonic Anhydrase.*Biochemistry* 1998:37:2096-2104.
15. Kennedy L, Baynes J W. Nonenzymatic glycosylation and chronic Complications of diabetes: an overview. *Diabetologia* 1984:26:93-98.
16. Pradeepa R, Mohan V. The changing scenario of the diabetes epidemic: Implications for India. *Indian J Med Res* 2002:116:121-132.

SP-13

COMPARATIVE STUDY OF DOXORUBICIN LIPOSOMES BY PHARMACOKINETIC MODELS

K. PRAKASH RAJ * AND K. KATHIRESAN,

Department of Pharmacy, Annamalai University, Chidambaram-608002, Tamil Nadu.
***Email-id :dr.kathiresan123@rediffmail.com**

ABSTRACT
Doxorubicin is an effective chemotherapeutic drug in cancer therapy. They are used for the treatment of variety of cancers. It is under the class of anthracycline antibiotics. From many pharmacokinetic data, the oral bioavailability of doxorubicin is may be only 5%. For that reason may deliver through injection that gives better bioavailability, but it may cause cardiac toxicity in clinical studies. For minimizing these problem, to design them in novel vesicular formulations such as liposomes, thermo liposomesetc. The aim of the study is to compare the pharmacokinetics of doxorubicin in different delivery such as bolus injection, continuous injection and liposomal delivery through some mathematical models. To predict the anti-tumour effectiveness, toxicity, area under the curve is to be computed. From this data, how liposomes delivery of doxorubicin is more effective are to be predicted.

Keywords: Bioavailability, bolus injection, antitumor.

INTRODUCTION

Doxorubicin is a natural product that is produced by different wild strains of *Streptomyces*. It is chemically a 1, 4-hydroxylated derivative of daunorubicin. It may be used therapeutically effective for variety of cancers but their major issues are very low oral bioavailability (5%) and in parenteral administration may causes cardiotoxicity[1,10]. Doxorubicin is commonly used to treat leukaemia's ,Hodgkin's lymphoma, as well as cancers of the bladder ,breast ,stomach ,lung , ovaries ,thyroid ,soft tissue sarcoma ,multiple myeloma etc. liposome encapsulated form of doxorubicin (doxil) may use for the treatment of Kaposi's sarcoma (AIDS-related cancer)[4]. For this recent advancements may achieve the delivery of doxorubicin with limits of toxicity through many vesicular systems. The aim of this study is to compare the delivery of doxorubicin in the form bolus injection, continuous infusion, and liposomal delivery through mathematical models[7].

From its recent studies found to be the liposomal preparations of doxorubicin should be superior to bolus injections are shown in rat models[6]. Because the effectiveness of doxorubicin against many tumours but it may cause cardiotoxicity, which may limits the patient lifetime dose per unit body surface area to around 450 to 550 mg/m^2.

The pharmacokinetic profiles may show that doxorubicin is transported passively across the cell membrane[2,9]. The efflux of doxorubicin is increased by p-glycoprotein which may involves the combination of active and passive transport. Drug distribution in tumours is usually in terms of concentrations in three compartments such as intracellular, extracellular and vascular. The plasma protein binding of doxorubicin is up to 75%. Only free doxorubicin is available to enter the tumour calls and kill them. The initial $t_{1/2}$ of doxorubicin is 4.75 minutes are studied in population of patients with breast cancer. Variations in plasma clearance rates may depend on tumour size.[1,8]

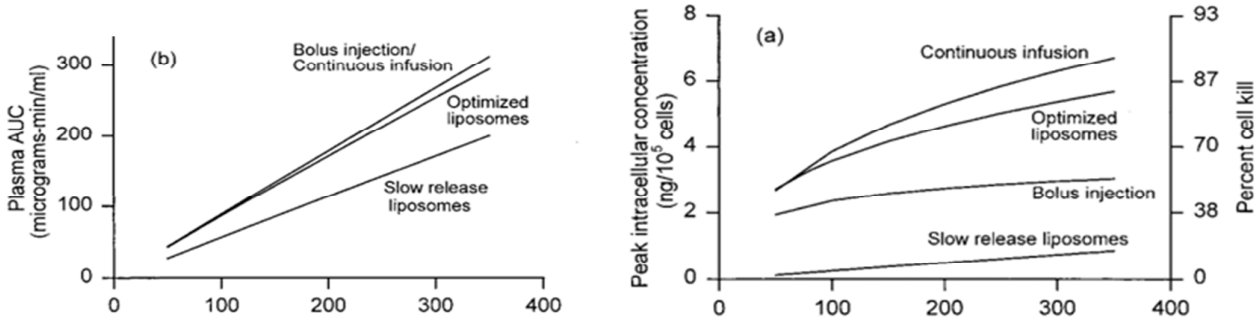
Model parameters:From various mathematical modelling, to obtain following pharmacokinetic data in various patient populations with breast cancer[1,2,5]

Symbol	Description	Value
MW	Molecular weight of doxorubicin	544
L	Typical spacing between micro vessels in tumour	200µm
D_t	Diffusivity of doxorubicin in tumour	$1.6*10^{-6}$cm/s^2
D	Total dose of doxorubicin injected	100-285mg (free), 20-350mg(liposomes)
S_t	Vascular (surface) density in tumour	200 cm^{-1}

P	Tumour vascular permeability for doxorubicin	$1.0*10^{-4}$cm/s
V_B	Total blood volume in body	5.0 L
V_t	Total tumour volume	50L
d_c	Cell density	$6*10^8$cells/ml
Ø	Volume fraction of extracellular space (tumour)	0.4
$t^{\alpha}_{1/2}$	Initial plasma half-life of doxorubicin	4.75 min
A	Time constant for doxorubicin in plasma	$0.693/ t^{\alpha}_{1/2}$
A	Inverse volume of distribution in plasma	0.13/L
V_{max}	Rate constant in kinetics for cellular transmembrane transport	$0.28ng/(10^5$ cells)/min
K_e	Michaelis constant for cellular transmembrane transport	0.219
K_i	Michaelis constant for cellular transmembrane transport	1.37 ng $/(10^5$ cells)
P_L, P^0_L	Tumour vascular permeability of liposomes	$3.4 * 10^{-7}$ cm/s
t_d	Duration of hyperthermia	60 min
t_h	Time after injection when hyperthermia starts	120 min
E	Enhancement factor for P_L45 C	1-100
A_1	Plasma pharmacokinetic parameter for liposomes	6.9µg/ml
A_2	Plasma pharmacokinetic parameter for liposomes	12.2 µg/ml
k_1	Plasma pharmacokinetic parameter for liposomes	0.00502 min^{-1}
k_2	Plasma pharmacokinetic parameter for liposomes	0.00025 min^{-1}
D_G	Dose corresponding to A_1, A_2, k_1,	50 mg/m^2
τ_{re}, τ_{re}^0	Time constant for liposome rupture in tumour extracellular space	24 h
τ^h_{re}	Time constant for liposome rupture in tumour extracellular space at 45 C	0.72 min
τ_{iv}, τ^0_{iv}	Time constant for liposome rupture in plasma	24 h
τ^h_{iv}	Time constant for liposome rupture in plasma at 45 C	0.72 min

Previous pharmacokinetic modelling for doxorubicin are assumed to be the plasma drug concentration is a good predictor for anticancer activity. The relative performance of antitumor activity of doxorubicin in different treatment modalities, schedules, and dosages are assessed by three indices such as peak intracellular drug concentration in the tumour, plasmaAUC, and peak plasma concentration. Drug delivery and toxicity resulting from various modes are shown in figure 1 as a function of total injected dose. From this figure 1a the bolus injection of doxorubicin (100mg) it may produces cardiotoxicity and low peak intracellular concentration. But in continuous infusion may give less toxicity and maximum plasma drug concentration. So compare to bolus injection, continuous infusion of doxorubicin is more effective. Figure 1b shows the correlation between the toxicity and plasma drug concentration and figure 1c may give the low peak plasma concentration in continuous infusion is believed to be less cardiotoxicity. [1]

In this figure may also compares the continuous infusion with liposomal delivery in two cases such as "slow release", and "optimized", for each dose to give maximum peak plasma. [3]



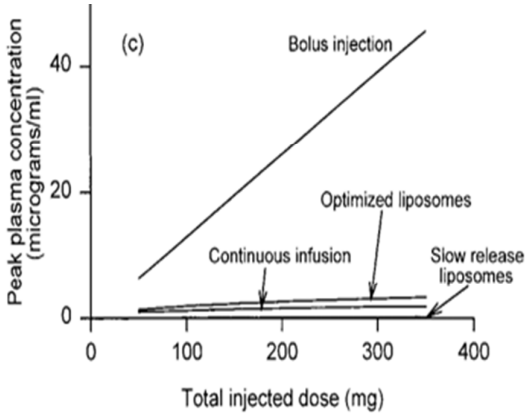


FIGURE 1: comparison of drug delivery and toxicity for various modes such as bolus injection, continuous infusion and liposomal delivery (slow release and optimized liposomes) are shown. (a) Maximumtumour intracellular concentration,(b) toxicity measured by plasma AUC,(c) toxicity measured by peak plasma concentration.

Concentration of doxorubicin. In these two cases, slow release liposomes are offer reduced toxicity, but it may reduce the efficacy of doxorubicin. Optimized liposomes are slightly less effective than continuous infusion, but it may offers rapid absorption across the cell membrane[8].

CONCLUSION:

The liposomal delivery of doxorubicin is very effective and reduces in cardiotoxicity compare to bolus injection and continuous infusion of doxorubicin. Because the peak intracellular concentration of doxorubicin is maximum in liposomal drug delivery. In this the toxicity is to be correlate with plasma drug concentration. So liposome drug delivery of doxorubicin is less risk of cadiotoxicity in cancer patients.

References:

1. A mathematical model for comparison of bolus injection, continuousinfusion, and liposomal delivery doxorubicin to tumor cells, *Ardith W. EI-KAREH and Timothy W.Secomb, Department of Physiology, University of Arizona, Tucson, AZ 85724-5051.*
2. VoraJ, and boroujerdi M (1996). Pharmacokinetic – toxic dynamic relationships of Adriamycin in rat; prediction of butylatedhydroxyanisole-mediated reduction in anthracyclinecardiotoxicity. *J pharm pharmacol*48, 1264-1269.
3. De valeriola D (1994). Dose optimization of anthracyclines. *Anticancer Res* 14, 2307-2313.
4. Guyton AC (1971*). Textbook of medical physiology (4thEd) W.B. Sounders,* Philadelphia.
5. SouthworthRW and Deleeuw SL (1965). Digital computation and numerical methods. McGraw-Hill, New york, NY.
6. Huang SK, Lee K-D, HongK, Friend DS, and Papahadjopoulos D (1992). Microscopic localization of sterically stabilized liposomes in colon carcinoma-bearing mice. *Cancer res* 54, 987-992.
7. Mayer LD, Tai LCL, KO DSC, MasinGinsberg RS, Cullis PR, and Bally MB (1989) influence of vesicle size, lipid composition, and drug-to –lipid ratio on the biological activity of liposomal doxorubicin in mice, *cancer res* 49, 5922-5930.
8. Lim HJ, Masin D, Mcintosh NL, Madden TD, and Bally MB (2000) Role of drug release and liposome –mediated drug delivery in governing the therapeutic activity of liposomal mitoxantrone used to treat human A431 and LS180 solid tumors. *J Pharmacol Exp Ther*292, 337-345.
9. Jain RK (1987), Transport of molecules in the tumorinterstitium; a review .Cancer Res 34, 2814-2822.
10. Shepherd JT, and Vanhouttle PM (1980). *The Cardiovascular Systems:Facts and Concepts.* Raven press, New York, NY.

SP-14

TRANSLATION OF PREDICTIVE IN-VIVO ANIMAL MODELS TO CLINICAL TRIALS

PADMAJA SANTOSH KORE*, CHAITALI SHASHIKANT WAGHMARE[1]

Department of Pharmacology, P. E. Society's Modern College of Pharmacy, Yamunanagar, Nigdi, Pune, India.

ABSTRACT:
Many resources are used during the development of new cancer therapeutics. The in vivo models should be selected which will improve this process. Genetically engineered mouse models (GEMM) of cancer had been improved in technical sophistication which summarize the human cognate condition, had a perceptible impact on our knowledge of tumourigenesis. However, the application of GEMMs ease the development of innovative therapeutic and diagnostic approaches has been lagged behind. GEMMs that summarize human cancer give an additional opportunity to enhance drug development and should complement the role of widely used engraftment tumour models.

KEY WORDS: Animal models, preclinical trial, translational studies, cancer studies.

INTRODUCTION

There exist a poor correlation between preclinical therapeutic findings and the eventual efficacy of these compounds in clinical trials during many years [1, 2]. Cell-based in-vitro systems and in-vivo animal models were the two universal methods used in preclinical testing. In cancer biology, cultured cells in-vitro were used for examination of chemotherapeutics and targeted therapeutics, which are responsible for early progress in cancer research. The major advantages, at a low cost to set experimental conditions and environmental factors, and to manipulate any target easily [2]. The major disadvantages, as the inability to replicate the three dimensional tumour structure, absence of tumour microenvironment and artificial levels of growth factors and cytokines in cell culture media [3]. Cell lines were incorporated into xenografts, in which cells are injected subcutaneously in immunocompromised mice, which are the effort to improve relevance of model. The main part of research in many laboratories are the murine models, as they are the most accessible animal model. In cancer biology, the usefulness of different types of animal models in preclinical compound testing has extensive reports which highlights the advances by employing these systems [4, 5]. Many advanced techniques of testing compounds have being developed due to increase in number of new drug targets and targeted agents. In mice, therapeutic efficacy studies does not have important factors in early stage of clinical trials, for e.g.; pharmacokinetics and pharmacodynamics. Pharmacokinetic studies shows how the body affect the drug and as well as the exact drug delivery of therapies. They show the factors such as absorption, distribution, metabolism and excretion. Pharmacodynamic studies shows how the drug affect the body by exposing whether the drug change its molecular target in tumour and surrogate tissue, and delineates the associated cell biological effects. The large investment by the private and public sectors is the development of antineoplastics, however there is limitation of availability of predictive preclinical systems hide our ability to select the therapeutics, which may be succeed or fail during clinical trials. In this article, different types of animal models were used to test novel therapeutics and chemotherapies, which discuss the strengths and weakness of each in this view.

Different types of animal model used in therapeutic examination:

The most usual animal model system recently used in oncology drug development and discovery continue the implantable or engraftment models, in which cultured human (xenografts) or mouse (allograft) cells or tumour tissue explants are grafted into recipient immuno-deficient or immune-competent mice. These models were broadly used in academic and pharmaceutical industry research settings to prioritise compounds for clinical testing [1, 6]. Subcutaneous implantable models render the ability to rapidly examine large cohorts of relatively uniform tumours whose growth and drug response can be easily determined. These models are inexpensive, convenient and easy to use and their behavior is different than the human cancer. When these models were used in the drug discovery, many compounds show compatible and

compelling anticancer activity in particular implantable model systems, but sometime it fails in further stages of clinical development [7, 8].

Xenograft models:
In xenograft animal models, utilization of human cells or tissue fragments require the use of immunocompromised mice which enable engraftment and it was been established for every human cancer for some extent. These make use of only few human cell lines that grow quickly and are sensitive to chemotherapy [7]. These models are useful to evaluate the direct effect of humanized monoclonal antibodies such as trastuzumab & bevacizumab immunomodulatory effects are disrupted.

Syngeneic models:
In these model, murine cell lines were injected subcutaneously in murine- competent murine model. It is a model which avoids the immune-deficiencies observed in other xenograft models [2]. There was a poor correlation between therapeutic activities of compounds tested in syngeneics or cell based assays and their efficacy in humans, potentially to innate differences in the biology of human and mouse cells [9].Neither cell based studies nor xenograft models accurately reconstruct the complex interactions between tumour and host is the basic problem. Tumours are composed of complex masses of neoplastic cells, fibroblastic, and vascular compartments. In some tumour types, stromal cells outnumber tumour cells are present [10]. In xenograft system these diversity is altered and diminished [11]. The various changed features in xenograft models were deranged tumour tissue architecture, lack of normal tissues nearby and the disruption of lymphatic and vascular supply and immune cells [3]. As they are inexpensive and easy to use, they were widely used in academics and industries [12, 13].

Orthotopic models:
Orthotopic model is a specialized version of xenograft model in which tumour tissue or cancer cells were transplanted to the orthotopic site. These models are more technically challenging to generate, they have advantage of examining effects on the microenvironment and on metastatic spread [14]. There are instances where subcutaneous and orthotopic models were compared, in which thorough investigation were done which has advantages of orthotopically transplantable tumours over simple subcutaneous models [15]. For eg. Parameters are chemosensitivity and vascularization are affected by tumour microenvironment [16].

Tumour graft models:
In thesemodel the grafting is done from patient derived tumours into immunocompromised mice as a tool in preclinical drug development [17]. These patient derived xenograft (PDX) models were used to test novel therapeutics, and it is also used to evaluate markers of response and resistance, and used to select drugs to treat individual patients. They also has some drawbacks, which includes variable transplantation failure rate, high labour costs and with ongoing passages between mice, and higher mutation rate away from parent tumour. These overall leads to increase costs compared with normal xenograft models [18, 19].

Genetically engineered mouse models (GEMMs):
In these model tumour generation occurs in situ, in proper tissue compartments and thus enabling complex process to be modelled. It is reasonable to expect that genetically engineered mouse models carry the genetic signature of native malignancy which recapitulate the biological manifestations of cancer in addition to clinical behaviour, and has an alternative to traditional preclinical assays [20]. These GEMMs models were used in drug evaluation in preclinical trials.

Preclinical therapeutic testing:
The main purpose of preclinical therapeutic testing is to check or predict whether a particular compound will be successful in the clinical trial. Encouraging preclinical results, most drugs are found to be ineffective late in their development, with only a small percentage (5%) of patients in phase 1 clinical trials [23]. Inaccurate use of tumour models, there were many other reasons in which preclinical studies fail to predict the clinical activity. Differences in Pharmacokinetic and Pharmacodynamic, drug delivery, and tumour heterogeneity might all contribute to discordant results. These failures were costly for scientists and drug industries. Preclinical models were able to provide information on mechanism of action, potential pharmacodynamic biomarkers, which includes biomarkers for prognostic and diagnostic endpoints, toxicity, off-target activity and resistance mechanism. Pharmacokinetic and Pharmacodynamic data were used to inform Phase 0 and

Phase 1 trial design. It remains uncommon to had comparable information which is related to tumour pharmacokinetic and this is related to pharmacodynamic when the drug is to be evaluated in the clinical trial. It is often unknown whether the selected drug therapy actually reaches the target, whether it's inhibiting pathways or impacting tumour cell biology and clinical trial designs need to be modified which ensure that these data were collected. Predictive biomarkers guided therapy which was already done in patients with breast cancer [24] and GIST [25] and tested using 'all comer' approach. Many novel agents were investigated and had been found to be ineffective late in their development [23, 26]. Clinical trials had developed which incorporate predictive molecular biomarkers in an early stage, which enable potential enrichment for patients to get benefits from drugs [26,27]. These approach has advantages of multiple cancers for which predictive molecular biomarkers were not available. Validated assay identification can measure a biomarker of target activation or target inhibition was often challenging. Identification of such biomarkers offer the promise from animal models, which accelerate their evaluation in clinical trials.

Preclinical trial design:

Many important aspects of early stages of clinical trials were incorporated in this design, which translated from preclinical studies.

1. Detection of tumour: Before enrolment tumour must be detected and can be done by regular palpation at the potential site of the tumour, or high resolution imaging. Imaging modalities include ultrasound, magnetic resonance imaging and computerized tomography scans.
2. Enrolment: Mice can be enrolled when tumour is detected. Incorporation of stratification, similar to that used in clinical trial, encompassing criteria, such as age, sex, tumour location and health status. Enrolment suitability and stratification should be confirmed. The mouse can be initiated into a variety of different studies. Such as:

(i) Short-term intervention studies: Establish parameters, such as therapeutic dosing regimen, serum and tumour pharmacokinetic (PK) and pharmacodynamics (PD), and effects on basic tumour cell properties such as proliferation rate and apoptosis. At this point potential biomarkers of interest should be examined.
(ii) Survival studies: Determine ability of drug to improve overall or progression- free survival.

3. Assessment of response: Animals must undergo serum biomarkers or serial imaging, to measure response. Reproducible imaging modalities, such as high resolution ultrasound ((a) & (b)), dynamic-contrast enhanced magnetic resonance imaging (DCE-MRI) (c) and positron emission tomography- computerized tomography PET- CT.
4. Endpoints: Predetermined timed endpoints with an aim to understand mechanisms of action. In survival studies animals remain on treatment until they require sacrifice due to pre-established morbidity endpoints. Pharmacokinetic and Pharmacodynamic effects can be examined and correlated with short term study results to examine potential mechanisms of resistance.
5. Clinical trial: Aspects like development preclinical biomarkers, novel and potential imaging methods, dosing schedule along with endpoints should be included in translation to early stage clinical trials.

Predictive in vivo models:

There were multiple measurements which predict response to novel therapeutics shown by in vivo models. Firstly, the model response to the standard treatments were assessed [21]. Malignancy which shows limited effective therapeutic option, agents used far should be evaluated for their lack of response. The model which generally responds to therapeutics which fails to have an impact on the corresponding human cancer, will not be effective at predicting responses to new agents. Xenografts had been poor at predicting responses to novel therapeutics is the one reason [7]. Genetically engineered mouse model ideally shows reasonable penetrance and tumour latency which recapitulate the histological appearance of human cancer. Genetically engineered mouse model not accurately captured the complex interplay between tumour cells, microenvironment and cooperating genomic changes when tumour latency was too short. If any model takes long time to produce tumour, then it became impractical for therapeutic assessments. Newer technologies can be used to overcome some of the limitation posed by traditional GEMMs, such as non- germline GEMMs [28]. There are facile ways to assess tumour progression and response to therapeutics. Serial imaging was performed to assess therapeutic response in human trials, therefore functional imaging in preclinical trials had been lagged behind. Many groups investigated different imaging modalities in GEMMs, such as dynamic contrast enhanced magnetic resonance imaging (DCE-MRI), positron emission tomography computer tomography (PET-CT) and high resolution ultrasound and best techniques which depend on the

model under examination [21]. The rapidly expanding field also steer the way for superior imaging modalities to permit earlier radiological assessment of therapeutic effects in human cancer [29,30]. Recent years of specific difficulty has the ability to accurately study metastases in different models. Orthotopic models and GEMMs do metastasise to specific organs of choice but potentially at a lower rate than the corresponding malignancies[31]. To study the role of spontaneous cancer metastases and examine effects of therapeutic agents in solid tumours, models were established [31]. It was shown that in mouse model of pancreatic cancer that tumours metastasise at an early stage and the majority of patients was present with metastatic disease [32,33]. These all results has clear implications for the treatment of pancreatic cancer[34]. In the preclinical evaluation of anticancer targets, GEMMs of cancer should be used along with cell culture-based xenograft and transplant model systems, as no model of cancer was perfect. The knowledge obtained from each system, help to understand the novel therapeutics more than any system alone. These all was encouraged and performed by industry and academia.

Preclinical to Clinical Transition:

Many novel therapeutics was tested on various GEMMs of cancer. Different types of drugs, such as receptor kinase inhibitors, rapamycin analogue, angiogenesis inhibitors and prostaglandin inhibitors shows preclinical efficacy[7]. In the treatment of lung adenocarcinoma epidermal growth factor was applied successfully in the clinic[35]. Results from GEMMs depend on the genetics of lung adenocarcinoma revealed same successes[36,37].KRAS-driven GEMM of lung adenocarcinoma has revealed significant preclinical responses with the combination of a HSP90 inhibitor and rapamycin[38]. There has been remarkable development and effective new treatments in acute promyelocytic leukaemia (APL), which creates the accurate GEMMs that has been developed for this disease. APL was curable, based on the genetic criteria of their disease, patients were stratified to the treatments[39]. In this process, the GEMMs of APL were fundamental, and it was used as preclinical predictive engines, with results translated into highly successful clinical trials [40]. In the field of pancreatic neuroendocrine tumours (NETs), significant achievements was also been well documented. Preclinical therapeutic trials which was well designed investigated the use of sunitinib, and other kinase inhibitors, in the genetically engineered RIPTAG mouse model [41-43]. These results in the development of Phase 1/2 trials in NET tumours and also successful in Phase 3 trials, which are set to change the face of treatment for some tumours[44, 45]. These first Phase 3 therapeutic success stories directly translated from results in GEMMs of cancer. The most renowned story which involves inhibitors of farnesyl transferase (FTIs), developed as inhibitors of Ras processing [46]. These drugs caused regression of HrasG12V-induced mammary tumours [47], but unfortunately in the RAS gene these results did not translate to patients whose tumours harboured mutations [48]. For this failure evaluation of preclinical studies has permitted further into the possible reason. Patients with KRAS mutations are resistant to the effects of FTIs, and those with HRAS mutations. The majority of human cancers have mutations in the KRAS oncogene rather than HRAS [49]. Additionally, an elegant study was performed using Kras-driven GEMMs [22]. In the treatment of lung and pancreatic adenocarcinoma, this study examined the efficacy of chemotherapeutics, EGFR, and vascular endothelial growth factor (VEGF) inhibitors, which clearly shows an excellent correlation between the results in the GEMMs and clinical trial results achieved both positive and negative[22]. These correlation was analysed retrospectively and multiple comprehensive preclinical endpoints and methods were used, which guides the way for therapeutic advances and translation to clinical trials. In different cancer types, multiple early stage clinical trials were underway, which was designed with the knowledge of successful results from preclinical studies using GEMMs. For successful translation of novel therapeutics, a thorough understanding of therapeutic mechanisms, preclinical models and early stage clinical trials was required. When GEMMs show response, and clinical trials fail to show efficacy, these would be answerable to the question as to why the translation has failed. These was completed when clinical trials in question were designed with scientific rationale and biomarker driven endpoints.

CONCLUSION

During drug development process, selection of most appropriate in vivo model was essential which enable accurate modelling of therapeutic efficacy. Development of innovative preclinical trials using sophisticated animal models that summarize the human malignancies which might able to advance the field of drug discovery and improve success rates for potential novel therapeutics in clinical trials. Close collaborations between industry and academia, drug transfers and enabling animal between organisations, divulging of expert knowledge were required. Ultimately, this lead to swifter, successful, translation to the clinical trial, which in long term would be cost effective compared with failure of therapeutic in its development.

REFERENCES:

1. Johnson JI, Decker S, Zaharevitz D, Rubinstein LV, Venditti JM, Schepartz S, Kalyandrug S, Christian M, Arbuck S, Hollingshead M, Sausville EA. Relationships between drug activity in NCI preclinical in vitro and in vivo models and early clinical trials. British journal of cancer. 2001 May; 84(10):1424.
2. Suggitt M, Bibby MC. 50 years of preclinical anticancer drug screening: empirical to target-driven approaches. Clinical Cancer Research. 2005 Feb 1; 11(3):971-81.
3. Frese KK, Tuveson DA.Maximizing mouse cancer models.Nature Reviews Cancer. 2007 Sep; 7(9):654.
4. Jonkers J, Berns A. Conditional mouse models of sporadic cancer. Nature Reviews Cancer. 2002 Apr; 2(4):251.
5. Ocana A, Pandiella A, Siu LL, Tannock IF. Preclinical development of molecular-targeted agents for cancer. Nature reviews Clinical oncology. 2011 Apr; 8(4):200.
6. Teicher BA. Tumor models for efficacy determination. Molecular cancer therapeutics. 2006 Oct 1; 5(10):2435-43.
7. Sharpless NE, DePinho RA. Model organisms: The mighty mouse: genetically engineered mouse models in cancer drug development. Nature reviews Drug discovery. 2006 Sep; 5(9):741.
8. Peterson JK, Houghton PJ. Integrating pharmacology and in vivo cancer models in preclinical and clinical drug development.European journal of cancer. 2004 Apr 1; 40(6):837-44.
9. Society for Clinical Trials Board of Directors. The Society for Clinical Trials opposes US legislation to permit marketing of unproven medical therapies for seriously ill patients. Clinical Trials. 2006 Apr; 3(2):154-7.
10. Olive KP, Jacobetz MA, Davidson CJ, Gopinathan A, McIntyre D, Honess D, Madhu B, Goldgraben MA, Caldwell ME, Allard D, Frese KK. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science. 2009 May 21.
11. Cook N, Olive KP, Frese K, Tuveson DA. K-Ras-driven pancreatic cancer mouse model for anticancer inhibitor analyses.Methods in enzymology.2008 Jan 1; 439:73-85.
12. Hambardzumyan D, Amankulor NM, Helmy KY, Becher OJ, Holland EC. Modeling adult gliomas using RCAS/t-va technology.Translational oncology. 2009 Jun 1; 2(2):IN6.
13. Voskoglou-Nomikos T, Pater JL, Seymour L. Clinical predictive value of the in vitro cell line, human xenograft, and mouse allograft preclinical cancer models. Clinical Cancer Research. 2003 Sep 15; 9(11):4227-39.
14. Bibby MC. Orthotopic models of cancer for preclinical drug evaluation: advantages and disadvantages. European journal of cancer. 2004 Apr 1; 40(6):852-7.
15. Hoffman RM. Orthotopic metastatic mouse models for anticancer drug discovery and evaluation: a bridge to the clinic. Investigational new drugs. 1999 Nov 1; 17(4):343-60.
16. Fidler IJ, Ellis LM. The implications of angiogenesis for the biology and therapy of cancer metastasis.Cell. 1994 Oct 21; 79(2):185-8.
17. Garber K. From human to mouse and back: "tumorgraft" models surge in popularity.
18. Dong X, Guan J, English JC, Flint J, Yee J, Evans K, Murray N, MacAulay C, Ng RT, Gout PW, Lam WL. Patient-derived first generation xenografts of non–small cell lung cancers: promising tools for predicting drug responses for personalized chemotherapy. Clinical Cancer Research.2010 Feb 23:1078-0432.
19. Hidalgo M, Bruckheimer E, Rajeshkumar NV, Garrido-Laguna I, De Oliveira E, Rubio-Viqueira B, Strawn S, Wick MJ, Martell J, Sidransky D. A pilot clinical study of treatment guided by personalized tumorgrafts in patients with advanced cancer. Molecular cancer therapeutics. 2011 Jun 14: molcanther-0233.
20. Van Dyke T, Jacks T. Cancer modeling in the modern era: progress and challenges. Cell. 2002 Jan 25; 108(2):135-44.
21. Olive KP, Tuveson DA. The use of targeted mouse models for preclinical testing of novel cancer therapeutics. Clinical Cancer Research. 2006 Sep 15; 12(18):5277-87.
22. Singh M, Lima A, Molina R, Hamilton P, Clermont AC, Devasthali V, Thompson JD, Cheng JH, Reslan HB, Ho CC, Cao TC. Assessing therapeutic responses in Kras mutant cancers using genetically engineered mouse models. Nature biotechnology. 2010 Jun; 28(6):585.
23. Roberts TG, Goulart BH, Squitieri L, Stallings SC, Halpern EF, Chabner BA, Gazelle GS, Finkelstein SN, Clark JW. Trends in the risks and benefits to patients with cancer participating in phase 1 clinical trials. Jama. 2004 Nov 3; 292(17):2130-40.
24. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. New England Journal of Medicine. 2001 Mar 15; 344(11):783-92.
25. Demetri GD, Von Mehren M, Blanke CD, Van den Abbeele AD, Eisenberg B, Roberts PJ, Heinrich MC, Tuveson DA, Singer S, Janicek M, Fletcher JA. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. New England Journal of Medicine. 2002 Aug 15; 347(7):472-80.
26. Yap TA, Sandhu SK, Workman P, De Bono JS. Envisioning the future of early anticancer drug development.Nature Reviews Cancer. 2010 Jul; 10(7):514.
27. Carden CP, Sarker D, Postel-Vinay S, Yap TA, Attard G, Banerji U, Garrett MD, Thomas GV, Workman P, Kaye SB, de Bono JS. Can molecular biomarker-based patient selection in Phase I trials accelerate anticancer drug development? Drug discovery today. 2010 Feb 1; 15(3-4):88-97.

28. Heyer J, Kwong LN, Lowe SW, Chin L. Non-germline genetically engineered mouse models for translational cancer research. Nature Reviews Cancer. 2010 Jul; 10(7):470.
29. Brindle K. New approaches for imaging tumour responses to treatment.Nature Reviews Cancer. 2008 Feb; 8(2):94.
30. Seaman ME, Contino G, Bardeesy N, Kelly KA. Molecular imaging agents: impact on diagnosis and therapeutics in oncology. Expert reviews in molecular medicine. 2010 Jul; 12.
31. Francia G, Cruz-Munoz W, Man S, Xu P, Kerbel RS. Mouse models of advanced spontaneous metastasis for experimental therapeutics. Nature Reviews Cancer. 2011 Feb; 11(2):135.
32. Rhim AD, Mirek ET, Aiello NM, Maitra A, Bailey JM, McAllister F, Reichert M, Beatty GL, Rustgi AK, Vonderheide RH, Leach SD. EMT and dissemination precede pancreatic tumor formation. Cell. 2012 Jan 20; 148(1):349-61.
33. Haeno H, Gonen M, Davis MB, Herman JM, Iacobuzio-Donahue CA, Michor F. Computational modeling of pancreatic cancer reveals kinetics of metastasis suggesting optimum treatment strategies. Cell. 2012 Jan 20; 148(1-2):362-75.
34. Tuveson DA, Neoptolemos JP. Understanding metastasis in pancreatic cancer: a call for new clinical approaches. Cell. 2012 Jan 20; 148(1-2):21-3.
35. Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C, Zhang S, Wang J, Zhou S, Ren S, Lu S. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. The lancet oncology. 2011 Aug 1; 12(8):735-42.
36. Politi K, Zakowski MF, Fan PD, Schonfeld EA, Pao W, Varmus HE. Lung adenocarcinomas induced in mice by mutant EGF receptors foundin human lung cancers respondto a tyrosine kinase inhibitor orto down-regulation of the receptors. Genes & development. 2006 Jun 1; 20(11):1496-510.
37. Ji H, Li D, Chen L, Shimamura T, Kobayashi S, McNamara K, Mahmood U, Mitchell A, Sun Y, Al-Hashem R, Chirieac LR. The impact of human EGFR kinase domain mutations on lung tumorigenesis and in vivo sensitivity to EGFR-targeted therapies.Cancer cell. 2006 Jun 13; 9(6):485-95.
38. De Raedt T, Walton Z, Yecies JL, Li D, Chen Y, Malone CF, Maertens O, Jeong SM, Bronson RT, Lebleu V, Kalluri R. Exploiting cancer cell vulnerabilities to develop a combination therapy for ras-driven tumors. Cancer cell. 2011 Sep 13; 20(3):400-13.
39. Lallemand-Breitenbach V, Guillemin MC, Janin A, Daniel MT, Degos L, Kogan SC, Bishop JM. Retinoic acid and arsenic synergize to eradicate leukemic cells in a mouse model of acute promyelocytic leukemia. Journal of Experimental Medicine. 1999 Apr 5; 189(7):1043-52.
40. Pandolfi PP. APL as a paradigm in biomedical research: a journey toward the cure. In Acute Promyelocytic Leukemia 2007 (pp. 1-2).Springer, Berlin, Heidelberg.
41. Pietras K, Hanahan D. A multitargeted, metronomic, and maximum-tolerated dose "chemo-switch" regimen is antiangiogenic, producing objective responses and survival benefit in a mouse model of cancer. Journal of Clinical Oncology. 2005 Feb 10; 23(5):939-52.
42. Paez-Ribes M, Allen E, Hudock J, Takeda T, Okuyama H, Viñals F, Inoue M, Bergers G, Hanahan D, Casanovas O. Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. Cancer cell. 2009 Mar 3; 15(3):220-31.
43. Chiu CW, Nozawa H, Hanahan D. Survival benefit with proapoptotic molecular and pathologic responses from dual targeting of mammalian target of rapamycin and epidermal growth factor receptor in a preclinical model of pancreatic neuroendocrine carcinogenesis. Journal of Clinical Oncology. 2010 Oct 10; 28(29):4425.
44. Yao JC, Shah MH, Ito T, Bohas CL, Wolin EM, Van Cutsem E, Hobday TJ, Okusaka T, Capdevila J, De Vries EG, Tomassetti P. Everolimus for advanced pancreatic neuroendocrine tumors. New England Journal of Medicine. 2011 Feb 10; 364(6):514-23.
45. Raymond E, Dahan L, Raoul JL, Bang YJ, Borbath I, Lombard-Bohas C, Valle J, Metrakos P, Smith D, Vinik A, Chen JS. Sunitinib malate for the treatment of pancreatic neuroendocrine tumors.New England Journal of Medicine. 2011 Feb 10; 364(6):501-13.
46. James GL, Goldstein JL, Brown MS, Rawson TE, Somers TC, McDowell RS, Crowley CW, Lucas BK, Levinson AD, Marsters JC. Benzodiazepine peptidomimetics: potent inhibitors of Ras farnesylation in animal cells. Science. 1993 Jun 25; 260(5116):1937-42.
47. Kohl NE, Omer CA, Conner MW, Anthony NJ, Davide JP, Desolms SJ, Giuliani EA, Gomez RP, Graham SL, Hamilton K, Handt LK. Inhibition of farnesyltransferase induces regression of mammary and salivary carcinomas in ras transgenic mice. Nature medicine. 1995 Aug; 1(8):792.
48. Cohen SJ, Ho L, Ranganathan S, Abbruzzese JL, Alpaugh RK, Beard M, Lewis NL, McLaughlin S, Rogatko A, Perez-Ruixo JJ, Thistle AM. Phase II and pharmacodynamic study of the farnesyltransferase inhibitor R115777 as initial therapy in patients with metastatic pancreatic adenocarcinoma. Journal of Clinical Oncology. 2003 Apr 1; 21(7):1301-6.
49. Cox AD, Der CJ. Ras history: The saga continues. Small GTPases. 2010 Jul 1; 1(1):2-7.

SP-15

REVIEW ARTICLE ON HALF CORD PARALYSIS: TRANSVERSE MYELITIS

MOHAMMED RAFIQ BAIG*, Dr.G.SUSMITHA
Department of Pharmacy Practice, Bhaskar Pharmacy College, Hyderabad- 500075, India.
E-mail Id: susmi.aswi@gmail.com

ABSTRACT:
Acute transverse myelitis (ATM) is an etiologically heterogenous syndrome with acute onset, in which inflammation of spinal cord results in neurologic deficits, manifesting as weakness, sensory loss and autonomic dysfunction. The chance of occurrence is 1-4 people in 1 million[1], it can be associated with infection of bacteria, virus, fungi and auto immune disorders which constitute as 40% of etiology, rest 60% is idiopathic (unknown). It can affect persons from age 10 to 70 years. By treatment it can be cured if diagnosed very soon if not it is nearly complete treatable and can maintain it with medications and physiotherapy.

INTRODUCTION:
Transverse Myelitis is defined as inflammation of the myelin sheath of neurons[4] present in the spinal cord. This inflammation may be through the length i.e. longitudinal (or) it can be perpendicular i.e. transversely, so based on the inflammation the name is given.In either way of inflammation, it causes some of the sensory and motor signal obstruction due to inflammation of myelin sheath which results in non-functioning of some of the body parts because of improper signaling through it.

ETIOLOGY AND OCCURENCE:
There are many idiopathic ways by which Transverse myelitis can occur and among those known are infections through various kind of microorganism and autoimmune disorder[2].Transverse myelitis is rare condition occurring to an individual in which a individual looses control over posture of body and voluntary actions.

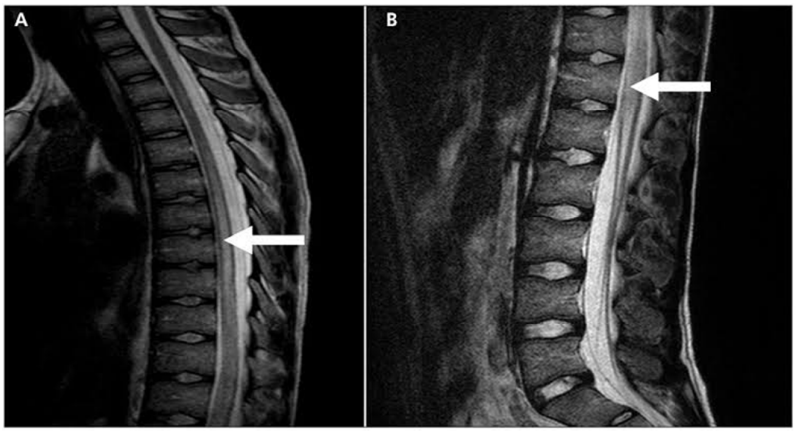
SYMPTOMS:
Rapid onset of weakness, bladder dysfunction and alteration of sensory and motor function.[5]

PATHOPHYSIOLOGY:
The progressive loss of protective fatty myelin sheath around nerves in the affected or infected spinal cord occurs for unclear reasons following infections and autoimmune response of the body. Infection may cause inflammation of spinal cord where as auto immune response may demyelinate the neurons of spinal cord which causes non-functioning or improper functioning of motor and sensory functions of body[1].

TREATMENT:
The recovery from the Transverse myelitis differs from person to person depending on the underlying cause. Few populations recover within 2-12 weeks of treatment some take longer than that and some patient may not show any sign of improvement.
If it is treated in early, patient felt completely or nearly complete recovery from the condition[3].
Treatment include medications depending upon the cause and other suggestions like physiotherapy, plasmapheresis.

CASE:
CLINCAL PRESENTAION:
A 13 years old female patient came to a tertiary hospital with chief complaints of following cough associated with sputum, fever (low grade) with intermitted chills and rigors, and blebs on gluteal mass.
When she came her posture was not good, she was malnourished, and had anemia, she lost control for walking and was unable to all kind of activity from lower region of body.
DIFFRENTIAL DIAGNOSIS:

Blood analysis, MRI scanning, Lumbar puncture, Sputum test were performed to diagnose the condition of patient.[6]Which shows anemia, inflammation of spinal cord, kind of infection (increase WBC), and bacteria was identified as gram negative (klebsiella pneumonia)[3] respectively for test above done.



MRI SAMPLE FOR INFLAMMATION OF SPINAL CORD

Treatment followed up for case:

Rx:

The combination of drugs {INJ.AUGMENTIN (AMOXICILLIN+CLAVULANIC ACID) -(250/125 mg)TAB.AZEE (AZITHROMYCIN)-250 mg} to avoid any secondary infection and also to control the infection spread in the body, and to maintain temperature {TAB.DOLO (ACETAMINOPHEN)-650mg}. INJ.OPTINEURON (MULTIVITAMIN AND FOLIC ACID)-1 AMP IN 100ml saline was given to make patient nourish, DEBRIDASE CREAM for dressingsregular dressings to treat blebs occurred on gluteal mass, physiotherapy for frequent change in posture. Later INJ AUGMENTIN was changed to TAB.AUGMENTIN (250/125).and after some improvement AMIKACIN was added.

DISCUSSION:

The medication amoxicillin + clavulanicacid(it inhibits bacterial cell wall synthesis and hence help in destruction of it to reduce infection) used for lowering down the infection initially and azithromycin(it interfere with protein synthesis and hence decreases its population) was used for preventing any secondary upper respiratory tract infection[2].Acetaminophen (it reduced prostaglandin in the brain and thereby reduces fever by its action) is used for lowering of excess body temperature and optineurin (multivitamin and folic acid) was used for treating anemia and malnourished condition of patient physiotherapy was done in order to make normal movement till treatment get compete and help in recovery of motor and sensory stimulation in the parts regular dressing was necessary for the blebs on gluteal mass with ointment to recover skin and avoid infections[1].Apart from this the AMIKACIN(It bind to 30s ribosome subunit and causes misreading of genetic code) was used for treating klebsiella pneumonia bacteria in order to recover and prevent further infection of it to spinal cord and as well as another target region[4].

CONCLUSION:

Through this review article I conclude that "The Transverse Myelitis" can be cured nearly completeif diagnosed early. proper caring of all the areas of etiology and the drugs such as amoxicillin,clavulonic acid, azithromycin and amikacin plays major role in treating the main cause of infection to spinal cord and hence it is important to understand all perspective of a condition before we start treating it.

REFERENCES:

1. Transverse myelitis consortium Working Group: Proposed diagnostic criteria and nosology of acute transverse myelitis. Neurology.2002, 59: 499-505.
2. Agmon-Levin N, Kivity S, Szyper-kravitz M, Shonenfeld Y: Transverse myelitis and vacciinesL a multi analysis. Lupus. 2009,18:1198-1204.10.1177/0961203309345730.
3. Ueda M, Kanamori A, Mihara T, Haea H, Mutoh T: A case of acute disseminated encephalomyelitis (ADEM) following treatment for pneumococcal meningoencephalitis [in Japanese]. Rinsho Shinkeigaku. 2009, 49L96-99.10.5692//clinicalneurol.49.96.
4. C.W. Lees, A. I. Ali, A. I. Thompsn et al.,"the safety profile of anti-tumour necrosis factor therapy in inflammatory bowel disease in clincal practice: analysis of 620 patient-years follow-up," Alimentary Pharmacology and therapeutics, Vol.29, no.3,pp.286-297, 2009.
5. Wingerchuck DM, Lennon VA, Lucchinetti CF, Pittock SJ, weinshenker BG. The spectrum of neuromyelitis optica. Lancet Neurol 6(9):805-815, 2007.

SP-16

MUSHROOM THERAPY-PREVENTING THE ADR'S OF ANTI NEOPLASTIC AGENTS- A REVIEW

R.SRINIDHI*, DR. G. SUSHMITHA
Department of Pharmacy Practice, Bhaskar Pharmacy College, Hyderabad- 500075, India.
E-mail Id: susmi.aswi@gmail.com

ABSTRACT:
Cancer is perilous and is reason for quietus all over the creation, espousing long term repercussion for a life time. Hence many drugs have been hatched in anticipation of cancer nevertheless engendering reactions. So an auxiliary remedy for the carryover of anticancer drugs is to avail oneself of mushrooms. As mushrooms bear active compounds alike β-glycans, β-proteoglycans,lectins, triterpenes, ergosterols, glutamine, arginine, they are urging compromising effect in chemotherapy. Widely utilized mushrooms are of genus *Cordyceps, Fomes, Laricifomes, Ganoderma, Grifola, Lentinula, Piptoporus, Inonotus, Agaricus.* Yet an investigation stipulate that the organic food along with periodic ingestion of mushrooms declines the threat of emerging cancer.

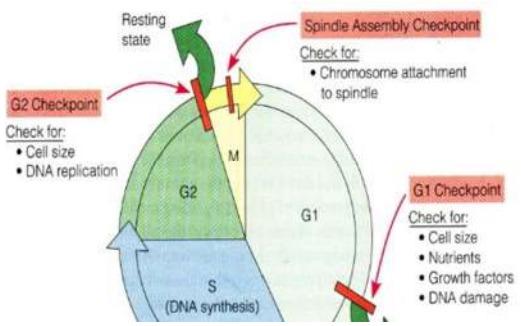
KEYWORDS: Cancer, Quietus, Auxiliary remedy, Mushrooms, Clinical trials, Potency, Anti tumor effects.

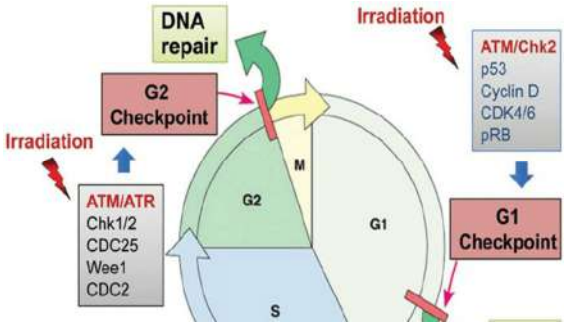
INTRODUCTION:

The gauge of attack of cancer in 2019 is about 1 million and fatal rate is estimated to be 6 lakh. Unfortunately highest fatal rates is due to uterine cancers and next place occupies the lung and skin cancer separately(U.S Department of Health and Human services, NIH,NCI). As there is huge increments in fatal rates steadily, it is important to decrease mortality rates to some extend by proper medical adherence. But the majority of consequences rising from the therapy mainly radiotherapy and chemotherapy, results in impairing and depleting the patient's immune system and triggering several unwanted effects[1].Keeping all these in mind, certain alternative remedy like *biotherapy*have been approached. Biotherapy includes monoclonal antibodies, cancer vaccines, interferons, interleukins, colony-stimulating factors, gene therapy, non-specific immune modulating agents[1]. Apart from all these, use of medicinal mushrooms dug a path in fields of therapeutics. These mushrooms not only have anti-cancer properties, but also can act against cardiovascular diseases, diabetes, act as anti-viral, anti-bacterial, anti-parasitic, anti-inflammatory, nephroprotective, neuroprotective and hepatoprotective properties[1].But medicinal mushrooms gained attentiveness due to its immune modulatory and immunostimulatory properties. Hence these are regarded as *immunomodulators or biological response modifiers.*

Cell cycle and regulation:

1) *Cell cycle checkpoints*: It is a stage at which the cell examines internal and external conditions and decides whether to cause cell division or not. There are many checkpoints but the most important are G1, G2, M check points.
2) *Cell cycle regulators:* The proteins that regulate the cell cycle are called as CCR's. They are cyclins, CDK's, APC/C complex, retinoblastoma protein, p53 protein and p21 protein.





Despite the redundancy and overlapping levels of cell cycle control, errors do occur which may lead to generation of tumor (uncontrolled cell growth) or cancer. In order to treat cancer certain anti neoplastic agents. They include alkylating agents, purine and pyrimidine analogues, antibiotics, vincristine, vinblastine, cisplatin, carboplatin. Though they inhibit the cancer cell growth they leave some toxicity.

Side effects: Bone marrow depression, Lymphocytopenia, Stomatitis, GI disturbances like nausea, vomiting, diarrhoea, haemorrhage, Alopecia, Foetal death, Carcinogenicity, Hyper uricemia, Inhibition of gonadal cells.

An ultimate target to treat the ADR's of anti-neoplastic agents is to make use of medicinal mushrooms. Some of the genus of medicinal mushrooms include- *Cordyceps, Fomes, Laricifomes, Ganoderma, Grifola, Inonotus, Lentinula, Piptoporus, Phellinus, Agaricus*[2]

1) Composition of mushrooms: It includes high molecular weight bioactive metabolites and low molecular weight metabolites.

a) *High molecular metabolites:* β-glucans, Lentinan, Schizophyllan, Terpenoids, Maitake D-fraction, Polysaccharide-protein complex, Bioactive mushroom proteins.

i) *β-glucans:* This bio-active compound is found in *Agaricus subrufescens.*These species have β-glucans which are resistant to acid in the stomach. These glucans binds to receptors of stomach and gets activated to release lysozyme, oxygen radicals and nitrogen oxides. This activates the leucocytes and increases its production[3].

Treatment: Treating the side effects of stomatitis, bone marrow depression.

Dose: 20-40 mg IV once or twice weekly for at least 1year

ii) Lentinan:Lentinula edodes have lentinan which inhibits the nuclear factor kappa B (NF-kB) thus suppressing aromatase activity(Mattila et al. 2001), thereby increases the gonadotropin secretion and stimulates the Leydig cells and Sertoli cell function[3].

*Treatment:*Inhibition of gonadal cells.

*Dose:*oral – 8g/day for 6 months, IV - 2-10mg on a weekly schedule

iii) Schizophyllan: This compound is present in *Schizophyllum commune*. Its mechanism is quite same[3].

*Treatment:*In case of inhibition of gonadal cells, loss of appetite, weakness, dryness of mouth and throat (in case of head and neck cancer).

iv) *Terpenoids:* This compound is present in *Ganoderma lucidum.* It acts by terminating the NF-kB thereby cancelling the expression of matrix-metalloproteinase namely MMP-2, MMP-9 (which is the main reason for serious haemorrhagic conditions)[3].

*Treatment:*Used in treatment of haemorrhage.

Dose: 8mg/kg per day for 8 weeks

v) *Polysaccharide-protein complex:*This complex comprises of glycoproteins, glycopeptides, proteoglycans, where krestin is the main component of polysaccharide. Hence recognised as *polysaccharide krestin*[4]. This is seen in ***Agaricus subrufescens.*** This acts by suppressing the substances of immune system that causes cancer[4]. This treats maximum side effects of anti-neoplastic agents such as lymphocytopenia, bone marrow depression and other carcinogens.

Dose : 3gm/day orally up to 7 years.

Maitake D-fraction: This is seen in *Grifola frondosa.* It acts by inhibiting cyclooxygenase pathway[4]and helps in treating haemorrhage but is ineffective in treating nausea and vomiting, in fact itself causes nausea and vomiting.

Dose: 200-250 mg or 500-2,500 mg powder daily

Low molecular metabolites: Cordycepin, Ergosterol, Grifolin, Irofulven, Polyphenolic compounds (stryrylpyrone class of phenols).

*vi) Cordycepins:*This is in *Cordyceps species,* and act by interfering the m-RNA translation pathway thus inhibiting the protein synthesis[5]. This is found to be more effective in treating the side effects of chemotherapeutic drugs such as gynaecomastia, bone marrow depression, hyperuricemia.
Dose : 1000-3000 mg daily with meals either in single dose or multiple doses *Ergosterol:*This is found in *Ganoderma lucidium.* It acts by inhibiting the transcription of MDR1 gene and interfering the P-glycoprotein expression[5]. It is used in treating the affected gonadal cells.
*Dose:*300 micro molars for 48 hours

vii) Grifolin: This is found in *Albatrellus confluens.* It acts by inhibiting the ERK1/2 or ERK5 pathway in G1 phase of cell cycle[6]. It is used to treat certain side effects of anti-cancer drugs.
Dose: 30ml IM for 24 hours

viii) *Irofulven:* This is found in *Ompholotus illudens.* It acts by interferingthe DNA synthesis and cell cycle arrest in S phase[6]. It treats side effects that are mentioned above.
*Dose:*0.45mg/dl IV for 21 days

ix) *Hispolon:* This is found in *Phellinus linteus.*It acts by inducing the ROS mediates caspase pathway programmed cell death, which leads to cytochrome c release and improper function of mitochondria[6]. It is an antioxidant and treats some of the above mentioned side effects.
*Dose:*20mg/kg for 7 days

CONCLUSION:

Through this review, I conclude that mushrooms play a major role in treating the sideeffects of many anti neoplastic agents. It was found that about 73% of the patients were cured with side-effect caused by ANA. Those who are allergic to mushrooms, can go for alternative therapy bearing herbal products.

REFERENCES:

1) Abercrombie M, Ambrose EJ(1962) The surface properties of cancer cells: a review. Cancer Res 22:525-548
2) Ajith TA, Janaradhanan KK (2003) Cytotoxic and anti-tumor activities of a polypore macro fungus, Phellinus rimosus (berk) Pilat. J Erhnopharmacol 84:157-162
3) Alexander J, Raymond E, Kachi MO, Brain EC, Lokiec FO et al (2004) Phase I and pharmacokinetic study of Irofulven administered weekly or biweekly in advanced solid tumor patients. Clin Cancer Res 10:3377-3385.
4) Akiyama H, Endo M, Matsui T, EMI N, Kawamoto Y, Koike T, Beppu H. Agaritine from Agaricus Blazei Murrell induces apoptosis in leukemic cell line U937. Biochim Biophys Acta. 2011;1810:519-525.
5) Shang EC, Weiss S, Kaeler G. The influence of early supplementation of parenteral nutritional on quality of life and body composition in patients with advanced cancer. JPENJ Parenter Enteral Nutr. 2006;30:222-32
6) The American Heritage Science Dictionary. Published by Houghton Mifflin, 2002. 704p

SP-17

AQUAGENIC URTICARIA

P.SHRUTHI REDDY*, CH. PRAVALYA*, Dr.G. SUSMITHA
Department of Pharmacy Practice, Bhaskar Pharmacy College, Hyderabad- 500075, India.
E-mail Id: susmi.aswi@gmail.com

Abstract
The other term used for Aquagenic Urticaria is water allergy or water urticaria . It is rarely diagnosed form of physical urticaria. This usually occurs when the skin corner in contact with water (in some patients even tears, sweat) and causes wheals. They are pin head-small pea sized wheals surrounded by variable sized erythematousflares following skin contact with water on face, neck and trunk, irrespective of temperature or source, pathogenesis of aquagenic urticaria is unknown.

KEYWORDS:Aquagenic urticaria, water, physical urticaria, diagnosis

INTRODUCTION

Water is found everywhere in our daily lives and it is harmless, but for some patients water is the main problem. Such person is unable to bear a few minutes of moisture on his body and by chance if he comes in contact with water he develops severe symptoms of allergy like itching, redness on skin. This type of allergy is regarded as Aquagenic urticaria.

DEFINITION:

Aquagenic urticaria is rare form of physical urticaria caused when skin comes in contact with water, irrespective of temperature and source, causes wheals[1,2]. Wheals are small punctate lesions are located mainly on upper body(neck,trunk,shoulder,arm,back)[3].

EPIDEMIOLOGY:

Aquagenic urticaria is first seen in shelly and rawnsley in 1964[4]. Nearly 100 cases reported in literature, most commonly seen in females during puberty or post puberty and some of them reported itching too. There have been reports of childhood onset disease, some are sporadic in nature, while several cases of familial disease[6].

CAUSES

Some of the researches are still working to know the causes of aquagenic urticaria. some of them are presence of Chemical additive in water, like chlorine causes development of wheels. Allergy like symptoms are experienced from the rash is due to release of histamine. Due to allergic reaction, immune system release histamine in response to fight against the harmful substance[5]. Histamine causes allergy like symptoms and affects parts of the body.

Pathogenesis

The exact mechanism of aquagenic urticaria is not known. The mechanism of aquagenic urticaria of first reported case Shelly and rawnsely in 1964 is hypothesized that when water comes in contact with sebum or sebaceous glands of skin it produce a toxic component, leads to degranulation of mast cells and release of histamine leads to formation of urticarial lesions[4]. Some of them proposed ,that it is due to change in osmatic pressure cause indirect action of urticaria[8]. Another mechanism is presence of water-soluble antigens in the epidermis which diffuse and dissolve into dermis and causes release of histamine from mast cells[7]. Recent studies proposed that they are completely independent of histamine release ,based on reports of several patients with aquagenic urticaria[5].

Clinicalpresentations

Symptoms develops within 30minutes after contact with water irrespective of temperature and source. The pruritic wheals of(1-3mm diameter) surrounded by(1-3cm) erythematous flares which usually last less than 1hour[4] and located on the neck, upper trunk and arms. Other symptoms include pruritus, burning sensation,

inflammation, or reading of skin and uncomfortable prickling wheals disappear within 30-60 minutes[1]. Some patients may have systemic symptoms such as wheezing, headache, respiratory distress, lightheadedness and palpitation but are rarely seen[6].Some uncommon clinical presentation are also found. Urticaria is thought to occur in response to any form of Aquagenic urticaria , so there have been report of patient who had reactions depending on salinity of the water. For example, a patient reacted with tap water, snow and sweat but can swim in the ocean without urticaria[6]. In patients who have decreases thickness of stratum corneum following epilation/cutaneous exposure to organic solvents has worse urticarial response to water[3]. Aquagenic urticaria associated with systemic disease, the urticarial response is more often considerable,consisting of large edematous plaques rather than classic punctate perifollicular wheals[6].

DIAGNOSIS

Physical urticaria	Provocation test
Pressure urticaria	A 6000 gm weight is applied to the skin for 20 minutes
Cold urticaria	An ice cube filled plastic bag is applied to patient's forearm for 20 minutes
Heat urticaria	A heated cylinder (50-55°c) is applied to the upper trunk for 30 minutes
Cholinergic urticaria	Exercise test until sweat warm bath -43°c
Aquagenic urticaria	Apply wet cloth of room temperature to upper body for 30 minutes -35°c

Diagnosis of Aquagenic urticaria is based on history of patient with water challenge test[3]. The test can be directed in a variety of ways, the standard method include use of cloth which is moistened at room temperature to the patients skin for 20 minutesshows positive response for aquagenic urticaria[10]. The temperature used for applicationof water to patients plays a key role because heat or cold exposure may deviate to other physical urticaria, gives a false positive response[3]. The mainprovocation in diagnosing Aquagenic urticaria is to differentiate this condition from other types of physical urticaria (eg :cholinergic urticaria, heat urticaria, cold urticaria, pressure urticaria, exercise -induced urticaria). Provocation test is used for this specific types are shown in the above table[2].

Laboratory analysis is used to differentiate aquagenic urticaria from immunoglobin -E mediated allergic reactions by the level of serum immunoglobin-E which is normal. When the patient is provisionally diagnosedwith angioedema along with urticaria we can explore the cause of angioedema by invigilating the Cl-.esterase inhibitors. Hereditary and acquired angioedema where only angioedema is seen should have normal levels of cl-esterase inhibitors. We should keep in mind that serum histamine levels cannot be increased when exposed to water all the time[3].

TREATMENT

As aquagenic urticaria is rare there is very limited data regarding the effectiveness of individual treatment[1]. Antihistamines are commonly used as first line therapy for aquagenic urticaria , they block H1 receptor and are non sedative , some of them are 1%diphenhydramine,ketotifen(5ml)[2]. First generation H1 antihistamines show sedative and anticholinergic side effects than therapeutic and anti puritic effect, last for 4-6 hours , so second generation anti histamines are used because they show less effect on CNS depression and has greater duration of action like fexofenadine(180mg)[9].Creamsandother topical agents severe as barrier. Between water and skin such as petroleum based products. They are applied before and after exposure to water to prevent water penetration into skin especially in pediatric patients to prevent side effect of anti-histamines[8]. Phototherapy is also used such as psoralens ultraviolet radiation A (PUVA) and ultraviolet radiation B used to resolve symptoms of aquagenic urticaria in few cases[3].Omalizumab an injectable medication commonly used to treat severe asthma but also reported as effective in few people with Aquagenic urticaria[2].

CONCLUSION:

Through this review, I conclude that since water is main source for everyone, unfortunately there are some people who suffers with water allergy. Use of anti histamines can give temporary relief but the goal to provide permanent cure is still under process. Many clinical trials is carrying out to provide effective therapy for patients suffering from Aquagenicurticaria.

REFERENCES:

1. Hyojin Kim, Department of dermatology, Busan Paul Hospital, Inje University College of Medicine, 75 Bokji-ro, Busanjin-guess, Business 47392, Korea
2. Hook Park, M. D., Department of dermatology, Uijeongbu St. Mary's Hospital, College of Medicine, The Catholic University of Korea, 65-1 Geumo-dong, Uijeongbu 480-130, Korea.
3. Jean S McGee Department of dermatology, Boston University School of Medicine, 609 Albany street, Boston, MA 02118,USA.
4. Shelley WB, Rawnsley HM. Aquagenic urticaria : contact sensitivity reaction to water. JAMA. 1964;189:895-898
5. Lying K, Nguyen L. Aquagenic urticaria : report of a case an review of the literature. Ann Allergy Asthma Immunology. 1998;80(6):483-485.
6. Fearfield LA, Gazzard B, Bunker CB. Aquagenic urticaria and human immunodeficiency virus infection:treatment with stanozolol. Be J Dermatology. 1997;137:620-622
7. Czarnetzki BM, Breetholt KH, Trips H. Evidence that water acts as a carrier for an epidermal antigen in aquagenic urticaria J Am Academy Dermatol 1986;15:623-627
8. Gallo R, Gonzalo M, CinottiE,Parodi A. Localized salt -dependent aquagenic urticaria :a subtype of aquagenic urticaria? Clinton Explain Dermatology. 2013;(7):754-757
9. Sibbald RG, Black AK, Easy RA, James M, Greaves MW . Aquagenic urticaria :evidence of cholingeric and histaminergic basis. Be J dermatology. 1981;105(3):297-302.
10. Zuberbier T, Aberer W, Asero R, et al. The EAACI/GA2LEN/EDF/WAO Guideline for the definition, classification, diagnosis and management of urticaria: the 2013 revision and update. Allergy 2014;69(7);868-887.

SP-18

PSITTACOSIS (PARROT FEVER)

B. LAKSHMINIKHILA*, Dr .G. SUSMITHA
Department of Pharmacy Practice, Bhaskar Pharmacy College, Hyderabad- 500075, India.
E-mail Id: susmi.aswi@gmail.com

ABSTRACT:
Psittacosis, otherwise parrot fever and ornithosis, is an infection which is caused by bacteria namely known as chlamydia psittaci. It leads to severe pneumonia and other health problems in humans. Chlamydia psittaci can infect different kinds of birds and is referred to as avian chlamydiosis. Most often human cases are seen after the exposure to infected parrot type of birds which are used as pets particularly cockatiels, budgerigars, macaws and also from non-psittadian species such as pigeons, sparrows, ducks, hens, gulls. The disease is contracted to the humans through the exposure bacteria present in feces and nasal discharges, shaded by infected birds.

INTRODUCTION:

Figure -1 Bird with psittacosis[12]

Definition: parrot fever and ornithosis are the other terms of psittacosis. Psittacosis is a respiratory tract infection which is clinically reported with influenza like symptoms (fever, headache, malaise, productive cough for 50% but delayed) and perhaps commonly known as simple community-acquired pneumonia (CAP) or influenza.[5]

- The term psittacosis is arrived because the major cause
- for the transmission is through the infected birds mainly identified to be psittacine (parrot fever) birds, especially cockatiels and budgerigars.[2]
- Among non-psittacine birds (pigeons, doves, poultry
- species such as hens, ducks, turkeys) are likely to be affected, hence ornithosis is the term for psittacosis.[2]

Etiology: Psittacosis (respiratory tract infection), chlamydial psittaci which is an obligate intracellular bacterium is the causative agent of infection and was 1st described in 1879.

- Chlamydial psittaci is a bacterial species, belongs to the family chlamydiaceae.[1]
- Although there are other sources that person may get affected, contact with the infected birds plays an important role and major risk factor for illness.[2]
- Genotype-A is indigenous among cockatoos, parakeets and Lories(psittaci forms) which is well known as zoonotic agent.[3]
- Genotype-B is indigenous for pigeons and doves.[3]
- Genotype C and D are mostly detected in non-psittacine birds.[3]
- But early studies says that Genotype E/B strain was identified in both parrots and humans but so far psittaci forms are most frequently affected by Genotype-A strain.[3]

Epidemiology: Psittacosis which is a rare zoonotic infection can affect any age group and gender but incidence tend to peak in middle age that is from 35-55 years. In 2018, 13 confirmed cases in Georgia and virgina, which are US secondary occupational exposure. [11,12]

TRANSMISSION:

The psittacosis is a respiratory infectious disease which is caused by a bacteria named chlamydial psittaci and is transmitted through psittacine birds (parrot type birds).

- Perhaps transmission of the disease is also been recorded by exposure to non-psittacine birds which includes pigeons, doves, hens, shorebirds.
- As discussed psittacosis an infection to human is caused by chlamydial psittaci, is a respiratory tract disease is caused by the inhalation of aerosolized bacteria from dried feces or respiratory tract secretions of the infected birds.
- Psittacosis is thought to be transmitted through person to person or can be explained as contagious in rare cases.
- Specific segregation remedies (private rooms, masks) are not mentioned as there is no documented proof of person to person transmission and there is no recommendation for droplet precautions.[8, 9,10]

PATHOPHYSIOLOGY:

- As mentioned, psittacosis is a respiratory infection which occurs by the inhaled aerosolized bacteria from avian faces, feather dust or respiratory secretions.
- Hypothetical and investigations say that the airflow in the respiratory bronchiole is slow moving and stops in the alveolar ducts.
- The submicron particles of about the size of 0.5large particles, 1 to 5p may be expected to affect proximally along the respiratory bronchiole.
- This structure is free of protective coverings like ciliated columnar epithelium and mucous layer in its distal portion, reason that virus can come into direct contact with the cells.
- As many other infections including psittacosis, respiratory bronchiole is the site of infection to begin.
- Lymphangitic spread plays a part.[14]
- The renewal of chlamydial psittaci bacteria from blood, liver, spleen re-emphasizes the systemic nature of this infection.[14]
- The organism can out last for many months in bird dander and faces, as the organism shows resistant towards drying.[15]
- The inoculum enters the blood stream via the lungs and enters the reticuloendothelial system, with secondary bacteremia leading to respiratory infection.[9, 10]

RISK FACTORS:

- Pigeon fancying.
- Contact with ill birds.
- Certain occupations like bird keeping or working in a pet shop and in most studies it is declared that the infection is likely to occur from infected birds and people affected are from pet shops who were working there as a keepers.
- Poultry forms.
- Vets
- Bird breeders and poultry processing plant workers.
- Zoo and park keepers.
- Street cleaners.
- Those working on building demolition or conservation where birds have been nesting.[8, 9,10]

CLINICAL MANIFESTATIONS:

Symptoms: The commencement of symptoms often needs an incubation period of at least 5 to 14 days but upon the serological testing longer periods have been recorded. The disease is mild in the initial stage later

turning non-specific illness to systemic illness with severity. Death of the person is seen in rare conditions. Community acquired pneumonia with flu like symptoms are traditionally observed. Non-productive cough, dyspnea, sore throat, epistaxis and pleuritic chest pain (rarely seen) are underlined respiratory symptoms. The disease may also cause other systemic illness related to gastrointestinal (abdominal pain, jaundice) neurological (severe headache, photophobia) dermatological (Horder spots – popularly known as facial macular rash and advent of rose spots which are seen in typhoid fever).[9,10, 13]

Signs:

- Pneumonia consolidation, can be a sign of a disease.
- There may be signs of Pericarditis, Endocarditis or Myocarditis.
- Meningitis, Encephalitis, seizures or Guillain-Barre syndrome may be rarely seen.
- Splenomegaly is commonly seen.[9, 10, 13]

DIAGNOSIS:

Traditionally, based on the analytical examination, the diagnosis has been reported. Using Micro-immunofluorescence (MIF) with paired sera which is more responsive than compliment fixation, tests give positive serological results. In case in point, the MIF still shows cross reactivity with other species of chlamydia. Due to the above following reasons, titer less than 1:128 is elucidated with a care.[1]

A true acute (obtained as close to the onset of symptoms) and convalescent (ideally taken 2-4 weeks later) specimen results are needed for proper presentation of the disease.

Modern diagnosis tests suggests molecular testing which involves Nuclei acid amplification for example PCR which has been increased in both authenticity and availability. Using an OmpA genotype- Specific real time PCR, CP. Psittaci genotype E/B was identified as the transmitted strain.[3]

The diagnosis is done by isolation of chlamydia Psittaci from sputum, plural fluid, by lumbar puncture or clotted blood during acute illness and before the treatment with antibiotics. All the collected swabs which are used for testing maintained at -80 degrees centigrade. [3]

DIFFERENTIAL DIAGNOSIS:

- Extrinsic allergic alveolitis (bird fanciers lungs)
- Pneumonia secondary to typical or atypical organisms such as legionella, mycoplasma and meningoencephalitis.
- Q-fever.
- Infective endocarditis.
- Influenza virus infection and bacterial pneumonia.[9, 10, 5]

MANAGEMENT:

- The antibiotics treating the infection caused by the bacterium chlamydia Psittaci as a first line of drug treatment are tetracycline (moderately used - oral doxycycline or tetracycline hydrochloride are used. If the disease in the patient is presented with acute illness, treatment with intravenous doxycycline hyclate are recommended).
- Normally, the patient is receptive towards the treatment within 24- 72 hours and the treatment is normally given for atleast 2 to 3 weeks to lower risk of relapse.
- Macrolide (Erythromycin and Azithromycin) are the considered to be good second line or alternative treatment for those who are contraindicated for tetracycline especially in children and pregnant women (shows teratogenicity).[1,6,7]

COMPLICATIONS:

- Relapse of disease after antibiotic therapy.
- Acute respiratory failure.
- Pericarditis.
- Myocarditis.

- Meningoencephalitis.
- Hemolytic anemia.
- Disseminated intravascular coagulation.
- Gullian-Barre syndrome.
- Gestational Psittacosis.[8, 9, 10]

CONCLUSION:

Through this review article, I conclude that Psittacosis which is a respiratory infective disease is treated with tetracycline and Macrolide. As there is no vaccine for Psittacosis till date, the spread of infection can be prevented by treating the infected people, infected birds with antibiotics and ensure that the environment in which they live is thoroughly cleaned and disinfected. If the disease is diagnosed at the early stage, the patient is treated with doxycycline. In some cases it may lead to severe pneumonia and rarely death.

REFERENCES:

1. J. of Avian Medicine and Surgery, 31(3):262-282(2017). https://doi.org/10.1647/217-265.
2. https://doi.org/10.1371/journal.pntd.0003318.
3. TaherHarkinezhad, KristelVerminnen, Caroline Van Droogenbrock, Daisy Vanrompay; https://doi.org/10.1097/jmm.0.471570
4. Published online 2016 Aug 29.doi:10.1136/bcr-2016-216879. Malcolm H. Mc Gavran, Charles W. Bread, Richard F Berendt and Robert M. Nakamura.
5. Anne-Marie Lonescu, DivyaKhare and Jay Kavi. Pii:bcr 2016216879. doi:10.1136 /bcr-2016-216879
6. San Raffaele Scientific Institue, Milan, Italy, Ferrari.andres@hsr.it Jclin Oncol.2012 Nov 1; 30(31):3903
7. BMJ Case Rep. 2016, 2016:bcr2016216879. Published online 2016 Aug 29. Doi:10.1136/bcr-2016-216879
8. Provikmo, occupational health services, Bruges, 8200 Belgium. Thomas.deschuyheller@provikmo.be
9. PLOS.one 2014 Feb 262(2):e89309.doi:10.1371/journal.pone.0089309.ecollection 2014
10. Stewardson AJ, Grayson ML; Psittacosis. Infect Dis Clin North Am. 2010 Mar24 (1); 7-25.doi: 10.1016/j.idc.2009.10.003.
11. Psittacosis; Public Health, England.
12. Psittacosis; The parrot society, UK.
13. Psittacosis; Health and Safety Executive (HSE).
14. 600MC GAVARANETAL.VOL.40, NO.6.
15. 658 VOL.40, NO.6.

SP-19

NUTRITIVE VALUES, DERMACOSMETIC PROPERTIES AND MEDICINAL PROPERTIES OF SPIRULINA

SONALI S. NIPATE*, SHRADDHA S. MAKESHWAR[1]

P.E.Society's,Modern College of Pharmacy,Nigdi, Pune-44.

ABSTRACT

Spirulina, blue- green free-floating filamentous microalgae that grows in alkaline water bodies. From the past 400 years, *Spirulina* was consumed as food. *Spirulina* is a well-known source of valuable food supplements, such as proteins, vitamins, amino acids, minerals, fatty acids, etc.The antioxidant, anti-inflammatory, antidiabetic activities of *Spirulina* were demonstrated in a large number of preclinical studies. It boosts the immunity and increases resistance to various infections. Thus, these multi beneficiary actions of spirulina make it an important natural product for the improvement of health of humans. In this review article an efforts are made to compile all the nutritive values, dermacosmetic properties and medicinal properties of *Spirulina* to serve as an easy source of literature.keywords: Anti-inflammatory, antidiabetic, dermacosmetic

INTRODUCTION:

Spirulina the blue green algae belong to the two genera namely Spirulina and Arthrospira, which consists of 15 different species.[1]*Spirulina Platensis* is the most commonly available, it is filamentous, multicellular, blue-green microalgae.[2] Spirulina is found to be magnificent source of macro and micro nutrients as per the chemical analysis. It is rich in proteins, vitamins, essential amino acids, essential fatty acids and dietary minerals. These loaded nutrients make spirulina an excellent beneficial for health, also a potential immunomodulant, anticancer, antioxidant, antiviral and antibacterial. It has positive effects in malnutrition, hyperlipidaemia, obesity, diabetes, heavy metal and chemical induced toxicity, inflammation, anaemia and damage caused due to radiation.[3-6]

Morphology of Spirulina:

Spirulina and *Arthrospira*are non-heterocyst, unbranched filamentous, genera of order *Nostacles* and family Osillatoriaceae. On observation under microscope, presence of two filaments in a single unit and absence of cellular septation are major points that differentiate *Spirulin*a from *Athrospira.*[7]*Spirulina Plantesis*is a photoautotrophic, alkaliphilic cyanobacterium and filamentous which belongs to Oscillatoriaceae and division Cyanophyta.[8]

Chemical composition of Spirulina:

Spirulina is rich in carbohydrates, protein, lipids, vitamins, minerals, pigments, and enzymes; however, it has achieved a great value in food, pharmaceutical and cosmetic industries. Branched polysaccharide structurally similar to the glycogen forms the major polymeric component of *S. Platensis*.[9]Anionic polysaccharide that is higher in molecular weight are been isolated from spirulina which have antiviral and immunomodulation activities.[10] Spirulina content 50 to 70% proteins, which is higher than meat, eggs, dried milk, grains and soybeans. It contains all the essential amino acids like leucine, valine and isoleucine.[3,11] Major protein in spirulina are phycobiliproteins, phycocyanin C and allophycocyanin. These proteins have significant health beneficial effects. 15 to 25% of dries weight of spirulina content phycocyanin. Phycocyanin is considered as the safe and natural food colorant used in chewing gums, confectionaries and dairy products.[12-15]The chromophore phycocyanobilin (PCB) present in spirulina is used as a supplement and is employed for various preventive and curative therapy in many diseases mediated by NADPH oxidase hyperactivity like cardiovascular diseases, diabetes, metabolic syndrome, allergic reaction, rheumatoid arthritis, cancer, Parkinson's and Alzheimer's diseases.[16]

Nutritive value of spirulina

Spirulina is one of the richest sources of proteins, it has five times greater protein content than meat. Spirulina comprises essential as well as non-essential amino acids, it contains highest amount of beta-

carotene the precursor for vit A. Spirulina contains vit B12 and is also the only vegetable source for vit B12. Spirulina serves as the source of the linoleic acid that is the precursor for body function regulating hormones.[17] Spirulina has been known to be good dietary source of vit A.

Nutrient profile

Nutrient content of spirulina when compared to other foods show, higher concentration of calcium than whole milk, protein than tofu, beta-carotene than carrots, and more iron than spinach. Also, more antioxidant and anti-inflammatory activity is seen in 3g spirulina than other fruits and vegetables.[18] General composition of spirulina: proteins- 60-69%; carbohydrates- 16-20%, lipids- 5-7%, minerals- 6-9%.[19] Health benefits of spirulina in addition to antioxidant and anti-inflammatory properties are protection of liver and kidney functions, improvement in blood quality and prevention of anaemia, reduction of blood pressure, prevention of liver and kidney damage, removal of heavy metals from body, and protection from UV radiation.[20]

Dermacosmetic properties

Skin is the largest organ of the body. A beautiful, flawless appealing skin is everyone's desire.[21-22] Recently spirulina extract has been widely used in the dermacosmetic formulations.According to the results obtained from the use of the formulations containing spirulina extract it is observed spirulina enhances the hydrophilic mantle and regeneration of the skin barrier which lead to the reduction of the transepidermal water loss.[23-24] It also reduces the oily skin appearance by regulation of the sebum production due to inhibition of 5-αreductase enzyme (responsible for sebum secretion) by linolenic acid.[25-26] Moisturizing effects of spirulina are also observed due to increase in water content in the stratum corneum and reduced transepidermal water loss.[27-28] Application of the formulation containing spirulina extract stimulated migration of the young cell from lower layers to the surface and peel off the surface cell contributing to the natural keratinization process or skin renewal process.[27-30] Post keratinization the skin formed was found to be uniform, homogenous and healthy.To summarize the effects on skin, spirulina hydrates the skin, improve skin texture by cell renewal process, enhances the skin microrelief, maintains the skin health.

Medicinal properties

Antiviral activity

Higher molecular weight anionic polysaccharides isolated from spirulina possesses the antiviral and immunostimulant effect, it also increases the TNF-α synthesis.[31-32] The active constituent's spirulina such as protein phycocyanin, protein bound pigments, GLA, sulphated polysaccharides and sulfolipids exert antiviral effect against herpes simplex, white spot syndrome virus, influenza A, human cytomegalovirus, mumps, measles and entrovirus.[33-38]

Antibacterial activity

The antimicrobial activity of spirulina is observed due to the linolenic acid (antibiotically- active fatty acid) present in its methanolic extract. It shows antibacterial activity against *Staphylococcus aureas, Escherichia coli, Candida albicans* and *Aspergillus niger.*[39]

Antioxidant activity:

In-vitro and in-vivo studies of spirulina indicates the antioxidant property of spirulina.[40-42] due to the high concentration of proteins, lipids, vitamins and minerals. Protein extract of *S. platensis* showed hydroxyl, peroxyl and free radical scavenging activity, inhibition of lipid peroxidation and reduction of oxidative stress.[41-43]

Immunostimulant activity:

Spirulina in response to the Concavalin A (Con A), enhances the production of antibodies.

It also increases the activated peritoneal macrophages and induce the spleen cell growth. Addition of spirulina to the spleen cell culture showed increased production of intelukin-1, antibodies andadditional effect on toll like receptor mediated cytokines production.[44-45]

Spirulina in anaemia:

Spirulina is rich in iron, 100g of spirulina contains 158% of daily iron requirement. Spirulina supplements benefit children older women as well as the pregnant women. Due to presence of iron, spirulina enhances the red blood corpuscles and increase in the mean corpuscular haemoglobin.[46-47]

Radiation protection effect:
Spirulina contains phytopigments such as carotenoids, chlorophyll and phycocyanin as well as polysaccharides; these constituents show radioprotection activity. Spirulina significantly enhances the antioxidant enzyme activity such as superoxide dismutase, glutathione reductase and catalase.[48-49]

Anti- inflammatory activity:
According to the research bilirubin inhibits NADPH activity. Chromophore phycocyanobilin is found to present in spirulina which is metabolised in mammalian cells to phycocyanorubin (homolog of bilirubin). Phycocyanorubin show inhibition of the enzyme complex. Spirulina exert a broad range of anti-inflammatory activity.[50-51]

Effects of spirulina in eye diseases:
Eye problems are caused due to vit A deficiency. Spirulina is rich in beta- carotene. Spirulina supplementation helps overcome vit A deficiency and effective in eye problems and prevent night blindness.[52-53]

Spirulina in hypertension and hyperlipidaemia:
Spirulina has high potassium and low sodium content; it has positive effect on blood pressure. Spirulina reduces systolic and diastolic blood pressure on oral administration C- phycocyanin present in spirulina is responsible for lipid lowering, it reduces the concentration of triacylglycerol and cholesterol which show hypolipemic effect.[53]

CONCLUSION

Spirulina has emerged as the wonder food supplement. Several leading organizations are utilizingthis beneficial action.Very few adverse effects havebeen reported with the use of spirulina which includeheadache, muscle pain, flushing of the face, sweating and difficulty in concentrating. Skin reactions have alsobeen reported in some individuals. The highly diversenutritive nature of spirulina together with its antioxidantand protective health benefits have been utilized invarious health related problems. The effectiveness ofspirulina is being very rapidly recognized and beingmade use of in the treatment and management ofvarious life-threatening diseases. The potential healthbenefits of spirulina must be adequately recognized andimplemented thus making full use of this nature's gift.

REFERENCES:

1. Hoseini, S.M., Khosravi-Darani, K. and Mozafari, M.R., 2013. Nutritional and medical applications of spirulina microalgae. *Mini reviews in medicinal chemistry*, *13*(8), pp.1231-1237.
2. Henrikson, R., 2009. Earth Food Spirulina: How This Remarkable Blue–Green Algae Can Transform Your Health and Our Planet. Ronore Enterprises. *Inc., Hana*.
3. Falquet, J. and Hurni, J.P., 1997. The nutritional aspects of Spirulina. *Antenna Foundation. Available online at: https://www. antenna. ch/wp-content/uploads/2017/03/AspectNut_UK. pdf (Accessed July 25, 2017)*.
4. Lee, J.B., Hayashi, T., Hayashi, K., Sankawa, U., Maeda, M., Nemoto, T. and Nakanishi, H., 1998. Further purification and structural analysis of calcium spirulan from Spirulina platensis. *Journal of natural products*, *61*(9), pp.1101-1104.
5. Lorenz, R.T., 1999. A review of Spirulina and Haematococcus algae meal as a carotenoid and vitamin supplement for poultry. *Spirulina Pacifica Technical Bulletin*, *53*, pp.1-14.
6. Soheili, M. and Khosravi-Darani, K., 2011. The potential health benefits of algae and micro algae in medicine: A review on spirulina platensis. *Current Nutrition & Food Science*, *7*(4), pp.279-285.
7. Hindák, F., 1985. Morphology of trichomes in Spirulina fusiformis Voronichin from lake Bogoria, Kenya. *Algological Studies/ArchivfürHydrobiologie, Supplement Volumes*, pp.201-218.
8. Usharani, G., Saranraj, P. and Kanchana, D., 2012. Spirulina cultivation: a review. *Int J Pharm Biol Arch*, *3*(6).
9. Gupta, R.S. and Changwal, M.L., 1992. Biotechnology of mass production of Spirulina and Arthrospira in fresh water. *proc: Spirulina ETTA National Sympodium. MCRC, Madras. Pp-125-128*.

10. Parages, M.L., Rico, R.M., Abdala-Díaz, R.T., Chabrillón, M., Sotiroudis, T.G. and Jiménez, C., 2012. Acidic polysaccharides of Arthrospira (Spirulina) platensis induce the synthesis of TNF-α in RAW macrophages. *Journal of applied phycology*, *24*(6), pp.1537-1546.
11. Gershwin, M.E. and Belay, A. eds., 2007. *Spirulina in human nutrition and health*. CRC press.
12. Azabji-Kenfack, M., Dikosso, S.E., Loni, E.G., Onana, E.A., Sobngwi, E., Gbaguidi, E., Kana, A.N., Nguefack-Tsague, G., Von der Weid, D., Njoya, O. and Ngogang, J., 2011. Potential of spirulina platensis as a nutritional supplement in malnourished HIV-infected adults in Sub-Saharan Africa: a randomised, single-blind study. *Nutrition and metabolic insights*, *4*, pp.NMI-S5862.
13. Moorhead, K., Capelli, B. and Cysewski, G., 2005. Nature's superfood: spirulina. *Cyanotech Corporation, Kailua-Kona, HI, USA*.
14. Thomas, S.S., 2010. The role of parry organic spirulina in health management. *India: Parry Nutraceuticals, Division of EID Parry (India) Ltd*.
15. Capelli, B. and Cysewski, G.R., 2010. Potential health benefits of spirulina microalgae. *Nutrafoods*, *9*(2), pp.19-26.
16. Grawish, M.E., Zaher, A.R., Gaafar, A.I. and Nasif, W.A., 2010. Long-term effect of Spirulina platensis extract on DMBA-induced hamster buccal pouch carcinogenesis (immunohistochemical study). *Medical Oncology*, *27*(1), pp.20-28.
17. Hamidah, A., Rustam, Z.A., Tamil, A.M., Zarina, L.A., Zulkifli, Z.S. and Jamal, R., 2009. Prevalence and parental perceptions of complementary and alternative medicine use by children with cancer in a multi-ethnic Southeast Asian population. *Pediatric blood & cancer*, *52*(1), pp.70-74.
18. Pinzani, M., Marra, F., Caligiuri, A., DeFranco, R., Gentilini, A., Failli, P. and Gentilini, P., 1996. Inhibition by pentoxifylline of extracellular signal-regulated kinase activation by platelet-derived growth factor in hepatic stellate cells. *British journal of pharmacology*, *119*(6), pp.1117-1124.
19. Delsin, S.D., Mercurio, D.G., Fossa, M.M. and Maia Campos, P.M.B.G., 2015. Clinical efficacy of dermocosmetic formulations containing Spirulina extract on young and mature skin: Effects on the skin hydrolipidic barrier and structural properties. *Clin PharmacolBiopharm*, *4*(144), p.2.
20. Wataru M, Katsumiy, Shojik; Carotenoid composition of Spirulina maxima. Bulletin of Japenese society of scientific fisheries 1986; 52(7): 1225-1227.
21. Mathew, B., Sankaranarayanan, R., Nair, P.P., Varghese, C., Somanathan, T., Amma, B.P., Amma, N.S. and Nair, M.K., 1995. Evaluation of chemoprevention of oral cancer with Spirulina fusiformis.
22. Layam, A. and Reddy, C.L.K., 2006. Antidiabetic property of spirulina. *Diabetologiacroatica*, *35*(2), pp.29-33.
23. Parikh, P., Mani, U. and Iyer, U., 2001. Role of Spirulina in the control of glycemia and lipidemia in type 2 diabetes mellitus. *Journal of Medicinal Food*, *4*(4), pp.193-199.
24. Cheong, S.H., Kim, M.Y., Sok, D.E., Hwang, S.Y., Kim, J.H., Kim, H.R., Lee, J.H., Kim, Y.B. and Kim, M.R., 2010. Spirulina prevents atherosclerosis by reducing hypercholesterolemia in rabbits fed a high-cholesterol diet. *Journal of nutritional science and vitaminology*, *56*(1), pp.34-40.
25. Gianeti, M.D., Gaspar, L.R., Bueno de Camargo Júnior, F., Campos, B.G.M. and Maria, P., 2012. Benefits of combinations of vitamin A, C and E derivatives in the stability of cosmetic formulations. *Molecules*, *17*(2), pp.2219-2230.
26. Hsiao, G., Chou, P.H., Shen, M.Y., Chou, D.S., Lin, C.H. and Sheu, J.R., 2005. C-phycocyanin, a very potent and novel platelet aggregation inhibitor from Spirulina platensis. *Journal of agricultural and food chemistry*, *53*(20), pp.7734-7740.
27. Iobst, S., Santhanam, U. and Weinkauf, R.L., 2016. Biotechnology in skin care (I): Overview. In *Biotechnology in Personal Care* (pp. 141-156). CRC Press.
28. Prasanna, R., Sood, A., Jaiswal, P., Nayak, S., Gupta, V., Chaudhary, V., Joshi, M. and Natarajan, C., 2010. Rediscovering cyanobacteria as valuable sources of bioactive compounds. *Applied Biochemistry and Microbiology*, *46*(2), pp.119-134.
29. Scotter, M.J., 2011. Emerging and persistent issues with artificial food colours: natural colour additives as alternatives to synthetic colours in food and drink. *Quality Assurance and Safety of Crops & Foods*, *3*(1), pp.28-39.
30. Hayashi, T., Hayashi, K., Maeda, M. and Kojima, I., 1996. Calcium spirulan, an inhibitor of enveloped virus replication, from a blue-green alga Spirulina platensis. *Journal of natural products*, *59*(1), pp.83-87.

31. Hoseini, S.M., Khosravi-Darani, K. and Mozafari, M.R., 2013. Nutritional and medical applications of spirulina microalgae. *Mini reviews in medicinal chemistry*, *13*(8), pp.1231-1237.
32. Mosulishvili, L.M., Kirkesali, E.I., Belokobylsky, A.I., Khizanishvili, A.I., Frontasyeva, M.V., Pavlov, S.S. and Gundorina, S.F., 2002. Experimental substantiation of the possibility of developing selenium- and iodine-containing pharmaceuticals based on blue–green algae Spirulina platensis. *Journal of pharmaceutical and biomedical analysis*, *30*(1), pp.87-97.
33. Romarís–Hortas, V., García-Sartal, C., del Carmen Barciela-Alonso, M., Domínguez-González, R., Moreda-Piñeiro, A. and Bermejo-Barrera, P., 2011. Bioavailability study using an in-vitro method of iodine and bromine in edible seaweed. *Food Chemistry*, *124*(4), pp.1747-1752.
34. Henrikson, R., 2009. Earth Food Spirulina, ;Ronore Enterprises, Inc: Hana, Maui, Hawaii.
35. Gershwin, M.E., Belay, A. *Spirulina in Human Nutrition and Health*, CRC Press, Taylor&Francis Group, Boca Raton: London, New York., **2008**, pp.1-27.
36. Mendiola, J.A., Jaime, L., Santoyo, S., Reglero, G., Cifuentes, A., Ibañez, E. and Señoráns, F.J., 2007. Screening of functional compounds in supercritical fluid extracts from Spirulina platensis. *Food chemistry*, *102*(4), pp.1357-1367.
37. De Mule, M.C., 1996. Bioactive substances from Spirulina platensis (Cyanobacteria). *Int. J. Experiment. Bot.*, *58*, pp.93-96.
38. Bermejo, P., Piñero, E. and Villar, Á.M., 2008. Iron-chelating ability and antioxidant properties of phycocyanin isolated from a protean extract of Spirulinaplatensis. *Food Chemistry*, *110*(2), pp.436-445.
39. Gad, A.S., Khadrawy, Y.A., El-Nekeety, A.A., Mohamed, S.R., Hassan, N.S. and Abdel-Wahhab, M.A., 2011. Antioxidant activity and hepatoprotective effects of whey protein and Spirulina in rats. *Nutrition*, *27*(5), pp.582-589.
40. Lee, J.B., Hayashi, T., Hayashi, K., Sankawa, U., Maeda, M., Nemoto, T. and Nakanishi, H., 1998. Further purification and structural analysis of calcium spirulan from Spirulina platensis. *Journal of natural products*, *61*(9), pp.1101-1104.
41. Hayashi, O., KATOH, T. and OKUWAKI, Y., 1994. Enhancement of antibody production in mice by dietary Spirulina platensis. *Journal of Nutritional Science and Vitaminology*, *40*(5), pp.431-441.
42. Hirahashi, T., Matsumoto, M., Hazeki, K., Saeki, Y., Ui, M. and Seya, T., 2002. Activation of the human innate immune system by Spirulina: augmentation of interferon production and NK cytotoxicity by oral administration of hot water extract of Spirulina platensis. *International Immunopharmacology*, *2*(4), pp.423-434.
43. Mohan, A., Misra, N., Srivastav, D., Umapathy, D. and Kumar, S., 2014. Spirulina, the nature's wonder: A review. *Lipids*, *5*, pp.7-10.
44. Branger, B., Cadudal, J.L., Delobel, M., Ouoba, H., Yameogo, P., Ouedraogo, D., Guerin, D., Valea, A., Zombre, C. and Ancel, P., 2003. Spiruline as a food supplement in case of infant malnutrition in Burkina-Faso. *Archives de pediatrie: organeofficiel de la Societefrancaise de pediatrie*, *10*(5), pp.424-431.
45. Hirahashi, T., Matsumoto, M., Hazeki, K., Saeki, Y., Ui, M. and Seya, T., 2002. Activation of the human innate immune system by Spirulina: augmentation of interferon production and NK cytotoxicity by oral administration of hot water extract of Spirulina platensis. *International Immunopharmacology*, *2*(4), pp.423-434.
46. Karkos, P.D., Leong, S.C., Karkos, C.D., Sivaji, N. and Assimakopoulos, D.A., 2011. Spirulina in clinical practice: evidence-based human applications. *Evidence-based complementary and alternative medicine*, *2011*.
47. Helliwell, K.E., Wheeler, G.L., Leptos, K.C., Goldstein, R.E. and Smith, A.G., 2011. Insights into the evolution of vitamin B12 auxotrophy from sequenced algal genomes. *Molecular biology and evolution*, *28*(10), pp.2921-2933.
48. McCarty, M.F., 2007. Clinical potential of Spirulina as a source of phycocyanobilin. *Journal of medicinal food*, *10*(4), pp.566-570.
49. Seshadri, C.V., 1993. *Large Scale Nutritional Supplementation with Spirulina Alga (LSNS): 1991-93*. Shri AMM MurugappaChettiar Research Centre.
50. Ramesh, S., Manivasgam, M., Sethupathy, S. and Shantha, K., 2013. Effect of spirulina on anthropometry and bio-chemical parameters in school children. *IOSR Journal of Dental and Medical Sciences*, *7*(5), pp.11-15.

51. Patricia, T.D., Aldo, F.H. and Marco, J.O., 2007. Antihyperlipemic and antihypertensive effects of Spirulina maxima in an open sample of mexican population: a preliminary report.
52. Nagaoka, S., Shimizu, K., Kaneko, H., Shibayama, F., Morikawa, K., Kanamaru, Y., Otsuka, A., Hirahashi, T. and Kato, T., 2005. A novel protein C-phycocyanin plays a crucial role in the hypocholesterolemic action of Spirulina platensis concentrate in rats. *The Journal of nutrition*, *135*(10), pp.2425-2430.
53. Guan, Y., Zhao, H.Y., Ding, X.F. and Zhu, Y.Y., 2007. Analysis of the contents of elements in Spirulina from different producing areas. *Guangpuxueyuguangpu fen xiGuangpu*, *27*(5), pp.1029-1031.

SP-20

MECONIUM ASPIRATION SYNDROME

K. ESTHER RANI*, K.SHVANI*, Dr G.SUSMITHA
Department of Pharmacy Practice, Bhaskar Pharmacy College, Hyderabad- 500075, India.
E-mail Id: susmi.aswi@gmail.com

ABSTRACT:
Meconium aspiration syndrome is common condition that is see in 8-25 % of all deliveries. Meconium is the first intestinal discharge of the newborn, which is composed of intestinal secretions such as bile, mucus and intestinal epithelial cells; this is formed during 3rd trimester of intrauterine life. In post-dated babies due the increased motilin, there is a Release of meconium into the amniotic fluid, making it contaminated and thereby it is aspired into the lungs of baby causing respiratory distress and so called meconium aspiration syndrome

INTRODUCTION:

It is a common cause of morbidity and mortality[9,1,4]seen in 8-25% of all deliveries, where the baby experiences respiratory distress due to aspiration of meconium stained amniotic fluid[5.] Usually the meconium is the first stool passed just after the baby is delivery i.e. within 24hrs[3]. It is composed of bile acids, desquamated skin, proteins, intestinal epithelial cells, lanugo, cholesterol and its precursors, lipids, enzymes. Enzymes include pancreatic phosholipaseA2[7.] 80-90 %[3,8] of water is also present it and it appears to thick, black greenish colored. The MAS is observed in both preterm and term babies but most commonly seen in term babies[2], where the gastric maturation of the baby results in the passage of meconium, due to increased gestational age. Passage of meconium was highly associated with birth asphyxia and resulted in a bad prognosis. Fetal distress is directly correlated with the APGAR SCORE, therefore meconium stained amniotic fluid along with low APGAR score[3], will ultimately lead to poor fetal outcome309.

ETIOLOGY:

The causes include prolonged labor, increased levels of motilin, maternal hypertension, [1]low APGAR score[,3] utero-placental insufficiency[3],oligohydramnios[1], ante partum hemorrhage.

PATHOPHYSIOLOGY:

Normally meconium passage from the fetus into amniotic fluid is prevented by lack of intestinal peristalsis, caused due to the low levels of motilin, but in case of postdated babies[8], due to transient parasympathetic stimulation due to the head compression, increase in motilin levels and cholinergic innervations. Apart from fetal maturation, meconium is also passed due to relaxation of anal sphincter which is caused by inutero-stress with hypoxia and acidosis.[3]

1.BALL-VALVE OBSTRUCTION:

When meconium enters the lungs, it causes air trapping thereby, results in mechanical obstruction to airways. Meconium migrates from proximal to distal airways during breathing movements and cause complete atelectasis[7], ventilation perfusion mismatch and air leak[2].

2. PNEUMONITIS:

Within few hours of meconium aspiration into the airways, there is an accumulation of macrophages and neutrophils, as the meconium acts as a chemoattractant. This further results in release of pro inflammatory mediators such as TNF-alpha, IL-8,RESULTING IN PARENCHYMAL INJURY AND CAUSES TOXIC PNEUMONITIS[2,8].

3. SURFACTANT INACTIVATION:

The bile acids and cholesterol which are the components of meconium will interfere with the lung surfactant by changing its viscosity and ultrastructure thereby; results in surfactant in inactivation .meconium presence

can also decrease the surfactant production by decreasing the levels of surfactant proteins A and B causing harm to type-II pneumocytes[4].

4. PULMONARY HYPERTENSION:
Release of vasoactive inflammatory substances, results in pulmonary vasoconstriction and chronic hypoxia leads to abnormal muscularisation which contributes to perinatal maladaptation of pulmonary circulation ultimately leads to a condition pulmonary hypertension[7].

5. INFECTION:
Risk of intra amniotic infection both clinical and culture prove may be observed as the meconium enhances the in-vitro bacterial growth[2].

6. UMBILICAL VESSEL DAMAGE:
Meconium has detrimental effect on placental and umbilical tissues resulting in vascular necrosis, vasoconstriction and ulceration; tissue injury may vary from mild inflammation of the lung placental membrane to severe focal injury to the umbilical vessel[2].

DIAGNOSIS:
APGAR SCORE- This score is commonly used to diagnose MAS[1,4].
A-APPEARANCE (Skin color)
P-PULSE (Heart rate)
G-GRIMACE RESPONSES (Reflexes)
A-Activity (Muscle tone)
R-RESPIRATORY (Breathing rate and effort)

Apgar sign	2	1	0
Appearance	Normal color all over.	Normal color but hands and feet are bullish.	Bluish-gray or pale all over.
Pulse	Normal	Below 100 beats per minute.	Absent.
Grimace	Strong cry.	Feeble.	Absent.
Activity	Full movement.	Some flexion.	Absent.
Respiration	Normal breathing	Slow or irregular breathing	Absent.

LUNG ULTRASONOGRAPHY:
During this, the lung is divided into three Regions (front, back, lateral) using anterior and posterior axillary lines as Boundaries while performing the scan of each region, the hand piece is held perpendicular or parallel to the ribs. The lung ultrasonography examinations are performed by one physician whereas the clinical data is collected by another physician. The ultrasound is carried out in MAS patients on admission to neonatal intensive care unit or within 2hr after receiving mechanical ventilation. Usually the neonatal lung, pleural lines and lines appears to be clear smooth, parallel and equally space but in MAS, the lung upon observation appears to be large areas of pulmonary consolidation with air bronchogram, blurred pleural lines and disappearance of A-lines. Some other observations include atelectasis and pleural effusion.[10]

ECHOCARDIOGRAM:
To assess the cardiac function and to evaluate PPH[4].

ARTERIAL BLOOD GAS TEST:
The known Normal partial pressure of oxygen is 75 to 100mmHg and partial pressure of CO2 is 38 to 42mmHg. A blood sample is taken from the artery of an infant by inserting a small needle through the skin to reach the artery for evaluating hypoxemia (PaO2< 50mmHg) or Hypercarbia (PaCo2 > 60mmHg)[2].

TREATMENT:
SURFACTANT:It is either given in bolus or lavage form clinical studies shows that the use of surfactant lung lavage therapy improved oxygenation but not overall mortality. It is currently used in30-50% of ventilated infants of MAS. At times it may be used in combination with extracorporeal membrane

oxygenation. It helps in activation of surfactant proteins thus prevents lung injury.[6].it removes meconium particulate from the lung [5].

HIGH FREQUENCY VENTILATION:

It is especially used in infants those with significant atelectasis, where application of higher mean air pressure (around 25cm) with recruitment maneuver at moderate frequency(6-8Hz) was found to be beneficial. According to the large neonatal databases, 20-30% of infants requiring ventilation and intubation with MAS were treated with high frequency ventilation which also shows clinical advantage in infants with severe PPH[2].

CORTICOSTEROIDS:

Beractant alone or in combination with Budesonide can be given where Budesonide dose is 0.25mg/kg. Methyl prednisone at a dose of 0.5mg/kg can be used[2].Pulmonary and systemic inflammation is a characteristic feature in MAS infants.so use of steroids will improve the pulmonary function by decreasing the inflammation and the hospital stay. According to the data of animal studies, the inhaled budesonide has shown a promising response when administered intra tracheal alone or with surfactant.

ANTIBIOTICS:

Gentamycin and Ampicillin are used in combination or Amikacin in a dose of 15mg/kg per dose of 24 hourly IV/3 days. AMeta ta analysis of three recent clinical trials, the use of antibiotics did not significantly reduce the risk of culture proven sepsis, duration of hospital stay and mortality, therefore their role has to be reevaluated by well -designed clinical trials[2,6]

INHALED NITRIC OXIDE:

Large RCTS has shown an appreciable efficacy of NO, in term infants with mild to severe PPH. Since it is a selective pulmonary vasodilator, it reduces the hypoxia induced pulmonary arterial vasoconstriction and thereby prevents PPH in MAS infants[2].Administration of N-Acetyl cysteine with the surfactant will prevent neutrophil migration, oxidative stress, lung edema by inhibiting Il-8.

PREVENTION:

Elective induction of labor at 41 weeks of gestation will help in prevention of maturation induced intrauterine meconium passage. Hence the infant will not be exposed to increased gestational age[2]. Early diagnosis: Early diagnosis should be done by ultrasound and regular observation of fetal heart rate.Amnio infusion is a technique of instilling the isotonic fluid into the amniotic fluid and that replaces the meconium stained amniotic fluid due to the umbilical cord compression, the fetal heart decreases the amnio infusion will results in increasing the amniotic fluid volume, thereby helping preventing the cord compression.

CONCLUSION:

As Meconium aspiration syndrome is the major cause of mortality in infants, due to respiratory distress, before we go for the treatment it is important to take preventive steps such as Elective induction of labor at 41 weeks of gestation and early diagnosis. Since there is an accumulation of meconium in airways and surfactant inactivation.Lavage surfactant therapy is the most effective treatment which improves oxygenation. Corticosteroids must be used only when MAS is severe, since there is an increased risk of adverse effects. The role of antibiotics has to be evaluated and then administered to the infants as their effect is not fully understood.

REFERENCES:

1) International journal of reproduction, Contraception, Obstetrics and Gynecology Sundaram R et al. Int J ReprodContraceptObstet Gynecol. 2016 Aug; 5(8):2503-2506
2) Indian J Pediatr (October 2016) 83(10): 1125-1130 SubhashChettri
3) International Journal of Research of Medical Sciences Ranee D et al. Int J Res Med Sci. 2017 Oct;5(10):4319-4323
4) Chand S, Salman A, Abbassi R, et al. (September 05, 2019) Factors Leading To Meconium Aspiration Syndrome in Term and Post-Term Neonates. Cureus 11(9): e5574.

5) Journal Of Perinatology (2020) 40:56-62 Julia D. Lopez
6) Journal of Perinatology (2016) 36, S48-S53 K Jain, VK Paul.
7) Journal of Perinatology (2008) 28, S3-S7
8) InternationalJournalofContemporaryPediatricsSaranappa SSB et al. Int J ContempPediatr. 2019 May;6(3):1330-1335
9) International Journal of Contemporary Pedi0atrics Kamble MB et al. Int J ContempPediatr. 2019 Jan;6(1):144-149
10) Journal of international medical research 2016 , Vol. 44(6) 1534 – 1542

SP-21

CURRENT MODERN DAY TRENDS OF "PRE CLINICALTRIALS''

[1]HAFSA SIDDIQUA*, [2] RUBINA KAUSER, [3]J.SUJATHA ,[4]DR.SEEMA TABASSUM, [5]DR. THATIKONDA KEERTHI,[6] DR. SHEHVAR BANU

[1]Assistant Professor, Department of Pharmaceutical Analysis & Quality Assurance
[2] Assistant Professor, Department of Pharmaceutical Analysis & Quality Assurance
[3] Assistant Professor, Department of Pharmaceutical Chemistry
[4]AssisstantProfessor,Pharm.D,[5]AssisstantProfessor,Pharm.D,[6]AssisstantProfessor,Pharm.D
ST.Pauls College Of Pharmacy, Turkayamjal, Hyderabad-501510
Email-id : hafsasiddiqua333@gmail.com

ABSTRACT :
Investigation Initiated Studies (IIS) is the need of the hour to compliance with the present trends in pharmacological research. IIS includes broad spectrum of studies, clinical trials of new drugs. It is beneficial for the progressive growth of fundamentals of drug efficacy, toxicity and pharmacokinetics data of drugs to anticipate the safety profile of the products. As per the guidelines of ICMR, at every organizational level the assessment of the quality of the generated data should be ensured. Auditing , monitoring, safety of the intervention and the quality of the data generated should be established by every institution as per the guidelines of Indian Council Of Medical Research (ICMR) . The Ethics Committee (EC) can effectively use its tools of monitoring and audit to affirm and ensure the ICMR guidelines. Thus we can infer that in modern times the current trends in Pharmacological research will make the conduct of the academic research far more arduous for the researchers to enhance the fulfillment of the rules laid by EC unlike earlier times, where only a small number of research institutes were indulged in clinical trials.[1,5]

Keywords: Investigation Initiated Studies (IIS), ICMR, clinical trials.

1.INTRODUCTION:

Pre – clinical tests are performed on animal subjects in order to assess if the expected and hoped treatment really works and whether it is safe to test on human beings. It helps to determine the effective dose, the toxic dose, pharmacological action etc., There are numerous rampantly spreading diseases. The methodology of finding a new cure for it is both expensive and time consuming, without any assurance of success it may cost about billions of dollars to investigate about them. In the pre clinical studies on an average Scientists starts with 10,000 companies and it requires about 7 years of time to investigate about them. Once the research proceeds to clinical trial stage, it takes about 6 more years of time. At the end of all the research done, if the new drug developed has unwanted cell toxicity characteristics along with the desired therapeutic effect then it has to be abandoned. Thereby there is a desperate need for the development of the modern and innovative trends such that unwanted toxicity is anticipated at much earlier stage in drug development process, so that it would effectively safe time, energy , money spent on the research , scientists and the institutions .[2,3,4]

2.DISCUSSIONS:

In the present trends researchers use good laboratory practices (GLP) for preclinical laboratory studies. These set of regulations assure the proper execution of all the basic requirements like Quality Assurance Program, Personnel and Organization, Facilities (both for test systems and reference), Apparatus and reagents, Standard Operating Procedures (SOP), Performance of study, reporting of results while performing the pre-clinical trials. The supervision of quality assurance system is employed throughout the studies to ensure the safety of the FDA regulated products. [6]

Plug and Play upstream Development:
By the optimization of in-silico codon, we can establish stable pools. After choosing the appropriate clone we acquire good quality material, thus highly stable cell line is produced, a MASTER CELL BANK can be established and the documentation is made ready for IND filing . These sort of advanced techniques helps to

isolate high producing clones by saving the time, energy and money. The various other advanced techniques are cell type selection, expression of vector, stable pool generation, single cell cloning.

	Conventional Technique	Modern Technique
Expression of Vector	4 weeks	4 weeks
Stable pool generation	11 weeks	2 weeks
Single cell cloning	13 weeks	7 weeks
TOTAL TIME	25 weeks	13 weeks

Thus the current method is cost effective and money saving. In the modern research small RNA molecules in the body can turn off or on by the genes. This sort out off or on by genes. This sort of study was performed on worms. This achievement was acknowledgement & awarded the Nobel prize in 2006.This is unlike a basic research which involves explore novel facts about how things work which consumes a great deal of time, in order to know a biological event.[6]The simplified techniques of pre-clinical trials involves the following simple steps :

Figure-1 Steps in new drug discovery[7]

This steps are further simplified with the aid of the following techniques in the modern world:
CHOZN platform: It offers simple, speedy pick up and piling of clones of high production quality.

- It offers a constant platform to maintain more than 90%of clones and 75% titer of m about 60 generations.
- It has reliable record for sourcing and austere manufacturing.
- It supports cell line engineering and potent cell line selection by optimizing media and feed.
- Faster clone selection while isolating producing cell lines through targeted gene modification.
- Accurate set of c GMP produces chemically specified production media to enhance growth of r-proteins from the producing clones [7]

RIGHT FIRST TIME:
It strikes a perfect balance between efficiency & speed so as to affirm high productivity and eliminate the delays in clinical trials. The significance of Do It Right The First Time (DRIFT) comes from the base that a JIT production system is majorly reliant on the movement of parts and information along the production process. Eventually, if there is the bare minimum error at one of the stages of production, it will be reflected on the entire production process. By "doing it right the first time" an organization can run an efficient production process.[8,14]

FAST TRACK PROCESS:
It parallelly executes the clone selection and the process development and saves up to 10 weeks time .Fast track drug development is the methodology to develop and review the drugs for the treatment of the serious conditions. It helps the patients to acquire the new drugs to the patients at a much earlier pace. The study of fast track drugs comes under the FD &C act Section 506, which is further sub divided under 3 sub-sections like Section 506 (a), Section 506 (b), Section 506(c).[9,13]

3.CONCLUSION

A simple, rapid, cost effective and accurate methods are developed for the pre clinical studies**.** Prior to the conduction of the clinical trials, valid and sound investigation is done. With the proper plan of work and research the actual pre clinical tests are performed with careful auditing and monitoring. Appropriate documentation is maintained and a 'master cell bank' is created which is ready for IND filing. Good lab practices and Good Manufacturing Practices are strictly employed and executed .small RNA molecules are used as a power buttons to switch on or switch off the genes. Novelty in the techniques are introduced such as CHOZN platform, right first time, fast track process and seamless integration which are a boon to the industry of clinical trials. Such sort of innovations are profusely welcomed in the near future. Such novelty in the stream of research assures the timely accomplishment of the work with the accuracy and precision and also it will be extremely cost-effective. Cell line development and the analytical development are done simultaneously.[15]

REFERENCES:

1. New clinical trial rules : Academic trials and tribulations, (ISCR) Perspectives in Clinical Research Vol 10/ Issue 3 / July –September 2019
2. Research Journal Of Pharmacy and Technology,Volume 12 August 2019
3. Indian Journal Of Pharmacology,Volume 51,Issue 3,May –June 2019
4. International Journal Of Pharmacy and Drug Research,Volume 8, No.1,January-June 2019
5. https://www.slideshare.net/mohankumaryadav8/preclinical-trials-1647469
6. wikipedia.org/wiki/Good_laboratory_practice
7. https://www.slideshare.net/mohankumaryadav8/preclinical-trials-1647469
8. https://www.sigmaaldrich.com/safe/biological-manufacturing/chozn-platform.html
9. https://www.investopedia.com/terms/d/drift.asp://
10. www.businessdictionary.com/definition/right-the-first-time.html
11. https://www.investopedia.com/terms/d/drift.asp
12. https://www.profil.com/knowledge-center/trial-stages
13. https://www.fda.gov/patients/drug-development-process/step-2-preclinical-research
14. https://bmcneurol.biomedcentral.com/articles/10.1186/1471-2377-9-S1-S2
15. https://en.wikipedia.org/wiki/Preclinical_development

SP-22

A CAUSATIVE RISK OF COLORECTAL CANCER BY RED MEAT

MARIYA BEGUM*, GAZALA RAHMAN, DR.G SUSMITHA
Department of Pharmacy Practice, Bhaskar Pharmacy College, Hyderabad- 500075, India.
E-mail Id: susmi.aswi@gmail.com

ABSTRACT:
Colorectal Cancer is the 2ND Most Leading cause of death .It is 2nd most common in Women & 3rd Most Common in Men. It occurs due to changes in the Genes that lies in Colon & Rectum due to which there is no control on Cell Growth that may result in escape of Growth (or)Fast Growth which becomes Tumor leading to COLORECTAL CANCER. These genetic changes builds up over the years,& by the age of 50-60 people starts with development of POLYPS , which are small growths on the lining of the intestinal tract that develops into cancer,and these few of polyps becomes causative agents for Cancer.Major Risk of Colorectal Cancer is due to RED MEAT or PROCESSED MEAT, since it contains Large amount of Iron ,Creatine ,Minerals such as Zinc & Phosphorus,and vit B (niacin,Vit B12,thiamin & riboflavin, Also red meat is a source of Lipoic Acid, other related risk factors are:Age above 45yrs, Inflammatory Bowel Disease(IBD), Family history ,Diet-high Fat diet, low fiber, Smoking ,Sedentary lifestyle, History of Polyps, Obesity .The Signs & Symptoms of Colorectal Cancer Includes : A Persistent change in bowel habbits including diarrhea or constipation or change in consistency of your Stool. Rectal bleeding or blood in your stool, persistent abdominal discomfort, such as gas ,Cramps, or Pain.Weakness or fatigue , Unexplained weight loss.Mostly the Diagnosis of Colorectal Cancer is done by Colonoscopy, Tissue Biopsy during Sigmoidscopy .However Preventive measures when taken will reduce the risk of colorectal Cancer by reducing the intake of Red Meat or Processed Meat in diet,changing the sedentary lifestyle , doing physical activity, Avoiding Smoking &Alcohol consumption, Diary Foods, Fried Foods ,including dietary Fiber.

KEYWORDS: **Red Meat, Processed Meat,Colorectal Cancer, Polyps Formation,Sedentary Lifestyle.**

INTRODUCTION:

Colorectal Cancer , also known as Bowel Cancer.It is a is a disease originating from epithelial cells lining the colon or rectum of the GIT,most frequently as a result of Mutations that are inherited or acquired and most probably occur in the Intestinal crypt stem cell. Current research shows there are certain chemicals in red &Processed meats-both added & naturally occurring-that cause these foods to be carcinogenic.For example, When a chemical in red meat called Haem is broken down in gut ,N-nitroso chemicals are formed &these have been found to damage the cells that lines the bowel,which can be called as bowel cancer. [9] These chemicals also form when processed meat is digested. In addition,the nitrite & nitrate preservatives used to preserve processed meat produce these N- nitroso chemicals &can lead to bowel cancer.The Major cause of Colorectal Cancer is due to productive intake of Red Meat over time which develops polyps in the intestine which and further becomes tumor in colon firstly and due to lack of appropriate measures further growths in rectum. A Subset of colorectal Cancer is characterized with deficient DNA mismatch repair. This phenotype has been linked to mutations of Genes such as MSH2, MLH1, &PMS2,these mutations results in so called high frequency microsatellite instability (H-MSI)which can be detected with an immunocytochemistry Assay.H-MSI is a hallmark of hereditary non polyposis Colon cancer syndrome(HNPCC,LYNCH SYNDROME)which accounts for about 6%of all colon cancer.H-MSI is also found in about 20% of sporadic colon cancer.[10]

Other important genes in colon carcinogenesis include the KRAS oncogene,Chromosome 18 loss of heterozygosity (LOH) leading to inactivation of SMAD4(DPC4)& DCC Tumor suppression genes. Chromosome arm 17p deletion &mutations affecting the p53 tumor suppression gene confer resistance to Programmed Cell Death(Apoptosis)& are thought to be late in colon carcinogenesis.[3]

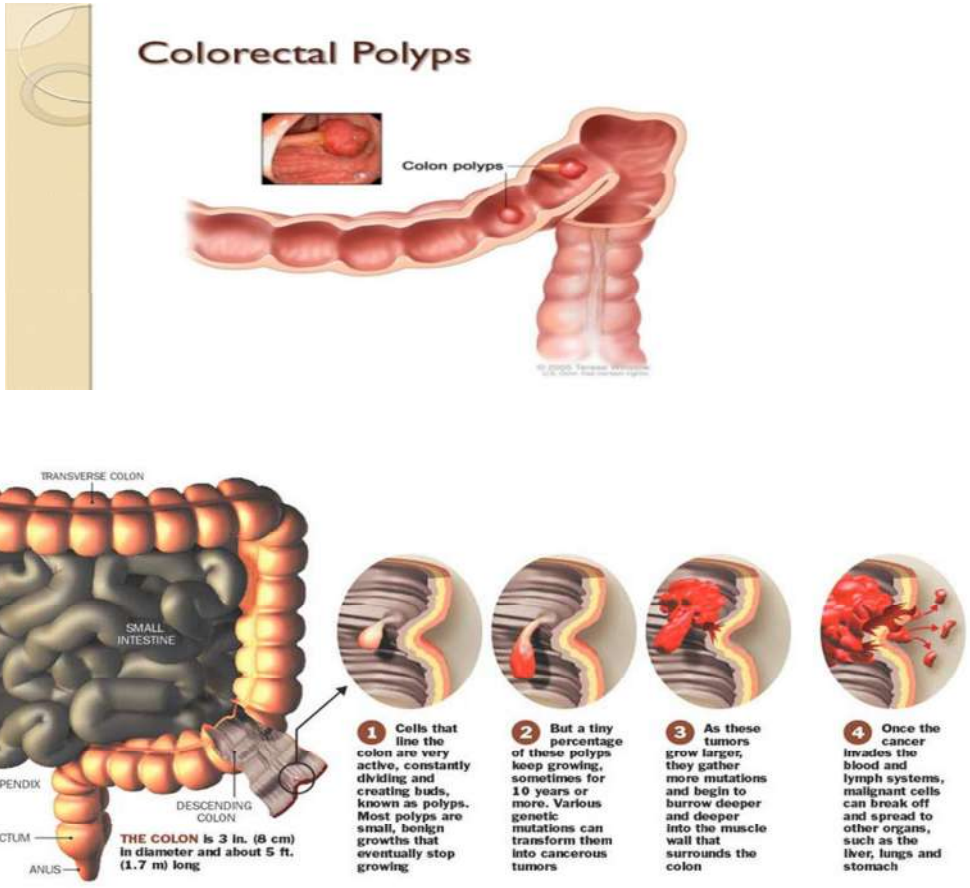


Figure 1:STAGES OF COLORECTAL CANCER[3]

ETIOLOGY:

Colon cancer is a multifactorial disease which contributes to various factors: Geneticfactors, environmental factors (including diet),& inflammatory conditions of digestive tract are all involved in the development of Colorectal Cancer. The genetic cause of colorectal cancer remains unknown,current research indicates that genetic factors have the greatest correlation to colorectal cancer.

Hereditary mutations of the APC gene are the cause of familial Adenomatous Polyposis(FAP),in which affected individuals carry an almost 100% risk of developing colon cancer by age 40yrs.

Hereditary nonpolyposis colon cancer syndrome (HNPCC,LYNCH SYNDROME) posses 40%risk of developing colorectal cancer,individuals with this syndrome has increased risk for Urothelial cancer & Endometrial cancer.[6]

LYNCH SYNDROME, is characterized by deficient mismatch repair (dMMR)due to inherited mutations in one of the mismatch repair genes such as hMLH1,hMSH2,hMSH6,hPMS1.

However Dietary factors are the subjects of intense &one of the ongoing investigations which reveals how red meat or processed meat and also sedentary lifestyle becomes the etiological factors for colorectal cancer.[3]

EPIDEMOLOGY:

The incidence & mortality rates vary markedly around the world. Globally CRC is the 3rd most common diagnosed cancer in males while women's contributes to 2nd most common cancer,with 1.8 million new cases & almost 861,000 globally deaths in 2018according to the World Health Organization GLOBOCAN database.

Colorectal cancer mortality rates are 47% higher in black men &34% higher in black women compared with whites.However from 2007 to 2016 death rates have been declined faster in blacks than whites narrowing

the racial disparity in both men & women.A review of surveillance ,epidemiology end result (SEER) Data found that US cases of colorectal cancer in persons aged 40-49yrs have increased significantly since 1995,with greater annual percentage of 29% of colorectal cancer occurringrisk.[8]

OBJECTIVE:

To reduce your risk of cancer, Cancer council recommends eating no more than 1 serve of lean red meat per day or 2 serves 3-4times per week. Red meat includes beef,lamp &pork. While processed meat oncludes bacon, ham,devon,frankfurts.[4]

- Cancer Council recommends moderate consumption of unprocessed lean meat.
- Avoid consuming more than 455g of cooked lean red meat each week which will affect and lead to colorectal cancer in a process.
- Limiting or avoiding processed meat such as frankfurts , salami bacon,and ham.which are high in fat and salt.
- Limiting consumption of burnt or charred meat and
- Choosing lean cuts of meat & chicken ,eating more fish & plenty of plant based foods such as fruits, vegetables,legumes,nuts &wholegrain cereals.[7]

CONCLUSION:

From the information provided we reach to the conclusions that red meat causes Colorectal cancer which is also called as bowel cancer.since the meat that we intake gets broken in the gut by the breakage of Haem which is broken down to N-nitroso chemicals are formed which damages the cell lining which is then leading to bowel cancer.So as to prevent the Colorectal cancer several preventive measures are taken into considerations.[12] The dietary recommendations are to reduce and avoid processed meat intake.Properly cooked meat should be suggested to avoid the risk of colorectal cancer.[5]

REFRENCES:

1. PDQ Adult Treatment Editorial Board. Colon Cancer Treatment–Health Professional Version. National Cancer Institute. Available at http://www.cancer.gov/types/colorectal/hp/colon-treatment-pdq. February 22, 2019; Accessed: May 21, 2019.
2. [Guideline] Desch CE, Benson AB 3rd, Somerfield MR, et al. Colorectal cancer surveillance: 2005 update of an American Society of Clinical Oncology practice guideline. J Clin Oncol. 2005 Nov 20. 23(33):8512-9. [Medline].
3. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, et al. Genetic alterations during colorectal-tumor development. N Engl J Med. 1988 Sep 1. 319(9):525-32. [Medline].
4. Meyerhardt JA, Niedzwiecki D, Hollis D, et al. Association of dietary patterns with cancer recurrence and survival in patients with stage III colon cancer. JAMA. 2007 Aug 15. 298(7):754-64. [Medline].
5. Pala V, Sieri S, Berrino F, et al. Yogurt consumption and risk of colorectal cancer in the Italian European prospective investigation into cancer and nutrition cohort. Int J Cancer. 2011 Dec 1. 129(11):2712-9. [Medline].
6. Tabung FK, Liu L, Wang W, Fung TT, Wu K, Smith-Warner SA, et al. Association of Dietary Inflammatory Potential With Colorectal Cancer Risk in Men and Women. JAMA Oncol. 2018 Jan 18. [Medline].
7. Cho E, Lee JE, Rimm EB, Fuchs CS, Giovannucci EL. Alcohol consumption and the risk of colon cancer by family history of colorectal cancer. Am J Clin Nutr. 2012 Feb. 95(2):413-9. [Medline]. [Full Text].
8. Siegel RL, Fedewa SA, Anderson WF, Miller KD, Ma J, Rosenberg PS, et al. Colorectal Cancer Incidence Patterns in the United States, 1974–2013. J Natl Cancer Inst. 28 February 2017. 109:[Full Text].

9. World Health Organization, International Agency for Research on Cancer. Colorectal Cancer: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012. International Agency for Research on Cancer. Available at http://gco.iarc.fr/today/data/factsheets/cancers/10_8_9-Colorectum-fact-sheet.pdf. Accessed: May 21, 2019.
10. Malvezzi M, Carioli G, Bertuccio P, Boffetta P, Levi F, La Vecchia C, et al. European cancer mortality predictions for the year 2018 with focus on colorectal cancer. Ann Oncol. 2018 Mar 19. [Medline]. [Full Text].
11. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin. 2019 Jan. 69 (1):7-34. [Medline]. [Full Text].
12. Campbell PT, Patel AV, Newton CC, Jacobs EJ, Gapstur SM. Associations of recreational physical activity and leisure time spent sitting with colorectal cancer survival. J Clin Oncol. 2013 Mar 1. 31(7):876-85. [Medline].

SP-23

A REVIEW ON SODIUM GLUCOSE CO-TRANSPORTER(SGLT-2) INHIBITORS

S.RAMYA*, Dr. G.SUSMITHA
Department of Pharmacy Practice, Bhaskar Pharmacy College, Hyderabad- 500075, India.
E-mail Id: susmi.aswi@gmail.com

ABSTRACT:
All over the world, incidence of diabetes is increasing day by day. Even after using proper medications, the target to stop this is not reached. Sadly, only half of the population is able to get control over it. Type- 2 diabetes adds the risk of cardiovascular and macro and micro vascular complications. SGLT-2 inhibitors are the latest group of drugs for treating Type - 2 diabetes having insulin independent action. The appreciable worth of using these drugs is that they show increment in urinary glucose excretion without causing hypoglycaemia and causes weight loss. This review highlights the physiology and pharmacology of SGLT-2 inhibitors.

INTRODUCTION:

Currently available anti-hyperglycaemic drugs work either by improving β-cell secretion of insulin(e.g.: sulfonylureas, and the new incretin agents) or by improving peripheral and hepatic insulin sensitivity (e.g.: metformin and pioglitazones). Even though, current anti-hyperglycaemic drugs have proven efficacy, they cause weight gain on long term use.[5]Recently a new class of anti-hyperglycaemic agents have been approved, which have better glycaemic control and improve insulin resistance in Type 2 DM patients. This new class of drugs are renal sodium linked glucose transporter-2 (SGLT-2) inhibitors which reduce the blood glucose levels by blocking renal glucose reabsorption system. SGLT-2 inhibitors acts by excretion of glucose in urine. They are indicated for glycaemic control and reduction of already ingested calories.

ROLE OF KIDNEYS IN GLUCOSE HOMEOSTASIS:

In normal individuals, glucose homeostasis is maintained by regulating production, reabsorption and utilisation of glucose. In spite of tight glucose regulation some individuals develop diabetes or hypoglycaemia[6]. Kidneys play a crucial role in filtration and reabsorption of glucose. Kidneys filter approximately 180gm of plasma glucose each day, of which 99% filtered plasma glucose is reabsorbed in proximal convoluted tubule. In normal individuals the reabsorption of glucose is 100% i.e., 180gm of filtered glucose is reabsorbed.[7] When there are hyperglycaemic conditions the amount of filtered glucose reabsorbed increase in proportion to the plasma glucose concentration. When the glucose levels exceeds the maximum capacity of the carrier proteins, which is usually 200mg/dl of plasma glucose concentration, glucose appears in the urine. In diabetes patients, the hyperglycaemia causes hyperfiltration of glucose by the kidneys and increases the luminal glucose levels, which exceeds the maximum reabsorption rate resulting in glucosuria.[8]

MECHANISM OF GLUCOSE TRANSPORT BY THE CARRIER PROTEINS ACROSS MEMBRANE:

Cell membrane is made up of lipids, which is impermeable to glucose, as glucose is a polar compound. So the glucose reabsorption in PCT and its transport across cell membrane needs carrier proteins which are located in cell membrane. Glucose enters the cell via two types of cell membrane associated carrier proteins: they are the facilitated glucose co-transporters(GULTs) and the active sodium coupled co-transporters (SGLTs). Secondary active sodium and glucose symporters – SGLT 1 and SGLT 2 helps in glucose transport through the apical membrane of intestinal and kidney epithelial cells. The active glucose transport is coupled with the downhill transport of sodium across the basolateral cells into the intracellular fluid. The energy produced by this transport is used in the concentration of glucose inside the cells. GLUTs catalyse the facilitated diffusion of glucose across the basolateral membrane. This process is independent of energy. GLUT-4 is primarily involved in insulin mediated glucose uptake in muscles and adipose tissue

MOLECULE	DOSE RANGE	ORAL BIOAVAILABILITY(%)
Dapagliflozin	5-10 mg once daily	78
Canagliflozin	100-300 mg once daily	65
Empagliflozin	10-25 mg once daily	N/a
Ipragliflozin	100-300 mg once daily	65

SGLT-2 CARRIER PROTEIN:

Six sodium – co transporters have been identified in the kidney (SGLT1 – SGLT6). SGLT-2 is majorly present in the brush boarders of epithelial cells in S1 segment of PCT of kidney and SGLT-1 is primarily present in the small intestine and distal S2/S3 segment of PCT of kidney and myocardium.[9] SGLT-2 inhibition results in decrease in blood glucose due to increase in the excretion of glucose through urine. This new class drugs also controls glucose levels by increasing insulinA9 sensitivity and uptake of glucose in muscle cells, decreased gluconeogenesis and improve first phase insulin release from beta cells.

MECHANISM OF ACTION:

Sodium – glucose co-transporter – 2 (SGLT-2) inhibitors acts by inhibiting the SGLT-2 carrier proteins in the PCT of kidney and prevents the reabsorption of glucose from glomerular filtrate and facilitate the excretion of filtered glucose through urine. The action of SGLT-2 inhibitors depend on the blood glucose levels and is independent of the insulin action. Therefore there are less risks of developing hyperglycaemia, unlike other anti-diabetes agents, which increase insulin secretion. This also has less risk of beta cell overstimulation and fatigue.[10] In addition there is calorie loss which is associated with excreted glucose and results in weight loss. Since the mechanism of action of this class of drugs depend on the renal glomerular tubular function, their efficacy is decreased in renal impairment patients.

INDIVIDUAL DRUGS:

PHLORIZIN:

It is the first known SGLT-2 inhibitor, which was separated from the root and bark of apple trees. Phlorizin is a non-selective SGLT inhibitor which blocks both SGLT-1 and SGLT-2. Phlorizin is not developed for human use because of its low availability and due its non-selective inhibition of SGLT-1 in the intestine causes GI disturbances like diarrhoea.[11]

DAPAGLIFLOZIN:

Dapagliflozin is an orally active SGLT-2 inhibitor. It is rapidly absorbed when taken orally. The peak plasma concentration is achieved within 2 hrs and its half life is approximately 16-17 hrs. The oral bioavailability of dapagliflozin is 78% and is having high protein binding. It is metabolised by uridine diphosphate glucuronyl transferase (UGT)1A9 and forms inactive metabolite. The approved dose of dapagliflozin is 10mg once daily, as monotherapy or as add on therapy to metformin, sulfonylureas, dipeptidyl peptidase 4 inhibitors and insulin. Dapagliflozin is specially indicated as an adjunct to diet and exercise to improve glycaemic control in adult with type 2 DM. In severe hepatic impairment the recommended dose is 5mg once daily taken in morning with or without food.[12] US Food and Drug Administration has approved dapagliflozin on January 2014.Dapagliflozin has shown, to reduce both HbA1c and fasting plasma glucose. Dapagliflozin administration led to significant placebo adjusted reactions in HbA1c of -0.58%,-0.77% and -0.89% in nearly 485 newly diagnosed, treatment-naïve T2DM patients controlled by diet and exercise administered 2.5, 5, 10mg of dapagliflozin respectively. The HbA1c change in the placebo group was -0.23%. Patients with oral diabetes agents for 6 weeks or more – metformin ≥1000mg and/or pioglitazones ≥ 30mg or rosiglitazone 4mg and/or at least 12 weeks of insulin and at least 6 weeks of a stable insulin dose at ≥50 units daily demonstrated mean changes in HbA1c of -0.70% for dapagliflozin 20mg at 12 weeks. When combined with insulin for 24 weeks, dapagliflozin 10mg dose provided statistically significant improvement in HbA1c, reduction in mean insulin dose(0.79%) and a statistically significant reduction in body weight compared with placebo in combination with insulin up to 2.4kg.

ADVERSE EFFECTS:

The most common adverse effects observed with SGLT-2 inhibitors is urogenital tract infections.[13] Glycosuria is a reason for development of genital, fungal and urogenital infections, most commonly vulvitis and vulvovaginitis in females and balanitis and balanoposthitis in males. Prevention for the infection is adequate perineal hygiene and treatment with anti-fungal agents. Volume depletion and orthostatic hypertension are presumed to occur as a result of osmotic diuresis, but incidence of this adverse effects has been observed to be minimal.

DRUG – DRUG INTERACTIONS:

SGlT-2 inhibitors can be used in combination with other anti-diabetic drugs and diuretics, but precautions should be taken when using along with loop diuretics because of potential risk of volume depletion. Concomitant use of canagliflozin with uridine glucosuryl transferase(UGT) inducer drugs, such as, rifampicin, phenytoin, phenobarbitone and ritonavir, should prompt an increase in dose from 100-300mg, if tolerated[14] canagliflozin metabolism is decreased by UGT enzyme inducing agents thereby decreasing peak plasma levels and reduced efficacy.[15]

THERAPEUTIC USES:

The goal of the treatment in Type 2 DM is to achieve near normal glycaemia to prevent the development and progression of vascular complications.[16] SGLT-2 inhibitors are a novel class of drugs with novel mechanism of action. SGLT-2 inhibitors can be used as monotherapy in initial therapy and also as combination therapy with other anti-diabetic drugs.

CONCLUSION:

Through this review I would like to conclude that SGLT-2 inhibitors are lower class of drugs which help in maintaining normal glucose level with less risk of developing hypoglycaemia. They are the attractive choice for combination with other anti-diabetic drugs as an add-on therapy because of their unique mechanism of action, independent of insulin, and additional benefits such as weight loss and in blood pressure. The drug should be used with caution in elderly patient who are prone to dehydration, genital infection and patient with reduce renal functions.

References:

1. Abdul GM, DeFranzo R. Dapagliflozin for the treatment of type 2 diabetes. Expert opin pharmacother. 201;14:1695-703.
2. Anjana RM, Pradeepa R, Deepa M, Datta V, Sudha R, Unnikrishanan A, etal. Prevalence of diabetes and prediabetes(impairing fasting glucose and/or impaired glucose tolerance) in urban and rural Council of Medical Research-India Diabetes(ICMR-INDIAB) study. Diabetology 2011;54:3022-27.
3. The emerging Risk Factors collaboration. Diabetes mellitus, fasting glucose, and risk of cause specific death. NEJM 2011;364829-41.
4. Wagman AS. Nuss JM. Current therapies and emerging target for the treatment of diabetes. CurrPharm Des 2001;7:417-450.
5. Wilding J. Thiazolidinediones, insulin resistance and ibesityl finding a balance. Int J Clin Pract 2006;60:1272-80.
6. Abdul GM, Defronzo R. Dapagliflozin for the treatment of type 2 diabetes. Expert opin pharmacother 2013;14:1695-703.
7. Wright EM renal Na(+)-glucose cotransporters. Am J Physiol Renal Physiol 2001;280:F10-F18
8. Marsenic O. Glucose control by the Kidney: an emerging target in diabetes. Am J Kidney Dis 2009; 53: 875-883.
9. Tahrani AA, Barnett AH. Dapagliflozin: a sodium glucose co-transporter 2 inhibitor in development for Type 2 diabetes. Diabetes Rep. 2010; 10: 101-107.
10. Nauck MA. Update on developments with SGLT-2 inhibitors in the management of Type 2 diabetes. Drug Des Devel Ther 2014; 8: 1335-80.
11. Ehrenkranz J, Lewis N, Ronald KC, Roth J. Phlorinzin: a review. Diabetes Metab Res Rev 2005; 21:31-8.
12. Dapagliflozin prescribing information, Astra Zeneca Pharmaceuticals, 2014.

13. Chao EC. Dapagliflozin: an evidence based review of its potential in the treatment of Type-2 diabetes. Core Evidence 2012; 7: 21-28.
14. Canagliflozin prescribing information, Janssen Pharmaceuticals. 2014. https://www.invokanahcp.com/prescribing-information.pdf.
15. Sarnoski-Brokavich S, Hilas O. Canagliflozin (invokana), a novel oral agent for Type-2 diabetes. P T. 2013;38:665-6.
16. Department of Pharmacology and Pathology, U P Rural Institute of Medical Science and Research, Saifai, Etawah-206130(U.P.),India.

SP 24

A REVIEW ON RECIPROCATION OF CANCER AND CIRCADIAN RHYTHMS: EMERGING THERAPY FOR CANCER.

S. SAI KEERTHANA*, Dr. G.SUSMITHA, DRr. A.V. KISHORE BABU, Dr. A. SRINIVASA RAO.
Department of Pharmacy Practice, Bhaskar Pharmacy College, Hyderabad- 500075, India.
E-mail Id: susmi.aswi@gmail.com

ABSTRACT:
Circadian rhythm is the natural cycle of physical, mental and behavior changes that the body goes through in a 24-hour cycle. Disruption of circadian rhythm is associated with a variety of human pathologies, including cancer. The Circadian clock controls the "On" and "Off" cycling of many functions that are important for cancer development. REV-ERB proteins are the key components of the clock machinery that repress biological functions that cancer cells depend on, such as cell division and cell metabolism. This suggests that pharmacological modulation of clock-related proteins may be suitable as an effective Anti-cancer strategy. The integration of circadian biology into cancer research and a better appreciation of this underlying fundamental biological phenomenon and its potential for chronotherapy could benefit many cancer patients worldwide.

Keywords:Circadian rhythm, REV-ERB Proteins, Cancer therapy, Chronotherapy.

INTRODUCTION:

Cancer is a condition in which abnormal cells divide uncontrollably and can invade the nearby tissues. It can also spread to other parts of the body through the blood and lymph systems. The treatment options include Surgery, Chemotherapy and Radiation therapy. Targeted therapies are also available for some types as of now. Cell physiology is regulated by 24-hour Circadian clock that are coordinated by the Suprachiasmatic nucleus, a hypothalamic pacemaker that will help the organism in adjusting to the environmental cycles.[1] Altered Circadian rhythms predict poor survival in cancer patients. The concept of treating cancer patients according to the biological clock is termed as Chronotherapy. It aims for administering the drugs at an appropriate time of the day to show maximum efficacy and minimum side effects. Several experiments have been conducted which reported positive associations between circadian clock and drug response in cancer patients.[2]The nuclear hormone receptors REV-ERB alpha and REV-ERB beta are found to be very crucial. Two REV-ERB receptor agonists i.e. SR9009 AND SR9011 have been developed with in vivo activity. They induced apoptosis of almost all the cells that had different mutations that drive cancer growth without causing any harm to the surrounding normal cells. In cells that had undergone oncogene induced senescence REV-ERB agonism blocked autophagy and induced apoptosis.[3,4]Thus, we summarize the link between the cancer and disruption of circadian rhythms.

CENTRAL AND PERIPHERAL CLOCK:

The circadian rhythms are hierarchical, and the central clock in the human body is composed of a biological pacemaker that is located in the brain's suprachiasmatic nucleus (SCN).[2]Peripheral clocks are synchronized by signals from the central clock are present in nearly all other tissues of the body. For synchronization, a major signal is the daily light–dark cycle that is detected by photoreceptors present in the mammalian retina, which relay signals to the SCN. These electronic and endocrine signalsgenerated are sent to organs and tissues in the body (peripheral clocks) via the endocrine and autonomic nervous systems, thus leading to synchronization. Peripheral cellular clocks also respond to other stimuli including temperature shock and glucocorticoids.[1,5]However, without external indicationor from a central clock, they lose synchronization with one another.

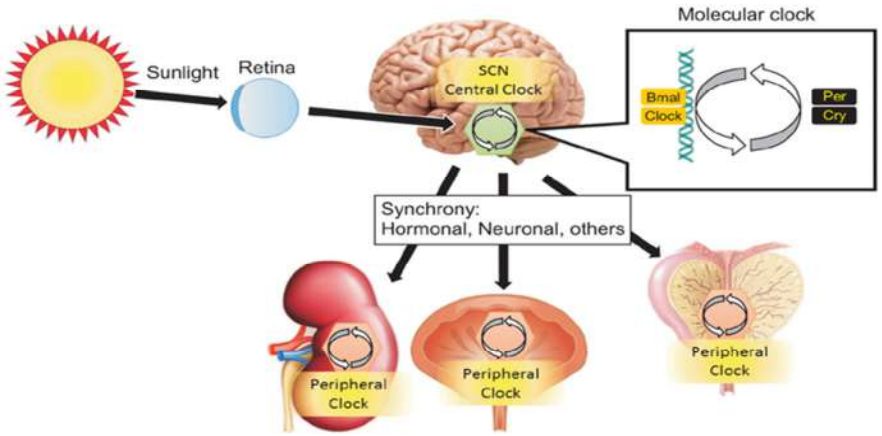


Figure-1Mechanism of central and peripheral circardian clock in mammals[1]

MOLECULAR REGULATION OF CIRCADIAN CLOCK:

Circadian clock works in an autonomous manner by the oscillatory patterns in the SCN. At its core, there are two intertwined transcription–translation negative feedback loops of genes and proteins that oscillate over a period of 24 hr in the cellular circadian machinery of mammals.[5,9]

The core molecular clock consists of a positiveloop (transcription factors BMAL1 and CLOCK),and a negative loop (period—PER andcryptochrome—CRY).[6]BMAL1 and CLOCK, thetranscription factors inthe positive loop heterodimerizeand bind to theE-box in the promoter region of target genes,resulting in expression of PER and CRY.[1]The transcription factors in the negative loop, PERand CRY heterodimerize for the repression ofBMAL1 and CLOCK expression.RORα and REV-ERBα, Constitute the second feedback loop which further control rhythms. The expression of BMAL1 is increased by RORα, while it is repressed by REV-ERBα.[9]

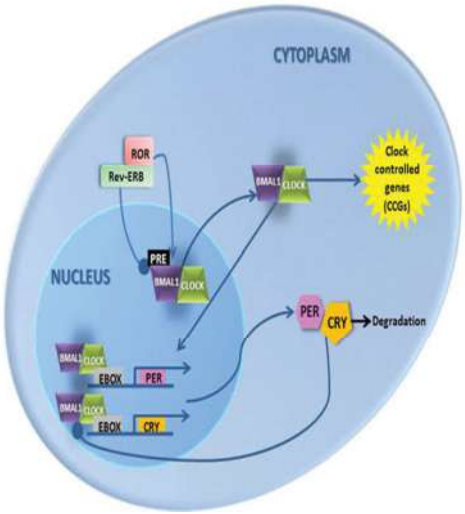


Figure -2Heme,a natural ligand for receptors[7]

LINK BETWEEN CLOCK DISRUPTION AND CANCER: [1]

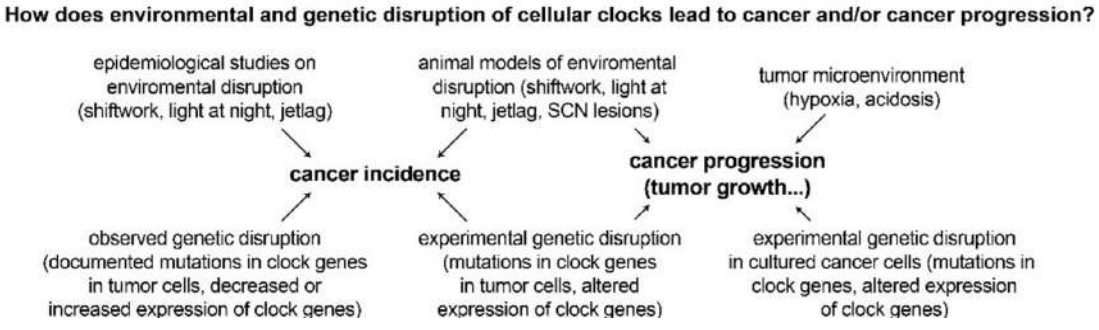
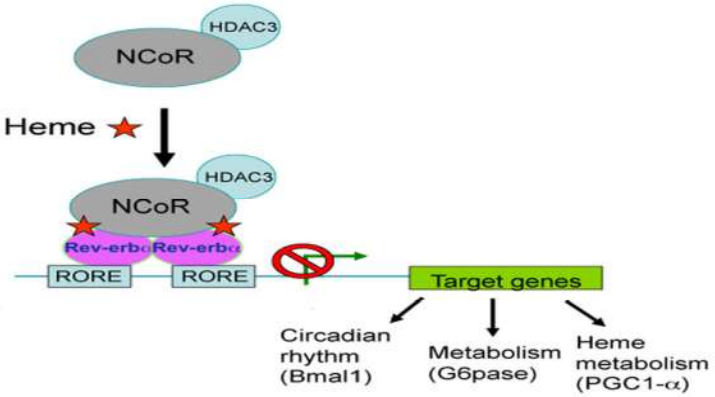


TABLE 1. ASSOCIATION OF CLOCK GENES AND PROTEINS WITH VARIOUS TYPES OF CANCER:[5]

CANCER TYPE (and effect)	TRIGGER	CIRCADIAN GENES/PROTEINS
Breast Cancer	Histone acetyltransferase activity	CLOCK(Protein)
Inhibits breast cancer cell proliferation and tumour growth	Expression	PER-1
Non-Hodgkin lymphoma	Genetic variations, functional polymorphism	CRY-2
Prostate cancer	Decreased expression levels	BMAL-1
Epithelial ovarian cancer	Low expression levels	CRY-1, BMAL-1
Chronic myeloid leukaemia	Downregulation	PER-1, CRY1-2
Endometrial cancer	Methylation	PER1-2, CRY-1

CIRCADIAN ANTI-CANCER DRUG TARGETS:

A number of drug targets have been emerged. One of the most important targets is REV-ERB Protein. REV-ERB proteins, which belong to the nuclear receptor superfamily have two variants i.e. REV-ERBα (NR1D1) andREV-ERBβ (NR1D2).[4]This superfamily consists of a large number of ligand-activated transcription factors. The natural ligand for these receptors is Heme.[7] Also, other synthetic agonists and antagonists have been recently designed. Two of the REV-ERB protein agonists i.e. SR9009 and SR9011claimed efficacy in blocking autophagy and triggering apoptosis in cancer cells.However, recent studies revealed that the REV-ERBα and REV-ERBβ variants are involved in the repression of common target genes and compensate for one another, indicating a more prominent function of REV-ERB proteins in circadian regulation.[8]Disruption of the circadian clock is associated with a variety of human pathologies, including cancer.Accordingly, the expression of several clock genes is perturbed in many tumours.[9]



Firure -3Anti cancer drug targets[7]

CHRONOTHERAPY:

The most important principle of chronotherapy against various forms of cancer is to maintain a balance between effectiveness and toxic effects of drugs.[11]The rhythmicity of several physiological processes is controlled by Circadian clock that in turn influence efficiency and tolerance of pharmacotherapy.[12] Biological parameters of a tumour, such as its growth kinetics, can affect the timing of optimal chronotherapy, indicating the importance of tailoring the therapy regimens for individual patients.[13] Various studies have indicated that toxicity and anticancer efficacy of anticancer drugs can be significantly modified by circadian stage of administration. The therapeutic index of drugs that target proliferation pathways and mimic or control rhythmicity increase susceptibility of cancer cells are improved when administered at specific times of day.[14]Timing of any therapy targeting a cancer cell pathway will work better if it is given when cancer cell proliferation is most active. The elucidation of links between drug pharmacokinetics and pharmacodynamics and clock genes result in the development of new therapeutic strategies.[15]

CONCLUSION:

To maintain a physiological homeostasis functioning of circadian clock is required.There is also a strong link between Cell cycle and proliferation to circadian rhythms.Thus, disruption in the clock oscillation patterns will damage the gene and protein expressions, leading to dysregulated cell proliferation and subsequent tumorigenesis. Tumour growth can be reduced by restoring normal circadian rhythms in tumour cells. The development of new drugs targeting both the core circadian machinery and REV-ERB proteins is an emerging area of research that may prove a useful anti-cancer strategy. Furthermore, timed administration of already established cancer treatments may increase their efficacy and improve current patient outcomes, suggesting an important role for the circadian cycle in drug delivery. While more work is required to understand how circadian rhythms and cancer interact, they are clearly an important factor that should be considered when studying the causes of cancer and developing new treatments.

ACKNOWLEDGMENT:

I would like to thank Dr.A.Srinivasa Rao (Principal, Bhaskar Pharmacy College) for providing computer laboratory facilities and accessibility for articles online. I would like to thank Dr.A.V.Kishore Babu [B.Pharm, Pharm.D (P.B), Head of the Department, Department of Pharmacy Practice] for his valuable suggestions.I would like to thank Dr.G.Sushmita (Pharm.D) for her expert advice and encouragement throughout this article.

REFERENCES:

1. Monica.N. Morgan, Sapir Dvuchbabny, Chloe-Anne Martinez, Bernadette Kerr. The Cancer clock is (not) ticking: Links between Circadian rhythms and Cancer. *Clocks & Sleep***2019**, *1*(4), 435-458
2. Tia Tyrsett Kuo, Andreas G. Ladurner. Exploiting the Circadian clock for improved Cancer therapy: Perspective from a cell biologist. Frontiers in Genetics **2011.**
3. Sulli G, Rommel A, Wang X, Kolar MJ, Puca F, Saghatelian A, et.al. Pharmacological activation of REV-ERB's is lethal in cancer and oncogene induced senescence. Nature **2018**:553, 351-5.
4. C De Mei, L Ercolani, C Parodi, M Veronesi, C Lo Vecchio.Dual inhibition of REV-ERBβ and autophagy as a novel pharmacological approach to induce cytotoxicity in cancer cells. Oncogene, **2015**,34,2597–2608.
5. Christos Savvidis, Micheal Koutsilieris. Circadian Rhythm Disruption in Cancer Biology. Molecular Medicine **2012**, 18, 1249–1260.
6. Gabriele Sulli, Michael Tun Yin Lam, Satchidananda Panda. Interplay between Circadian Clock and Cancer: New Frontiers for Cancer Treatment. Trends in molecular medicine, Volume 5, issue 8, **2019,**P475-494
7. Edna Matta-Camacho, Subhashis Banerjee.Structure of REV-ERBβ Ligand-binding Domain Bound to a Porphyrin Antagonist: Journal of Biological Chemistry, **2014**.
8. Burris T. P. Nuclear hormone receptors for heme: REV-ERBα and REV-ERBβ are ligand-regulated components of the mammalian clock. Mol. Endocrinol. **2008**,22,1509–1520.
9. Shearman LP, et al. Interacting molecular loops in the mammalian circadian clock. Science. **2000**, 288:1013–9.
10. Kiessling S, Cermakian N. Clock genes and cancer. In: Biological Timekeeping: Clocks, Rhythms and Behaviour. Kumar V (Ed.). Springer India, New Delhi, India, **2017**,481–500.
11. Dr. Francis Levi. Circadian chronotherapy for human cancers. The Lancet Oncology. VOLUME 2, ISSUE 5, MAY 01, **2001**, P307-315.
12. Dara Mohammadi. Chronotherapy: treating cancer at the right time. The Pharmaceutical Journal, 19 FEB **2016**.
13. Lévi F. From circadian rhythms to cancer chrono therapeutics. Chronobiology Int **2002**; 19: 1–19.
14. Marie-Christine Mormont Ph.D. Francis Levi M.D., Ph.D.Cancer chronotherapy: Principles, applications, and perspectives. American Cancer Society.Volume97, Issue 1,1 January **2003**, 155-169.
15. Matsuo, T.; Yamaguchi, S.; Mitsui, S.; Emi, A.; Shimoda, F.; Okamura, H. Control Mechanism of the Circadian Clock for Timing of Cell Division in Vivo. Science **2003**, 302, 255–259.

SP-25

OLFACTORY BULBECTOMY: A RENOWNED MODEL FOR DEPRESSION

GHODAKE SHWETA[1] AND KORE PADMAJA*

****P.E. Society's Modern College of Pharmacy, Yamunanagar, Nigdi, Pune-44, India***

ABSTRACT:
The olfactory bulbectomized (OB) rat has been proposed primarily as an animal model for depression. Certain behavioural changes were observed due to OBX such as increased in the hyperactivity when kept in an enclosed area also alterations in food enthused and conditioned taste aversion behaviour. Likewise some of the alterations in the noradrenergic, serotonergic, cholinergic, GABAergic and glutamatergic neurotransmitter systems were also witnessed. The varieties of immune changes are also observed. An enhanced night-time secretion of corticosterone level is observed in OBX rats, which is normally suppressed or could be either decreased by dexamethasone. However, many other behavioural, neurotransmitter and immune changes have been shown to be attenuated by chronic irrespective of acute antidepressant treatment. (TCA's) such as (amitriptyline), atypical mediators (example: mianserin), selective serotonin reuptake inhibitors (SSRI's) agent such as (paroxetine), reversible inhibitors of monoamine oxidase A (The well-knownmoclobemide), alsoputative antidepressant agents like 5-HT 1A agonists for example (zalospirone), non-competitive N-methyl-D-aspartate antagonists (MK-801) have demonstrated antidepressant-like activity during this model. The aim of this review is to revise the studies done using OBX model till date so as why is it the most renowned of all models for depression.

Keywords: Olfactory bulbectomy, depression, neurotransmitters, immune system, behaviour

INTRODUCTION:

Depression is a chronic, recurring and usually life-threatening illness which affects up to 17% of the totalworld population.Medications suggested for depression those which are most effective such as tricyclic antidepressants (TCA's), selective reversible inhibitors of monoamine oxidase-A, and specific serotonin-noradrenaline (NA) reuptake inhibitors are clinically employed for drug therapy. However, these drugs can induce a variety of side-effects including cardiac toxicity, hypopiesia, sexual dysfunction, body weight gain, and sleep disorder.[1]However, a significant population of depressed patients (20% to 30%) does not respond to these medications. This is partially due to our limited understanding of the precise neurobiological mechanisms associated with depression [2].Olfactory bulbectomy model is widely used for investigation of newer upcoming antidepressants.

History of Olfactory bulbectomy as a model to study clinical depression:

Investigation of rats with bilateral lesions of olfactory bulbs was cast-off in evaluating the effect of anosmia on learning performance. After awhilst, clear impairment in passive avoidance learning after OB was reported. Additional studies revealed supplementary OBX induced behavioral alterations in sexual behaviour [3]Certain evidences also shows epilepsy due to OBX. The behavioural changes of olfactory bulbectomized rats could be used to distinguish innovative anti-depressants and a year later the olfactory bulbectomized rat was accessible as a model for depression and the detection of newer anti-depressant for their efficacy[4].After its arrival as a predictive model for antidepressants, the study expanded in numerous directions. The brain related chemistry of animals was determined & slope of antidepressants grew up higher as compared to earlier ones. Presently, the OBX model is used as a predictive pharmacological model for testing the efficacy of antidepressant drugs as well as a model for learning the cognitive debility and neurodegeneration. [5]

Changes observed in olfactory bulbectomized rats or mice:

Behavioural changes:

The typical and the most widely accepted behavioural pattern in the OBX model of depression is the significant increase in locomotor/exploratory activity during the OFT [5]. Mice stereotypically exhibit less exploratory behaviour during the first few minutes of testing in a familiar open field arena. OBX mice also

showed chronic hyperactivity. Interestingly, altogether observations can be compared with clinical features that demonstrate a remarked cognitive decline in depressed patients [6].

Endocrine changes:

Surgical removal of olfactory bulbs has shown marked increase in ACTH as well as cortisone levels due to the altered activity of hypothalamus.[7]Handling of animals on other hand has a different effect on corticosterone levels in OBX animals as compared to that of the control ones.

In contrast to a study with mice did not show increased serum corticosterone levels after bulbectomy also, not necessary to show altered levels in rats too. [8]

Neurotransmitter changes:

Serotonergic system alterations have been observed to a great extent due to olfactory bulbectomy, not only decrease in levels but also alterations in various arenas of brain.

Not only the levels but also the rate of synthesis of serotonin found to be decreased. Administration of Memantine, a non-competitive NMDA receptor antagonist, for consecutive 28 days starting two days prior the OBX surgery prevented OBX-induced hyperactivity and moderately the loss of fear memory in rats. [9]

Immune changes:

Numerous changes have been observed after removal of olfactory bulbs such as lowering of lymphocytes along with decreased neutrophil phagocytosis along with increase in macrophage and monocyte activity. [10]

Another marked increase was found in the production of IL-1 and Tumor necrosis factor alfa when tested with lipopolysaccharide. [11]

Urinary metabolic disturbances:

There are certain evidences showing changes urinary metabolic profile associated with biomarkers was found to be helpful in estimating the diagnosis of agitated depression when administered with fluoxetine as standard. [12]

Involvement of olfactory bulbectomy (OBX) as a model in various studies:

We have overviewed as why olfactory bulbectomy (OBX) is known as versatile as a model which is being used since past few decades. Let us have a look on various studies related to depression in ascending order in which olfactory bulbectomy (OBX) plays a significant role in testing the efficacy of the drugs.The OBX model used in 1984, for testing of antidepressants and the change in the behaviour associated with surgical removal of olfactory bulbs. The changes in the behaviour as well as central neurotransmitter function followed with the bilateral ablation of the olfactory lobes of the rat represented according to them. Their aim to assess critical behaviour changes in olfactory induced rats and thereby, mechanisms of drugs associated to attenuate antidepressant effect. [13]OBX induced rat was well-validated animal model of stress-induced depression and was used for investigation of standardized extract of *Centellaasiatica L* showed reversal of physiological and behavioural changes followed by OBX induced depression in rats. [14] Olfactory bulbectomy in 2014 was used as a model of depression for potential effects of Panaxquinquefolium (PQ) along with nitric oxide modulators. Ablation of the olfactory bulbs leads to depression like symptoms and reversed the behavioural and cognitive changes when administerd with PQ extract. The result was found to be dose-dependent. [15]Application of OBX model was used to study the neurodegeneration and the associated cognitive decline. They stated that OBX induced rats and mice have different effects on BDNF & 5-HT2c receptor as well. But these two species may also differ on how the treatment is been given after induction of OBX.[16]OBX was also used to test the effectiveness of putative antidepressant agents. Evidences suggest that OBX leads to dysfunctioning of several of cellular processes within the hippocampal arena which may include decreased in dentate gyrus neurogenesis. [17] Review was widely done on OBX model for depression which includes frequently used olfactory bulbectomy in rats & mice. They stated with evidences that OBX model provide a tool for an investigation to the neurobiology of depression and significant mechanism of action for several antidepressants. [18]

CONCLUSION:

On removal of olfactory bulbs, rats and mice display behavioural deficits that lead in disruption of limbic and hypothalamic regions. Olfactory bulbectomy is one of the distinctive models of depression as it is the specific one which arises due to neurodegeneration process. This model is useful for wide range of

antidepressant and one can rely on OBX models which mimics the symptoms of depression. Animals show hyperactivity on removal of olfactory bulbs and hence, benefits for investigating efficacy of upcoming antidepressants. Numerous studies have been conducted using OBX models in order to find out the correlation between specific neurochemical behaviour. Investigating with OBX models have proven the testing of efficacy of antidepressants.Applications of olfactory bulbectomy as a model for depression have elevated its importance in the fields of neurobiology where the basis for behavioural, neurological and cognitive stands by. Due to the clear-cut concept of olfactory bulbectomy model, it has been the frequently used model for depression. Due to its moral results and non-tedious surgical technique as it can be performed in experimental laboratory itself also being cheap irrespective of requirement of skilled researcher for surgical purpose has gained limelight in the field of research.

References:

1. Freitas AE, Machado DG, Budni J, Neis VB, Balen GO, Lopes MW, *et al.* Antidepressant-like action of the bark ethanolic extract from *Tabebuiaavellanedae* in the olfactory bulbectomized mice. J Ethnopharmacol 2013; 145:737-45.
2. Nestler, Eric J., and Steven E. Hyman. "Animal models of neuropsychiatric disorders." *Nature neuroscience* 13.10 (2010): 1161.
3. Uher, R., and P. McGuffin. "The moderation by the serotonin transporter gene of environmental adversity in the etiology of depression: 2009 update." *Molecular psychiatry* 15.1 (2010): 18.
4. Wang, Qingzhong, et al. "The recent progress in animal models of depression." *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 77 (2017): 99-109.
5. Hendriksen, Hendrikus, et al. "The olfactory bulbectomy model in mice and rat: one story or two tails?." *European journal of pharmacology* 753 (2015): 105-113.
6. Cobb, J.A., O'Neill, K., Milner, J., Mahajan, G.J., Lawrence, T.J., May, W.L., Miguel-Hidalgo, J., Rajkowska, G., Stockmeier, C.A., 2016. Density of GFAP-immunoreactive astrocytes is decreased in left hippocampi in major depressive disorder. Neuroscience 316, 209–220
7. Breivik, T., Gundersen, Y., Myhrer, T., Fonnum, F., Osmundsen, H., Murison, R., Gjermo, P., von Horsten, S., Opstad, P.K., 2006. Enhanced susceptibility to periodontitis in an animal model of depression: reversed by chronic treatment with the antidepressant tianeptine.J.Clin.Periodl.33, 469-77
8. Freitas AE, Machado DG, Budni J, Neis VB, Balen GO, Lopes MW, *et al.* Antidepressant-like action of the bark ethanolic extract from *Tabebuiaavellanedae* in the olfactory bulbectomized mice. J Ethnopharmacol 2013; 145:737-45.
9. Watanabe, A., Tohyama, Y., Nguyen, K.Q., Hasegawa, S., Debonnel, G., Diksic, M., 2003. Regional brain serotonin synthesis is increased in the olfactory bulbectomy rat model of depression: an autoradiographic study. J. Neurochem. 85, 469–475.
10. Song, C., Earley, B., Leonard, B.E., 1996. Behavioural and immunological effects of the antihistamine terfenadine in olfactory bulbectomized rats. Eur. Neuropsychopharmacol. 6, 157–162.
11. Connor, T.J., Harkin, A., Kelly, J.P., Leonard, B.E.,2000.Olfactory bulbectomy provokes a suppression of interleukin-1beta and tumour necrosis factor-alpha production in response to an in vivo challenge with lipopolysaccharide: effect of chronic desipramine treatment. Neuro immune. 7,27-35.
12. Ozcan, Halil, et al. "Olfactory bulbectomy and raphe nucleus relationship: a new vision for well-known depression model." *Nordic journal of psychiatry* (2019): 1-7.
13. Jancsár S., Leonard B.E. (1983) The olfactory bulbectomized rat as a model of depression. In: Usdin E., Goldstein M., Friedhoff A., Georgotas A. (eds) Frontiers in Neuropsychiatric Research. Palgrave Macmillan, Londoz
14. Kalshetty, Padmaja, et al. "Antidepressant effects of standardized extract of Centellaasiatica L in olfactory bulbectomy model." *Biomedicine & Aging Pathology* 2.2 (2012): 48-53.
15. Rinwa, Puneet, and Anil Kumar. "Panaxquinquefolium involves nitric oxide pathway in olfactory bulbectomy rat model." *Physiology & behavior* 129 (2014): 142-151.
16. Hendriksen, Hendrikus, et al. "The olfactory bulbectomy model in mice and rat: one story or two tails?." *European journal of pharmacology* 753 (2015): 105-113.
17. Morales-Medina, J. C., et al. "The olfactory bulbectomized rat as a model of depression: the hippocampal pathway." *Behavioural brain research* 317 (2017): 562-575.
18. Wang, Qingzhong, et al. "The recent progress in animal models of depression." *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 77 (2017): 99-109.

SP-26

BIOMARKERS FOR NEUROPSYCHIATRICDISORDERS WITH RESPECT TO TRANSLATIONAL ANIMAL MODELS

PADMAJA KORE*, VARSHA SATAV[1]
P.E. Society's Modern College of Pharmacy, Yamunanagar, Nigdi, Pune-44, India

ABSTRACT:
Unravelling neuropsychiatric disorders with the aid ofanimal models possess a substantial challenge due to the subjective and overlapping symptoms of the disease. The symptoms themselves are varied and have complex neurobiological mechanism behind them, of which we have rudimentary understanding. Multifactorial etiology of such disorders contributes to unreliable diagnosis and inaccurate therapeutic regimen. Emergence of biomarkers will help in understanding the pathophysiology behind such debilitating disorders and aid in identify promising therapeutic targets. The drug targets hence found can be translated into therapeutic strategies. Statistical tools like genomics, proteomics and metabolomics along with the research domain criteria (RDoC) will lead to formation of robust animal models.

KEYWORD: Neuropsychiatric disorders, biomarkers, proteomics, genomics.

INTRODUCTION:

Neuropsychiatry endeavor's to understand abnormal behaviour and behavioural disorders with the help of various neurobiological and psychological–social factors. Neuropsychiatryprovides a bridge between the disciplines of psychiatry, neurology and neuropsychology [1]. Neuropsychiatric disorders are sometimes referred to as neurobehavioral disorders [2].
The complexity of neuropsychiatric disorders arises from the high level of etiologic heterogeneity and involvement of several multifactorial environmental and genetic factors [3].

Statusquo of the diagnostic tools and animal models for neuropsychiatric disorders

Current medical practice is standing on three pillars namely:-

1) The symptoms presented by the patient.
2) Diagnosis by the physician on the basis of the symptoms.
3) Prescribing an optimum therapeutic regimen.

From above, it can be concluded that the accurate diagnosis of any disorder plays a crucial role in providing the optimum therapeutic treatment.However in case of neuropsychiatric disorders; psychiatrists face an intricate amalgamation of emotional, behavioural and cognitive symptoms. Hence the diagnosis is often based on self-reported symptoms, behaviour of the patient and their mental history. In reality, these diagnostic evaluations are often incomplete, unstructured, open-ended, and prone to inaccuracy and may cause misdiagnosis of the patient [4].Translational animal models are essential for the bench-to-bedside journey of a potential drug molecule. Not only the animal models help assess the therapeutic efficacy of the drug molecule but also they help in understanding the underlying pathophysiology of the disease and then identify drug targets in accordance.As mentioned earlier, diagnosis of psychiatric disorders is largely based on the self-reported symptoms. However, self-reported symptoms like sadness, guilt, hallucination, etc. cannot be determined in animals. Some rational connections such as insomnia or abnormality in social behavior can be measured only to a certain estimate.
An ideal animal model should cover the etiology and pathophysiology of the disorder along with its symptoms and treatment in order to meet the following validation criteria.

1. Construct validity.
2. Face validity.
3. Predictive validity.

Biomarkers: towards a new era

Biomarkers are objective, quantifiable characteristics of biological processes used to indicate a pathological condition and/or pharmacological response to a therapeutic regimen[5].

Ideal characteristics of a biomarker in neuropsychiatric disorders[6]

1. Biomarkers used in psychiatric disorder must show diagnostic specificity. I.e. a diagnostic biomarker for a particular disorder must be specific only for that disorder (presence of a common biomarker in multiple disorders will defeat the entire purpose of using biomarkers in neuropsychiatric disorders.)
2. A recognized biomarker must have high reliability and reproducibility (for this conformation by at least two independent published studies is optimum.)
3. Patients of neuropsychiatric disorders are already under traumatic conditions. Hence to avoid exacerbation of existing symptoms by additional stress, invasive methods of collecting biological sample must be avoided.
4. However, most of the methods involved in sample collection of neuropsychiatric disorders are invasive (blood collection, analysis of cerebrospinal fluid, etc.) this increases the scope for discovery of biomarkers which can be collected by non-invasive methods (urine and stool analysis, saliva swab, etc.)

Sampling techniques of biomarkers

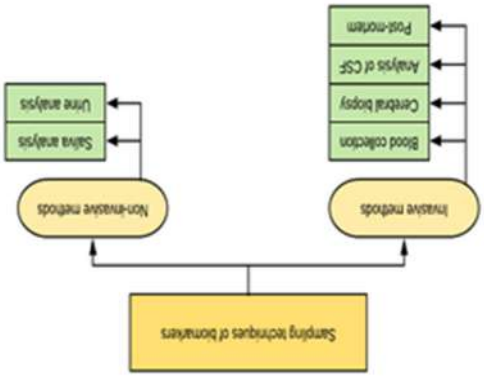


Figure: Various sampling techniques used for collection of biomarkers in neuropsychiatric disorders[8].

Blood collection

Collecting peripheral biomarkers from blood is by far the easiest invasive method. However, this technique has its own drawbacks. One such major drawback being the heterogeneous nature of the blood; which consist of blood cells, plasma, serum, etc. These components often affect the levels of biomarkers.

Example- Use of brain derived neurotrophic factor (BDNF) as a biomarker for depression. Various studies showed inconsistent resultsregarding the level of BDNF in depression[7, 8]. These inconsistencies were caused due to release of BDNF from platelets [9]. Such discrepancies can be avoided by finding various defining parameters for the composition of blood to be used.

Cerebral biopsy

Parkinson's disease is caused due the death of dopaminergic neuronal cells in substantia nigra[10]. But the pathophysiology of neurodegenerative disorders like depression does not include a single brain area; rather a network of multiple brain regions is involved in the dysfunction[11]. Hence selecting a brain region for performing biopsy can be challenging.

Analysis of CSF

Cerebrospinal fluid (CSF) is in close proximity to CNS and hence may more accurately reflect the on-going pathology of the CNS[12]. Analyzing the CSF might give a novel biomarker candidate.Example- increased α-synuclein levels in CSF may reflect extent of the synaptic loss in Alzheimer's disease[13].

Post-mortem

Biomarkers obtained post-mortem can be used as a starting point for further research. However, various technical factors like storage, method of extraction, etc. can affect the sample quality.

Salivaand urine analysis

Analysis of salivary samples is by far the most non-invasive method. Also the easy sampling technique avoids degrading the quality of the sample.

Example- Presence of alpha-synuclein (a-syn) is associated with Parkinson's disease[14].

Analysis of urine biomarkers can be intricate because multiple factors like kidney function, diet, age, medication, etc. are to be considered.

Tools of biomarker discovery

Study of proteins, metabolites, genes and lipids before and after chronic use of a drug in psychological disorders will help unravel the pathology of the disorder, help identify therapeutic targets and promote new drug discovery.

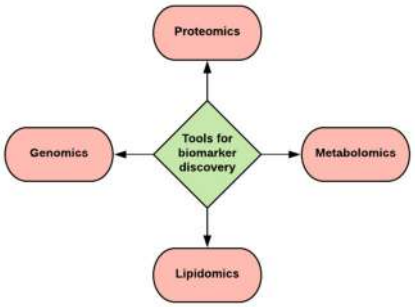


Figure: Various techniques for biomarker discovery in psychiatry[15]

Proteome is the total set of proteins expressed by a cell, a tissue or an organism at a given time under specific conditions and proteomics include the study of proteome[15].Till now proteomic study in psychiatry has been performed using samples of pituitary gland[16],fibroblasts[17],cerebrospinal fluid(CSF)[18],liver[19],etc.The substrates as well as the products of metabolism are known as metabolites and the profiling of metabolites is known as metabolomics [20].Similar as that of proteomics a wide range of samples can be analyzed.Lipidomics is a characterizing technique used for profiling lipids and their biological functions with respect to the regulation of genes and expressions of proteins involved in lipid metabolism [21].Since, psychiatric disorders are highly heritable; genomics was earlier the basis of biomarker discovery [22]. However, major drawback of genomics is that it cannot differentiate post-translational modifications in proteins[23]. The post-translational modifications have shown to form multiple protein products from the same gene [24-26]. Epigenomics is used to describe the variations in gene expression and serves as a perfect link between the disease,genetics and environment [27].

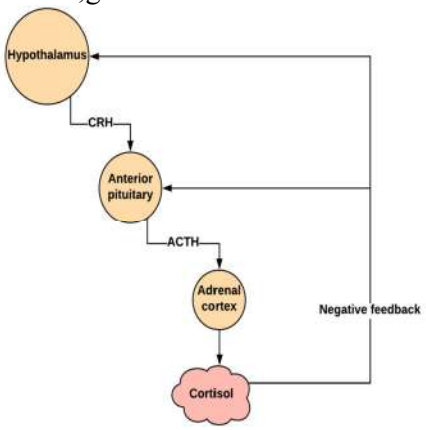


Figure: The hypothalamus-pituitary-adrenal axis[27].

(CRH-Corticotropin releasing hormone, ACTH-Adrenocorticotropic hormone)

The effect of trauma in early life affects the hypothalamus-pituitary-adrenal axis (HPA axis) alters the stress reactivity in psychiatric disorders [28]. Evidence of elevation in cortisol levels, hyperactive HPA axis along with increased volume of pituitary gland are seen in psychotic disorders [29, 30]. Atypical antipsychotics like Olanzapine inhibit activity of HPA axis [31] along with decrease in volume of pituitary gland in a dose-dependent manner [32] and hence support the stress- vulnerability model. All the above evidences suggest that dysfunction in HPA axis may be a significant biomarker in psychiatric disorders.

The research domain criteria (RDoC): joining the links together-

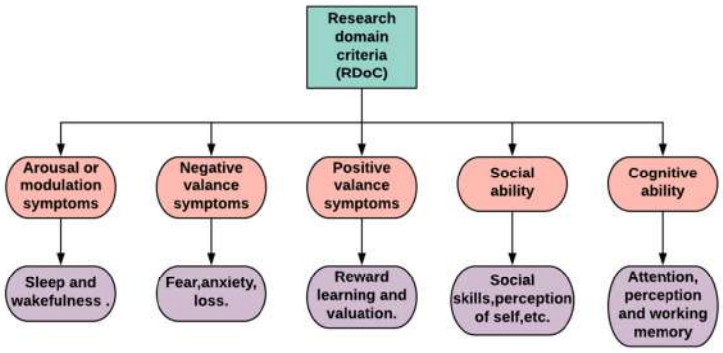


Figure: Framework of research domain criteria [33].

CONCLUSION-

Neuropsychiatric disorders possess a substantial challenge due to the subjective and overlapping symptoms of the disease. Multifactorial etiology of such disorders contributes to unreliable diagnosis and inaccurate therapeutic regimen. Emergence of biomarkers will help in understanding the pathophysiology behind such debilitating disorders and help identify promising therapeutic target

Reference

1. Sachdev P. International neuropsychiatric association. Neuropsychiatric disease and treatment. 2005 Sep;1(3):191.
2. Zasler ND, Martelli MF, Jacobs HE. Neurobehavioral disorders. InHandbook of clinical neurology 2013 Jan 1 (Vol. 110, pp. 377-388). Elsevier.
3. Schork NJ, Greenwood TA, Braff DL. Statistical genetics concepts and approaches in schizophrenia and related neuropsychiatric research. Schizophrenia bulletin. 2006 Oct 11;33(1):95-104.
4. Shear MK, Greeno C, Kang J, Ludewig D, Frank E, Swartz HA, Hanekamp M. Diagnosis of nonpsychotic patients in community clinics. American Journal of Psychiatry. 2000 Apr 1;157(4):581-7.
5. Strimbu K, Tavel JA. What are biomarkers?. Current Opinion in HIV and AIDS. 2010 Nov;5(6):463-6.
6. Sokolowska I, Wetie AG, Wormwood K, Thome J, Darie CC, Woods AG. The potential of biomarkers in psychiatry: focus on proteomics. Journal of Neural Transmission. 2015 Aug 1;122(1):9-18.
7. Piccinni A, Marazziti D, Catena M, Domenici L, Del Debbio A, Bianchi C, Mannari C, Martini C, Da Pozzo E, Schiavi E, Mariotti A. Plasma and serum brain-derived neurotrophic factor (BDNF) in depressed patients during 1 year of antidepressant treatments. Journal of affective disorders. 2008 Jan 1;105(1-3):279-83.
8. Bocchio-Chiavetto L, Bagnardi V, Zanardini R, Molteni R, Gabriela Nielsen M, Placentino A, Giovannini C, Rillosi L, Ventriglia M, Riva MA, Gennarelli M. Serum and plasma BDNF levels in major depression: a replication study and meta-analyses. The World Journal of Biological Psychiatry. 2010 Sep 1;11(6):763-73.
9. Karege F, Bondolfi G, Gervasoni N, Schwald M, Aubry JM, Bertschy G.Low brain-derived neurotrophic factor (BDNF)levels in serum of depressed patients probably results from lowered platelet BDNF release unrelated to platelet reactivity. Biological psychiatry.2005May1;57(9):1068-72.
10. Berg D, Postuma RB, Bloem B, Chan P, Dubois B, Gasser T, Goetz CG, Halliday GM, Hardy J, Lang AE, Litvan I. Time to redefine PD? Introductory statement of the MDS Task Force on the definition of Parkinson's disease. Movement Disorders. 2014 Apr;29(4):454-62.
11. Leistedt SJ, Linkowski P. Brain, networks, depression, and more. European neuropsychopharmacology. 2013 Jan 1;23(1):55-62.
12. Teunissen CE, Petzold A, Bennett JL, Berven FS, Brundin L, Comabella M, Franciotta D, Frederiksen JL, Fleming JO, Furlan R, RQ Hintzen MD. A consensus protocol for the standardization of cerebrospinal fluid collection and biobanking. Neurology. 2009 Dec 1;73(22):1914-22.
13. Llorens F, Schmitz M, Ferrer I, Zerr I. CSF biomarkers in neurodegenerative and vascular dementias. Progress in neurobiology. 2016 Mar 1;138:36-53.

14. Vivacqua G, Latorre A, Suppa A, Nardi M, Pietracupa S, Mancinelli R, Fabbrini G, Colosimo C, Gaudio E, Berardelli A. Abnormal salivary total and oligomeric alpha-synuclein in Parkinson's disease. PLoS One. 2016 Mar 24;11(3):e0151156.
15. Wilkins MR, Sanchez JC, Gooley AA, Appel RD, Humphery-Smith I, Hochstrasser DF, Williams KL. Progress with proteome projects: why all proteins expressed by a genome should be identified and how to do it. Biotechnology and genetic engineering reviews. 1996 Dec 1;13(1):19-50.
16. Krishnamurthy D, Levin Y, Harris LW, Umrania Y, Bahn S, Guest PC. Analysis of the human pituitary proteome by data independent label-free liquid chromatography tandem mass spectrometry. Proteomics. 2011 Feb;11(3):495-500.
17. Wang L, Lockstone HE, Guest PC, Levin Y, Palotas A, Pietsch S, Schwarz E, Rahmoune H, Harris LW, Ma D, Bahn S. Expression profiling of fibroblasts identifies cell cycle abnormalities in schizophrenia. Journal of proteome research. 2009 Nov 25;9(1):521-7.
18. Huang JT, Wang L, Prabakaran S, Wengenroth M, Lockstone HE, Koethe D, Gerth CW, Gross S, Schreiber D, Lilley K, Wayland M. Independent protein-profiling studies show a decrease in apolipoprotein A1 levels in schizophrenia CSF, brain and peripheral tissues. Molecular psychiatry. 2008 Dec;13(12):1118.
19. Prabakaran S, Wengenroth M, Lockstone HE, Lilley K, Leweke FM, Bahn S. 2-D DIGE analysis of liver and red blood cells provides further evidence for oxidative stress in schizophrenia. Journal of proteome research. 2007 Jan 5;6(1):141-9.
20. Johnson CH, Ivanisevic J, Siuzdak G. Metabolomics: beyond biomarkers and towards mechanisms. Nature reviews Molecular cell biology. 2016 Jul;17(7):451.
21. Han X, Gross RW. Global analyses of cellular lipidomes directly from crude extracts of biological samples by ESI mass spectrometry a bridge to lipidomics. Journal of lipid research. 2003 Jun 1;44(6):1071-9.
22. Witzmann FA, Arnold RJ, Bai F, Hrncirova P, Kimpel MW, Mechref YS, McBride WJ, Novotny MV, Pedrick NM, Ringham HN, Simon JR. A proteomic survey of rat cerebral cortical synaptosomes. Proteomics. 2005 May;5(8):2177-201.
23. Schwanhäusser B, Busse D, Li N, Dittmar G, Schuchhardt J, Wolf J, Chen W, Selbach M. Global quantification of mammalian gene expression control. Nature. 2011 May;473(7347):337.
24. Rozek LS, Dolinoy DC, Sartor MA, Omenn GS. Epigenetics: relevance and implications for public health. Annual review of public health. 2014 Mar 18;35:105-22.
25. Zhang TY, Labonté B, Wen XL, Turecki G, Meaney MJ. Epigenetic mechanisms for the early environmental regulation of hippocampal glucocorticoid receptor gene expression in rodents and humans. Neuropsychopharmacology. 2013 Jan;38(1):111.
26. Pariante CM, Dazzan P, Danese A, Morgan KD, Brudaglio F, Morgan C, Fearon P, Orr K, Hutchinson G, Pantelis C, Velakoulis D. Increased pituitary volume in antipsychotic-free and antipsychotic-treated patients of the AEsop first-onset psychosis study. Neuropsychopharmacology. 2005 Oct;30(10):1923.
27. Ryan MC, Sharifi N, Condren R, Thakore JH. Evidence of basal pituitary–adrenal overactivity in first episode, drug naive patients with schizophrenia. Psychoneuroendocrinology. 2004 Sep 1;29(8):1065-70.
28. Cohrs S, Röher C, Jordan W, Meier A, Huether G, Wuttke W, Rüther E, Rodenbeck A. The atypical antipsychotics olanzapine and quetiapine, but not haloperidol, reduce ACTH and cortisol secretion in healthy subjects. Psychopharmacology. 2006 Mar 1;185(1):11-8.
29. Nicolo JP, Berger GE, Garner BA, Velakoulis D, Markulev C, Kerr M, McGorry PD, Proffitt TM, McConchie M, Pantelis C, Wood SJ. The effect of atypical antipsychotics on pituitary gland volume in patients with first-episode psychosis: a longitudinal MRI study. Schizophrenia Research. 2010 Jan 1;116(1):49-54.
30. American Diabetes Association. Consensus development conference on antipsychotic drugs and obesity and diabetes. Diabetes care. 2004 Feb 1;27(2):596-601.
31. Horrobin DF. The membrane phospholipid hypothesis as a biochemical basis for the neurodevelopmental concept of schizophrenia. Schizophrenia research. 1998 Apr 10;30(3):193-208.
32. Horrobin DF. Phospholipid metabolism and depression: the possible roles of phospholipase A2 and coenzyme A-independent transacylase. Human psychopharmacology: Clinical and experimental. 2001 Jan;16(1):45-52.
33. NIMH » RDoC Matrix [Internet]. Nimh.nih.gov. 2019 [cited 3 February 2019].

SP-27

CADD IN ANTICANCER DRUG WITH RECEPTOR PDB ID (3RE2 PROTEIN)

TEJASWINI KUMARI DASH*[1]MRITYUNJAY BANERJEE[2], H. K.SUNDEEP KUMAR[3], A. SRINIVASA RAO[4]

*[*1]Research Scholar, Pharmaceutical Chemistry, Biju Patnaik University of Technology, Odisha*
*[*1] Assistant Professor, Bhaskar Pharmacy College, Yenkapally, Hyderabad*
[2]Associate Professor, Institute of Pharmacy & Technology (IPT), Salipur, Odisha
[3]Assistant Professor, Institute of Pharmacy & Technology (IPT), Salipur, Odisha
[4] Professor and Principal, Bhaskar Pharmacy College,Yenkapally, Hyderabad
Email ID:tdash153@gmail.com

ABSTRACT:
Cancer can be described as the uncontrolled growth of abnormal cells. Menin is a tumor suppressor protein that is encoded by theMEN1(multiple endocrine neoplasia 1) gene and controls cell growth in endocrine tissuesare present in cancers cells. 3RE2 proteins inhibitors play a vital role in deactivating cancer disease.[12]Some of the commonly used cancerdrugs are Imatinib, Sunitinib, Tandutinib etc.These drugs mainly work against the effects of kinase .Methods: The Protein- Ligand interaction plays a role in structural baseddrug designing. In our research work I have taken the Protein available in protein data bank PDB ID 3RE2& commercially available drugsSunitinib against cancer. The 3RE2was docked to the above said drug Sunitiniband the energy value obtained is -242.94 using the Argus Lab & Hex docking software. Results: Depending on the energy values we have chosen the marketed drug. We tried to improve the binding efficiency and steric compatibility of the drugs namely Several modifications were made to the groups which were interacting with the receptor molecule. This drug molecule was prepared using ACD ChemSketch and docked using Argus Lab &Hex version 5.1dockingsoftware.Discussion: Taking as a reference drug,several analog structure can be prepared to get a potent lead molecules.From this work we can improve the steric[3] compatibility and ADMET of the drugs.Further research is under process.

Keywords: **Cancer,Sunitinib,MEN1,Argus lab,Hexversion5.1**

INTRODUCTION:

Anticancer[1] chemotherapy ,targeted drug therapy are now a days a very active field of research, so it clearly indicate that there is a need of an updated treatment from the point of view of medicinal chemistry & drug designing.[1]In silico drug designing now a days is very updated technology to discover drug molecules with less consumption of materials &manpower.The basic target of our work is to develop some novel antineoplastic [10]compound & forward for further evaluation.Antitumour[2] chemotherapy,Menin is a tumor suppressor proteinnow a days a random target for cancer[7] chemotherapy.The effect of drug structure on MEN1activity will be seen in the docking studies.

OBJECTIVES OF WORK:

- Identification of cancer drug target.
- In silico analysis of compound library against drug.
- Selection of scaffold based in silico result.
- ADMET of chemical compound to check their suitability of drug.
- Docking of drug with a particular protein.

APPROACH:

The basic focus of current study is to elucidate the docking of antineoplastic[2] drug from the atomistic point of view of chemistry on the relationship between chemical structure & chemical & biochemical reactivity of antineoplastic agent aiming at rationalization of the action of drug in order to allow the design of new active molecules.

STUDY DESIGN:

- Selection of cancer drug target (PDB ID-**3RE2**)
- Selection of ligands.
- Assesment of drug like properties (ADMET).
- Docking studies of drug molecules against cancer drug target.

MATERIAL & METHOD:

Present study I have used bioinformatics tool.Bilogical data base like (PDB-PREOTEIN DATA BANK) & some softwares like HEX,.Hex is an intermolecular graphic programme for calculating & displaying feasible docking modes of pair of protein & DNA molecules & can show protein[3] ligand docking.PDB is a single world widearcheive of structural[7] data & biological macromolecule,established in *Brookheaven National Laboratories*.It contain structural info of macromolecules determined by X-Ray crystallographic NMR method.Molinspirationis a molecular editor programme for creating & modifying representation of chemical structure[6].In this I have checked

Lipnski rule of 5 of drug.

- Molecular weight must be less than 500
- No of OH & NH doner must be less than 5
- No of H accepter (-N,-O,-S)must be less than 10.
- Log P must be less than 5.

METHODS: IMAGE OF RECEPTOR (PDB ID-3RE2)

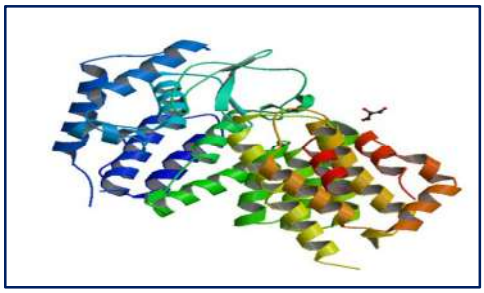
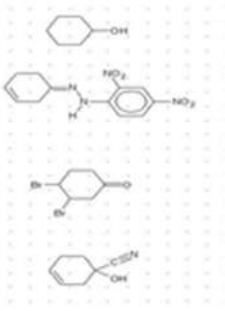


Figure – 1 IMAGE OF RECEPTOR (PDB ID-3RE2)[12]

3RE2

The structure of RECEPTOR (PDB ID-3RE2) which can be essential target for novel anticancer drug like SUNITINIB[11] was retrieved from RCSB PDB(3RE2).Using Chemsketch the structures of these drugs were sketched and by using Open Bable GUI we convert the cdx file to PDA file. The docking analysis of thecompound with3RE2 was carried by using HEX version 5.1 docking software.

1.**CHEMSKETCH**:It is a type of software in which we can sketch & can find IUPAC name of our chemical structure .[13]



2.**MOLINSPIRATION:**I have used this software to know the **ADMET** data of Marketed drug **SUNITINIB**.[11]

ADMET DATA OF MARKETED DRUG SUNITINIB

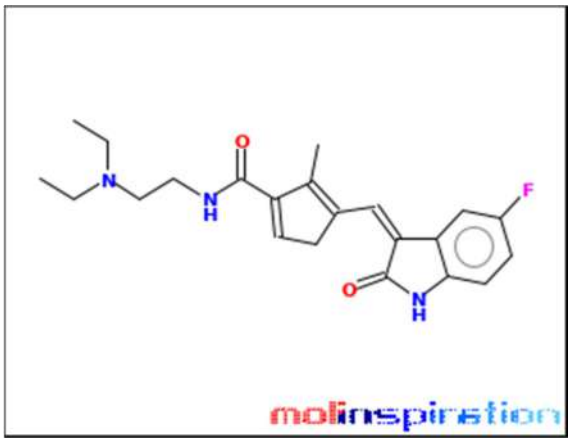


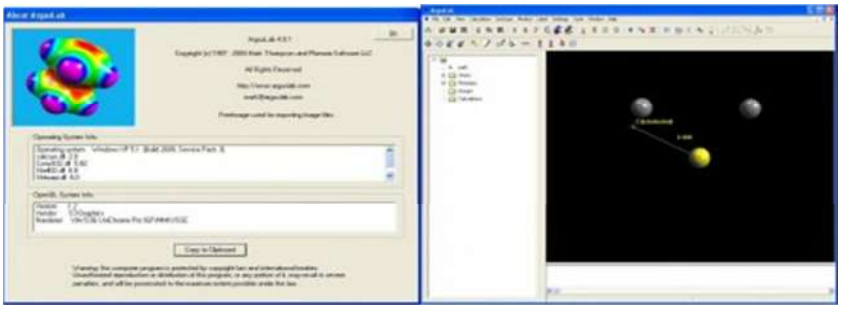
Figure 2: **ADMET DATA OF MARKETED DRUG SUNITINIB**[11]

Molinspiration propertyengine[14] v2018.10

miLogP 2.19
TPSA 65.20
natoms 28
MW 383.47
nON 5
nOHNH 2
nviolations 0
nrotb 7
volume 358.79

SUNITINIB

3. **ARGUSLAB** : It is a program to build graphic representations of molecular models. Using this program, we will be able to show molecular models to pupils& design matters by combining different elements. We will be able to include in our model several atoms, residues, groups and calculations.[15]

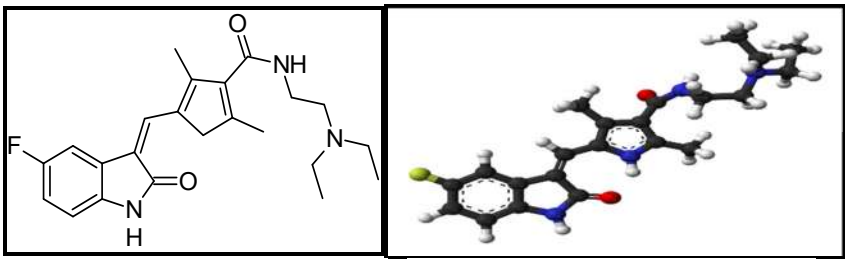


4.HEX version 5.1: It is a software in which both **Protein&drug molecule** are docking & giving result for the required data.

HEX version 5.1

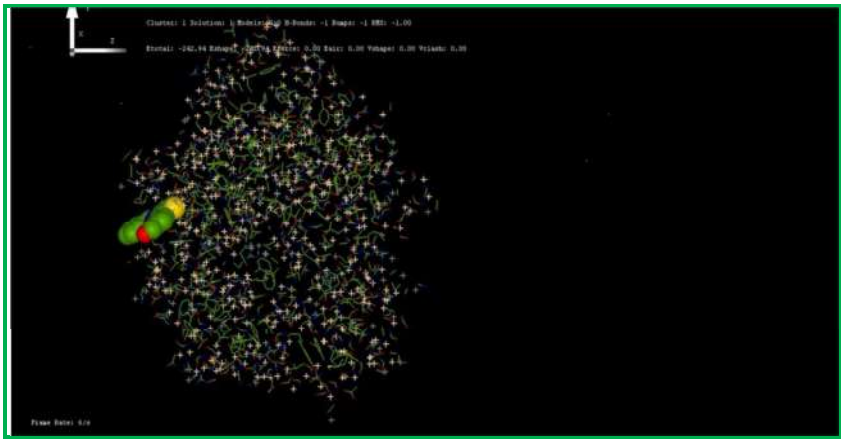
RESULT

STRUCTURE OF SUNITINIB



(Skeletal formula of sunitinib)**(**Ball-and-stick model of sunitinib)

DOCKING OF MARKETED DRUG SUNITINIB WITH 3RE2 PROTEIN



DOCKING 3RE2-SUNITINIB (-242.94)

DISCUSSION

Menin is a tumor suppressor protein[3] that is encoded by the MEN1 (multiple endocrine neoplasia 1) gene and controls cell [8]growth in endocrine tissues.Menin[12] also serves as a oncogenic cofactor of MLL (mixed lineage leukemia) fusion proteins in acute leukemias. Direct association of menin with MLL fusion proteins is required for MLL fusion protein-mediated leukemogenesis in vivo, and theinteraction has been validated as a new potential therapeutic target for development of novel anti-leukemia agents.[9] Here, we have seen theCADD methodology& docking study of RECEPTOR (3RE2)with SUNITINIB.It also provides essential structural information for development of inhibitors targeting the menin-MLL interaction as a novel therapeutic strategy in MLL-related leukemias.Here I have taken a marketed anticancer drug SUNITINIB & docked with 3RE2 protein to see the effect of drug.After docking a result came with energy value -242.94.Further research & development are under process.

Acknowledgements

Tejaswini Kumari Dash would like to express her sincere thanks to Biju Patnaik University of Technology,ODISHA authorities and Dr. A. Srinivasa Rao,Professor and Principal, Bhaskar Pharmacy College, Hyderabad for their encouragement and help during the research work.

References

1. Maojiang Wu, Qiming Sun, Chunhao Yang, Dongdong Chen, Jian Ding, Yi Chen, Liping Lin, YuyuanXie.Synthesis and activity of Combretastatin A-4 analogues: 1,2,3-thiadiazoles as potent antitumor agents.Bioorganic& Medicinal Chemistry Letters.Volume 17, Issue 4, 15 February 2007, Pages 869-873.
2. Joel R. Walker, Galal Alshafie, Hussein Abou-Issa, Robert W. Curley Jr.An improved synthesis of the *C*-linked glucuronide of *N*-(4-Hydroxyphenyl)retinamide.Bioorganic& Medicinal Chemistry Letters.Volume 12, Issue 17, 2 September 2002, Pages 2447-2450.
3. Nobutoshi Murakami, Satoru Tamura, Kouhei Koyama, Masanori Sugimoto, Ryuji Maekawa, MotomasaKobayashiNew analogue of arenastatin A, a potent cytotoxic spongeandepsipeptide, with anti-tumor activity.Bioorganic& Medicinal Chemistry Letters.Volume 14, Issue 10, 17 May 2004, Pages 2597-2601.
4. Izabela Brudziñska, Yuji Mikata, Makoto Obata, ChikaraOhtsuki, Shigenobu Yano
Synthesis, structural characterization, and antitumor activity of palladium(II) complexes containing a sugar unit.Bioorganic& Medicinal Chemistry Letters. Volume 14, Issue 10, 17 May 2004, Pages 2533-2536.
5. Alex W. White, Nicola J. Curtin, Brian W. Eastman, Bernard T. Golding, ZdenekHostomsky, Suzanne Kyle, Jianke Li, Karen A. Maegley, Donald J. Skalitzky, Stephen E. Webber, Xiao-Hong Yu, Roger J. GriffinPotentiation of cytotoxic drug activity in human tumour cell lines, by amine-substituted 2-arylbenzimidazole-4-carboxamide PARP-1 inhibitors.Bioorganic& Medicinal Chemistry Letters.Volume 14, Issue 10, 17 May 2004, Pages 2433-2437.
6. Jacek W. Morzycki, Agnieszka Wojtkielewicz, SławomirWołczyńskiSynthesis of analogues of a potent antitumor saponin OSW-1.Bioorganic & Medicinal Chemistry Letters.Volume 14, Issue 12, 21 June 2004, Pages 3323-3326.
7.Kwai-Ming J. Cheung, Thomas P. Matthews, Karen James, Martin G. Rowlands, Katherine J. Boxall, Swee Y. Sharp, Alison Maloney, S. Mark Roe, ChrisostomosProdromou, Laurence H. Pearl, G. Wynne Aherne, Edward McDonald, Paul WorkmanThe identification, synthesis, protein crystal structure and in vitro biochemical evaluation of a new 3,4-diarylpyrazole class of Hsp90 inhibitors.Bioorganic& Medicinal Chemistry Letters.Volume 15, Issue 14, 15 July 2005, Pages 3338-3343.
8. Mao-Cai Yan, Yang Liu, Hong Chen, Ying Ke, Qing-Chun Xu, Mao-Sheng ChengSynthesis and antitumor activity of two natural *N*-acetylglucosamine-bearing triterpenoid saponins: Lotoidoside D.Bioorganic & Medicinal Chemistry Letters.Volume 16, Issue 16, 15 August 2006, Pages 4200-4204.
9. Chang-Ming Sun, Lee-Gin Lin, Hsi-Jung Yu, Chih-Yu Cheng, Ya-Chuan Tsai, Chi-Wei Chu, Yi-Hui Din, Yat-Pang Chau, Ming-Jaw DonSynthesis and cytotoxic activities of 4,5-diarylisoxazoles.Bioorganic & Medicinal Chemistry Letters.Volume 17, Issue 4, 15 February 2007, Pages 1078-1081.
10.Pingping Tang, Fatemah Mamdani, Xiaoyi Hu, Jun O. Liu, Biao YuSynthesis of OSW saponin analogs with modified sugar residues and their antiproliferative activities.Bioorganic& Medicinal Chemistry Letters.Volume 17, Issue 4, 15 February 2007, Pages 1003-1007.
11.Sunitib structure , From Wikipedia, the free encyclopedia
12.3RE2 receptor ,RCSB Protein Data Bank,Crystal structure of Menin Reveals Binding site for Mixed Lineage Leukemia (MLL)J.Biol.chem.
13.chemsketch,ACD Labs.
14.Molinspiration,chem informatics software.
15. ArgusLab,A Molecular modeling graphics.

SP-28

A REVIEW ON CURRENT TRENDS IN INSULIN THERAPY: SCOPE AND FUTURE

C. PALLAVIGOUD, M. PALLAVI, DR. SRINIVASRAO, DR. KISHORE BABU
Department of Pharmacy Practice, Bhaskar Pharmacy College, Hyderabad- 500075, India.

ABSTRACT:
Diabetes Mellitus is a metabolic disorder characterized by elevated blood glucose levels.All patients with T1DM and Many patients with advanced type 2 diabetes mellitus (T2DM) require insulin to maintain blood glucose levels in the normal range. insulin injections via subcutaneous route are most commonly used and it is the standard route of administration but it isassociated with injection pain, needle phobia, lipodystrophy, noncompliance and peripheral hyperinsulinemia.To overcome thenoncompliance, various alternative and noninvasive insulin delivery methods have been developed

KEY WORDS: Diabetes Mellitus, Hyperinsulinemia, noninvasive insulin delivery.

INTRODUCTION:

Diabetes mellitus prevalence is increasing worldwide and insulin is necessary to maintain the blood glucose levels in the normal range in diabetes patients by various insulin delivery methods [1,2]. The most commonly used method of insulin administration is via subcutaneous routes such as vials, syringes, insulin pens and pumps. Alternative insulin delivery systems that are non invasive are currently available for the administration of insulinwhich includesoral, nasal,buccal, mucosal, transdermal, nasal, peritoneal. All these delivery systems are developed to provide predictable and effective lowering of glucose levels in the blood. [3,4]This review focuses on various insulin delivery techniques with its advantages and disadvantages in the management of Diabetes.

INSULIN DELIVERY TECHNIQUES: Insulin is administered through subcutaneous route by using vials, syringes and pens.[5]

CURRENT TRENDS IN INSULIN THERAPY: Vials, syringes, and insulin pens are currently used for delivering insulin among which insulin pens are user friendly and are less painful. Insulin pumps are also availablein current generation which is more user friendly with calculated doses and alarms.[6]

Sensor–augmented pump therapy:sensor-augmented pump therapy includesCGM (continuous Glucose Monitors) readings toadjust insulin delivery through insulin pump. SAP requires patient involvement for using CGM readings to adjust insulin pump delivery and patient to wake up to manage nocturnal hypoglycemia. It is designed to reduce the severity and duration of hypoglycemia.[7,8,9]

Advancements in insulin therapies:
Injectable Insulins:

Insulin degludec and VIAject: Insulindegludec is an ultra-long acting insulin used to reduce the blood glucose levels in diabetic patients.it has single amino acid[threonine] deleted from B-chain in the position 30 in comparison to human insulin, and is conjugated to hexadecanedioic acid via glutamic acid linker to lysine in the position 29 of beta chain[10] .It was developed by NovoNordisk under the brand name Tresiba.VIAject is an ultra fast acting insulin that absorbs more rapidly and it is used to reduce the postprandial oxidative stress and improves the endothelial functions. [11,12]

NEW TECHNIQUES TO DELIVER INSULIN:
1.INHALED INSULIN DELIVERY:

Inhaled insulin delivery is used to reduce the blood glucose levels by inhaling the aerosols in the emergency conditions like diabetic ketoacidosis. Advantages include more bioavailability and it avoids first pass metabolism [13]. EXUBERA is the First inhaled product approved by US FDA in 2006[14]AFREZZA is a powdered form of insulin which is delivered with an inhaler into the lungs where it is absorbed. Its onset of action is 15 minutes.[15]

2. ORAL INSULIN DELIVERY: Oral insulin delivery is more comfortable to the patients when compared to other delivery methods. It improves the glycemic control by using PLGA (Poly, lactic-co-glycolic-Acid). However, challenges remain. Bioavailability through the oral route is low due to its larger size, hydrophilicity and inactivation in the GI tract by proteolytic enzymes [16]. Hence certain formulations have been developed to improve the insulin bioavailability through oral delivery such as capsulin, IN-105, ORMD-0801[17]. A new approach to improve the insulin bioavailability is by combining the SNEDDS (Self Nano Emulsifying Drug Delivery Systems) and thiolated chitosan.[18]
Advantages: It is easy and convenient and has good patient compliance.
Limitations: It has low bioavailability and insulin passes through hepatic metabolism.[19]

3.BUCCAL INSULIN DELIVERY: Mucosal insulin delivery is less effective than parenteralroute due to the metabolism of insulin in the mucosal membrane and poor permeability. However, insulin delivery through buccal route has been emerged as an effective delivery site in recent years. The drug is delivered with the help of a spray into the buccal cavity where it gets absorbed and enters into the systemic circulationvia jugular vein and this route bypasses the first pass metabolism. [20]
Advantages: It avoids the destruction of acid labile insulin in the stomach, Eliminates the injection pain and improves the patient compliance and it also shows low enzymatic activity.
Limitations: It has small absorption sites and there is a chance of swallowing the delivery systems accidentally.
ora-lyn is a liquid formulation of human insulin that directly delivered into the buccal mucosa. It is the only buccal formulation marketed tillnow. It is formulated by Generex Biotechnology corporation using metered dosage applicator.[21]

4.NASAL INSULIN DELIVERY:
Intra nasal insulin delivery is most widely developed as an alternative to subcutaneous route its pharmacokinetics is similar to IVinjections. This method of insulin delivery is mostly used for postprandialhyperglycemiafor treating NIDDM and IDDM
Advantages: it has high vascularity and large absorptive surface
Limitations: insulin through this route is degraded by enzymes and has low bioavailability and has mucociliary clearance and absorption is not consistent.[22]
NASULIN was formulated by nastech pharmaceuticals company Inc. Its onset of action is about 10-15% with the bioavailability of 15-25%.Nasal insulin has effect on memory function (improves memory and preserves the cognition) as it crosses BBB.[23]

5.TRANSDERMAL INSULINDELIVERY:
Transdermal insulin delivery is the most attractive alternate to subcutaneous route of insulin as it is needle free.[24]

Advantages: it prevents insulin degradation and offers sustained release of insulin required by the patients and has large absorptive surface and high vascularity, Iontophoresis and sonophoresis techniques are usedand micro needle approach is used toincrease the insulin permeability. It has good patient compliance and it isconvenient to the patient.

Limitations: skin is relatively impermeable to insulin due to its large molecular weight and it also varies in dosing.[25]

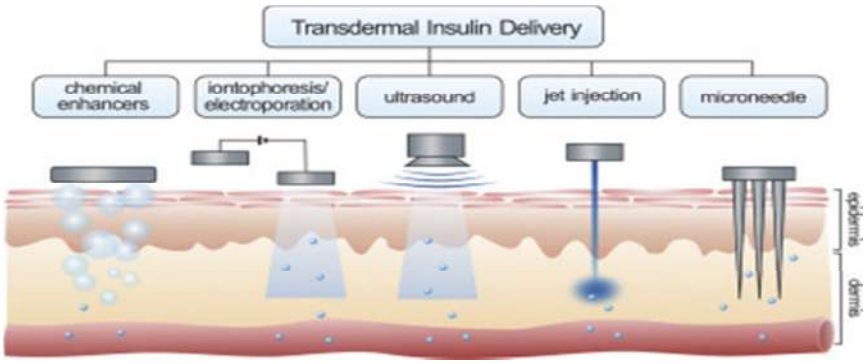


Figure –1 Transdermal Insulin Delivery[3]

6.ARTIFICIAL PANCREAS:

Artificial pancreas is developed to automatically and continuously monitor the blood glucose levels in diabetes patients mostly in type 1DM.These artificial pancreases includes continuous glucose sensors [26]. This is a physiological approach to provide regulation of blood glucose levels. Artificial pancreas uses insulin pumps under closed loop control system. Artificial pancreas will mimic the secretion of insulin and glucagon and changes the glucose levels in the blood stream. The main goal of this artificial pancreas is to maintain normal blood glucose levels by avoiding the risk of hypoglycemia[27]

CONCLUSION:

Current methods in insulin delivery has some disadvantages. In order to avoid these limitations many advancements in the insulin delivery methods are madeto improve the efficacy and to provide the predictable glycemic control. Such as insulin degludec is a long acting insulin and it is given less frequently and VIAject is a fast-acting insulin used in post prandial glycemic control. Inhaled insulin delivery has more bioavailability, oraland buccal insulin delivery is easy and patient friendly, nasal insulin delivery is mostly used for post prandial hyperglycemia. Needle free transdermal insulin delivery is an attractive alternative to subcutaneous insulin delivery. Artificial pancreas will continuously monitor and automatically regulates the blood glucose levels by avoiding the risk of hypoglycemia.

REFERENCES:

1.IDF Diabetes Atlas. 6th ed. Brussels, Belgium: International Diabetes Federation; 2013. [Last accessed on 2013 Sep 16]. The Global Burden. International Diabetes Federation
2.Rosenfeld L. Insulin: discovery and controversy. Clin Chem. 2002;48:2270–2288.
3.Martin AL, Martin MM. Continuous infusion of insulin vs repeated S.C. injections in the treatment of diabetic ketoacidosis in children. Acta Diabetol Lat. 1978;15:81–87.
4.Slama G, Hautecouverture M, Assan R, Tchobroutsky G. One to five days of continuous intravenous insulin infusion on seven diabetic patients. Diabetes. 1974;23:732–738.
5.The history of injecting and the development of the syringe. Exchange supply tools for harm reduction.[last accessed on 2013 sep 16]
6.Baruah MP. Insulin pens: the modern delivery devices. J Assoc Physicians India. 2011;59 Suppl:38–40.
7.Moser EG, Morris AA, Garg SK. Emerging diabetes therapies and technologies. Diabetes Res Clin Pract. 2012;97:16–26.
8.Garg SK, Voelmle MK, Beatson CR, Miller HA, Crew LB, Freson BJ, et al. Use of continuous glucose monitoring in subjects with type 1 diabetes on multiple daily injections versus continuous subcutaneous insulin infusion therapy: A prospective 6-month study. Diabetes Care. 2011;34:574–9.
9.Garg SK. Role of continuous glucose monitoring in patients diabetes using multiple daily insulin injection. Infusystems Int. 2009;8:18–21.
10.Danne T, Bolinder J. New insulins and insulin therapy. Int J Clin Pract Suppl. 2011;(170):26–30.
11.Hompesch M, McManus L, Pohl R, Simms P, Pfützner A, Bülow E, Flacke F, Heinemann L, Steiner SS. Intra-individual variability of the metabolic effect of a novel rapid-acting insulin (VIAject) in

12.Steiner S, Hompesch M, Pohl R, Simms P, Flacke F, Mohr T, Pfützner A, Heinemann L. A novel insulin formulation with a more rapid onset of action. Diabetologia. 2008;51:1602–1606.
13.Gänsslen M. Über inhalation von insulin. KlinWochenschr. 1925;4:71.
14FDA Approved Drug Products. [Last accessed on 2013 Oct 27].
15.Richardson PC, Boss AH. Technosphere insulin technology. Diabetes Technol Ther. 2007;9(Suppl 1):S65–72.
16.Oral Delivery of Insulin: Novel Approaches. [Last accessed on 2013 Nov 10].
17.Soares S, Costa A, Sarmento B. Novel non-invasive methods of insulin delivery. Expert Opin Drug Deliv. 2012;9:1539–58.
18.Sakloetsakun D, Dünnhaupt S, Barthelmes J, Perera G, Bernkop-Schnürch A. Combining two technologies: Multifunctional polymers and self-nanoemulsifying drug delivery system (SNEDDS) for oral insulin administration. Int J Biol Macromol. 2013;61:363–72.
19.Wajcberg E, Miyazaki Y, Triplitt C, Cersosimo E, DeFronzo RA. Dose-response effect of a single administration of oral hexyl-insulin monoconjugate 2 in healthy nondiabetic subjects. Diabetes Care. 2004;27(12):2868–2873.
20.Al-Waili NS. Sublingual human insulin for hyperglycaemia in type I diabetes. JPMA. 1999;49(7):167–169.
21.Heinemann L, Jacques Y. Oral insulin and buccal insulin: A critical reappraisal. J Diabetes Sci Technol. 2009;3:568–84.
22.Yaturu S. Insulin therapies: Current and future trends at dawn. World J Diabetes. 2013;4:1–7.
23.Salzman R, Manson JE, Griffing GT, Kimmerle R, Ruderman N, McCall A, et al. Intranasal aerosolized insulin. Mixed-meal studies and long-term use in type I diabetes. N Engl J Med. 1985;312:1078–84.
24.Prausnitz MR, Langer R. Transdermal drug delivery. Nat Biotechnol. 2008;26:1261–8.
25.Kanikkannan N. Iontophoresis-based transdermal delivery systems. BioDrugs. 2002;16:339–447.
26.Mastrototaro J. The MiniMed Continuous Glucose Monitoring System (CGMS) J Pediatr Endocrinol Metab. 1999;12 Suppl 3:751–758.
27.Cobelli C, Renard E, Kovatchev B. Artificial pancreas: past, present, future. Diabetes. 2011;60:2672–2682.

SP-29

TOPICAL APPLICATIONS OF RETINOIDS IN DERMATOLOGY

PADMAJA KORE*, SHRADDHA MAKESHWAR[1]
P.E. Society's Modern College of Pharmacy, Yamunanagar, Nigdi, Pune-44, India

ABSTRACT:
Retinoids are derivatives of vitamin A, found in both natural and synthetic from. Based on the molecular structure it is classified into three generation. Retinoids have been extensively studied worldwide. Retinoid is used to treat many skin conditions from acne to photoaging. The use of retinoids is done topically and systemically. Retinoids are used in cancer treatment and prevention. This article is a review of retinoid, its introduction, classification, and topical applications of retinoid in dermatology. Skin conditions such as acne vulgaris, rosacea, Psoriasis, Lichen planus, Ichthyosis, Darier's diseases, Skin aging and Skin pigmentation are been covered in the review.

Key words: Retinoid, isotretinoin, tretinoin, alitretinoin.

INTRODUCTION:

Retinoid were introduced to dermatology in 1980s. The pleiotropic effects of retinoids are been reported for its selective targeting.[1,2] The uses of retinoid in the dermatological field have been increased after its introduction due to the selective targeting of the certain skin structure, resulting in continuous broadening of the therapeutic range. The understanding of the retinoid function was cleared in late 1980s and early 1990s, after the identification of the retinoid binding protein.[3, 4] After the introduction to retinoids, it is been extensively used in for systemic and topical treatments in various diseases. Retinoids are naturally found as well as available in synthetic forms showing specific biological effects.[5]

Retinol (vitamin A) and retinoids:

Vit A has two fat soluble unsaturated isoprenoids, retinaldehyde and retinoic acid which are necessary for growth and differentiation of epithelial tissues.[6] Unlike retinol, retinoids are derivatives of retinol and involved in the pathogenesis of certain skin diseases such as acne and psoriasis.[6-8]

Classification of retinoids:

Retinoids is the derivative of vit A and classified into three generations depending upon the three substitution in its structural domain. The first-generation retinoids are naturally occurring non- aromatic retinoid which retained the cyclic structure of vit A; including retinol, retinal, isotretinoin, tretinoin and alitretinoin.[9] Second generation of retinoids has cyclic changes in the ring and are monoaromatic compound; it includes etretinate, motretinate and acitretin.[10, 11] Third generation of retinoids include adapalene, tazarotene and bexarotene

Dermatological application

Acne vulgaris

Acne vulgaris is the common skin problem. Retinoids are used in the treatment of the of acne vulgaris and related disorders as retinoids are comedolytic and anti- comedogenic. Retinoids are effective in both inflammatory and non- inflammatory acne treatment by normalizing keratinization and immunomodulation. The retinoids also have preventive effects. However, after the clearance of the acne continuation retinoid prevent acne reoccurrence.[12]Tretinoin, adapalene and tazarotene are the retinoids that are topically used in the treatment of acne vulgaris; where adapalene is more effective and stable than tretinoin.[13]

Rosacea

Rosacea is another skin condition similar to the acne vulgaris, it is the condition that causes the redness and little bumps filled with pus. Topical application of retinoid helps in the treatment of rosacea. According to the research adapalene has found to be effective in treating rosacea and reducing inflammatory

papules.[14]Later studies showed retinoids are more effective when used with metronidazole. Topical application of clindamycin with tretinoin helped reduction of erythema and telangiectatic.[15]

Psoriasis: Along with the anti-inflammatory and immunomodulatory properties retinoids also induce cell differentiation and cell proliferation inhibition. Also, retinoid modulate T- cells and polymorphonuclear cell and inflammatory cytokines expression.[16, 17]
Daily application of retinoid (Tazarotene) once in the evening help in the treatment of the psoriasis (condition that causes building of skin cells and forming scales and itching dry patches).[18]

Lichen planus: Retinoids are recommended by the World Workshop in Oral Medicine IV for the treatment of the oral lichen planus (OLP). As per the studies retinoids are used to treat both keratotic and erosive OLP.[19] Retinoids are not used in cutaneous lichen planus (CLP), but combination of tretinoin and azelaic acid are used topically to treat lichen planus pigmentaosus and facial dyspigmentation due to the peeling effect.[20]

Ichthyosis: Ichthyosis is a skin condition characterized by dry, scaly and thick skin. The retinoid promotes keratolysis and cell differentiation. Tretinoin, adapalene and tazarotene are used in the treatment of the ichthyosis. Along with ichthyosis retinoids also have positive effects on ectropion and pseudoainhum.[21]

Darier's diseases: Retinoid are found to be effective in the Darier's diseases both localized and generalized diseases.[22] Tazarotene had shown to have positive effects in some cases and no effect on other. However, adapalene is been reported to have potential effects in the localized Darier's diseases.[22]

Skin aging:A beautiful, flawless youthful skin is everyone's desire. However, skin aging is a huge hurdle as it causes wrinkles, pigmentation and loss of skin elasticity making it look dull. The 1984, research studies demonstrated that retinoid have efficient potential in skin aging and ever since retinoid are used in the treatment and prevention of photoaging in skin. Retinoid enhances collagen production suppresses collagen degradation and promote epidermal thickening. Also, retinoids increase elastin and fibrillin content in skin along with decreased level of melanin.It promotes angiogenesis and suppress atypia.[9] Tretinoin and tazarotene used reported and approved for the treatment of photoaging skin. According to the clinical manifestation retinoid use showed smother skin with lesser wrinkle lighten pigmentation and increased elasticity.

Premalignant and malignant lesions
Retinoid is widely recognized for the prevention and treatment of cancer. Retinoid induce cell differentiation and apoptosis also, inhibition of cell proliferation.[23]The retinoids are used for the use in treatment and prevention. Isotretinoin is used in Actinic keratosis[24], tretinoin in squamous cell carcinoma[25], tazarotene in basal cell carcinoma[26], bexarotene in cutaneous T-cell lymphoma[27], alitretinoin in Kaposi sarcoma[28] and isotretinoin in Muir- Torre syndrome.[29]

Skin pigmentation
Retinoid have potential effects on hyperpigmentation. Tropical application of tretinoin showed reduced epidermal melasma, actinic lentigines and superficial post-inflammatory hyperpigmentation due to keratolysis and cell differentiation.[3]

CONCLUSION: In conclusion, retinoid has a various potential therapeutic applications for various dermatological problems, and can be used as a potential target for various skin diseases.

References:

1. Millikan, L.E., 2016. *Drug therapy in dermatology*. CRC Press.
2. Orfanos, C.E., Zouboulis, C.C., Almond-Roesler, B. and Geilen, C.C., 1997. Current use and future potential role of retinoids in dermatology. *Drugs*, *53*(3), pp.358-388.
3. Korting, H.C. ed., 2001. *TherapeutischeVerfahren in der Dermatologie: Dermatika und Kosmetika; 124 Tabellen*. Blackwell-Wiss.-Verlag.

4. Orfanos, C.E., 1995. Retinoide–Wirksamkeit und Verträglichkeit der systemischenTherapie. *Dermatologie. Heutiger Stand. Stuttgart/New York, Thieme*, pp.301-308
5. IUPAC-IUB Joint Commission on Biochemical Nomenclature. Eur J Biochem 1982; 129: 1-5
6. Safavi, K., 1992. Serum vitamin A levels in psoriasis: Results from the first national health and nutrition examination survey. *Archives of dermatology*, *128*(8), pp.1130-1131.
7. Tang, G.W. and Russell, R.M., 1990. 13-cis-retinoic acid is an endogenous compound in human serum. *Journal of lipid research*, *31*(2), pp.175-182.
8. Matsuoka, L.Y., Wortsman, J., Tang, G., Russell, R.M., Parker, L., Gelfand, R. and Mehta, R.G., 1991. Are endogenous retinoids involved in the pathogenesis of acne?. *Archives of dermatology*, *127*(7), pp.1072-1073.
9. Mukherjee, S., Date, A., Patravale, V., Korting, H.C., Roeder, A. and Weindl, G., 2006. Retinoids in the treatment of skin aging: an overview of clinical efficacy and safety. *Clinical interventions in Aging*, *1*(4), p.327.
10. Wiegand, U.W. and Chou, R.C., 1998. Pharmacokinetics of acitretin and etretinate. *Journal of the American Academy of Dermatology*, *39*(2), pp.S25-S33.
11. Briggs, G.G., Freeman, R.K. and Yaffe, S.J., 2012. *Drugs in pregnancy and lactation: a reference guide to fetal and neonatal risk*. Lippincott Williams & Wilkins.
12. Gollnick, H., Cunliffe, W., Berson, D., Dreno, B., Finlay, A., Leyden, J.J., Shalita, A.R. and Thiboutot, D., 2003. Management of acne: a report from a Global Alliance to Improve Outcomes in Acne. *Journal of the American Academy of Dermatology*, *49*(1), pp.S1-S37.
13. Martin, B., Meunier, C., Montels, D. and Watts, O., 1998. Chemical stability of adapalene and tretinoin when combined with benzoyl peroxide in presence and in absence of visible light and ultraviolet radiation. *Br J Dermatol*, *139*(Suppl 52), pp.8-11.
14. Altinyazar, H.C., Koca, R., Tekin, N.S. and Eştürk, E., 2005. Adapalene vs. metronidazole gel for the treatment of rosacea. *International journal of dermatology*, *44*(3), pp.252-255.
15. Chang, A.L., Alora-Palli, M., Lima, X.T., Chang, T.C., Cheng, C., Chung, C.M., Amir, O. and Kimball, A.B., 2012. A randomized, double-blind, placebo-controlled, pilot study to assess the efficacy and safety of clindamycin 1.2% and tretinoin 0.025% combination gel for the treatment of acne rosacea over 12 weeks. *Journal of drugs in dermatology: JDD*, *11*(3), pp.333-339.
16. Carretero, G., Ribera, M., Belinchón, I., Carrascosa, J.M., Puig, L., Ferrandiz, C., Dehesa, L., Vidal, D., Peral, F., Jorquera, E. and Gonzalez-Quesada, A., 2013. Guidelines for the use of acitretin in psoriasis. *ActasDermo-Sifiliográficas (English Edition)*, *104*(7), pp.598-616.
17. Blair, H.A. and Scott, L.J., 2016. Alitretinoin: a review in severe chronic hand eczema. *Drugs*, *76*(13), pp.1271-1279.
18. Weinstein, G.D., Koo, J.Y., Krueger, G.G., Lebwohl, M.G., Lowe, N.J., Menter, M.A., Lew-Kaya, D.A., Sefton, J., Gibson, J.R., Walker, P.S. and Group, T.C.C.S., 2003. Tazarotene cream in the treatment of psoriasis: two multicenter, double-blind, randomized, vehicle-controlled studies of the safety and efficacy of tazarotene creams 0.05% and 0.1% applied once daily for 12 weeks. *Journal of the American Academy of Dermatology*, *48*(5), pp.760-767.
19. Petruzzi, M., Lucchese, A., Lajolo, C., Campus, G., Lauritano, D. and Serpico, R., 2013. Topical retinoids in oral lichen planus treatment: an overview. *Dermatology*, *226*(1), pp.61-67.
20. Wolff, M., Sabzevari, N., Gropper, C. and Hoffman, C., 2016. A case of lichen planus pigmentosus with facial dyspigmentation responsive to combination therapy with chemical peels and topical retinoids. *The Journal of clinical and aesthetic dermatology*, *9*(11), p.44.
21. DiGiovanna, J.J., Mauro, T., Milstone, L.M., Schmuth, M. and Toro, J.R., 2013. Systemic retinoids in the management of ichthyoses and related skin types. *Dermatologic therapy*, *26*(1), pp.26-38. Cooper, S.M. and Burge, S.M., 2003. Darier's disease. *American journal of clinical dermatology*, *4*(2), pp.97-105.
22. Cooper, S.M. and Burge, S.M., 2003. Darier's disease. *American journal of clinical dermatology*, *4*(2), pp.97-105.
23. Niles, R.M., 2000. Recent advances in the use of vitamin A (retinoids) in the prevention and treatment of cancer. *Nutrition*, *16*(11-12), pp.1084-1089.
24. Ianhez, M., Fleury Junior, L.F.F., Miot, H.A. and Bagatin, E., 2013. Retinoids for prevention and treatment of actinic keratosis. *Anais brasileiros de dermatologia*, *88*(4), pp.585-593.

25. Weinstock, M.A., Bingham, S.F., DiGiovanna, J.J., Rizzo, A.E., Marcolivio, K., Hall, R., Eilers, D., Naylor, M., Kirsner, R., Kalivas, J. and Cole, G., 2012. Tretinoin and the prevention of keratinocyte carcinoma (Basal and squamous cell carcinoma of the skin): a veterans affairs randomized chemoprevention trial. *Journal of Investigative Dermatology*, *132*(6), pp.1583-1590.
26. Bianchi, L., Orlandi, A., Campione, E., Angeloni, C., Costanzo, A., Spagnoli, L.G. and Chimenti, S., 2004. Topical treatment of basal cell carcinoma with tazarotene: a clinicopathological study on a large series of cases. *British journal of dermatology*, *151*(1), pp.148-156.
27. Heald, P., Mehlmauer, M., Martin, A.G., Crowley, C.A., Yocum, R.C., Reich, S.D. and Worldwide Bexarotene Study Group, 2003. Topical bexarotene therapy for patients with refractory or persistent early-stage cutaneous T-cell lymphoma: results of the phase III clinical trial. *Journal of the American Academy of Dermatology*, *49*(5), pp.801-815.
28. Walmsley, S., Northfelt, D.W., Melosky, B., Conant, M., Friedman-Kien, A.E. and Wagner, B., 1999. Treatment of AIDS-related cutaneous Kaposi's sarcoma with topical alitretinoin (9-cis-retinoic acid) gel. Panretin Gel North American Study Group. *Journal of acquired immune deficiency syndromes (1999)*, *22*(3), pp.235-246.
29. Spielvogel, R.L., DeVillez, R.L. and Roberts, L.C., 1985. Oral isotretinoin therapy for familial Muir-Torre syndrome. *Journal of the American Academy of Dermatology*, *12*(3), pp.475-480.

SP-30

SELF MEDICATION: RISKS, BENEFITS AND THE ROLE OF PHARMACIST

MADHAVI M *, SOHAN S THIPE, SHEFALI P NETKE, MUSKAN DOSANI,TUSHAR K SAMBRE.

Yashodhara bjaj colleg of pharmacy,mul road, Bangali camp,Chandrapur, Maharashtra, 442401.
Email-Id:madhumartha@gmail.com

ABSTRACT

Self-medication is the selection and use of medicines by individuals to treat self-recognized or self-diagnosed conditions or symptoms. Several benefits have been linked to appropriate self-medication, among them: increased access to medication and relief for the patient, the active role of the patient in his or her own health care and reduced (or at least optimized) burden of governments due to health expenditure linked to the treatment of minor health conditions. However, self-medication is far from being a completely safe practice, in particular in the case of non-responsible self-medication. The pharmacist in his professional capacity and in direct contact with patients can play a key role in helping people to make informed choices about self-care, in providing and interpreting the information available and in warning the patient to not to use or suggesting non-OTC drugs without consulting a doctor.

Key words:Self-medication, benefits, risks, pharmacist.

INRODUCTION

Self-medication or self-treatment is the treatment of self-diagnosed diseases, disorders or symptoms. In other words, it can be defined as intermittent or continued use of a medication that is prescribed by physician for chronic or recurrent symptoms.Medicines for self medication are often called Over the Counter (OTC) drug, which are available without a Doctor's prescription through pharmacies, mostly in the less developed countries. Self medication is different from country to country, and might be affected by nutrition, lifestyle, environmental factors, socioeconomic factors and education, law, availability of drugs and exposure to advertisements. Moreover, self medication does not mean the use of modern medicines but also of herbs. This type of habit is common from long past time.It is clear/common not only in developing countries but also in under developed countries. However, WHO is making issues telling that self-medication should always be taken in the right way and it must be controlled. [1, 2, 3] So Pharmacist can play a key role in giving advice to consumers on the proper and safe use of medicinal products intended for self-medication.[4]

REASONS OF SELF MEDICATION [5]

A number of reasons could be enumerated for self-medication like
Urge of self-care, Lack of time, Lack of health services, Financial, constraints, Ignorance, feeling of sympathy toward family members in sickness, Misbelieves, Extensive advertisement, Availability of drugs in other than drug shops

KINDS OF SELF MEDICATION

There are several kinds of self-medications which vary from culture to culture or nation to nation. Common people, in most parts of the world have strong believes in their regional traditional medicines, such as herbal medicines, acupuncture, Ayurveda and other traditional remedies which is often used as self-medication for their diseases.. Although, this kind of self-medication is legal and there is no prohibition for pharmacists to give the requested drugs but governments should aware their people about effects, side effects, cautions and interactions of these drugs by mass medias such as radio, TV or newsletter and internet.[6,7]

Table 1: List of drugs used for self-medication[8]

No.	CATEGORY	Drugs
1	Cough & Cold	Aminophylline, Camphor, Chlorphenaramine maleate, codeine phosphate, Dextromethorphan, EphedrineHcl,

		Eucalyptus oil, Menthol,XylometazolineHcl.
2.	Analgesics	Acetaminophen, Ibuprofen, Aspirin, Camphor.
3.	Antipyretics	paracetamol
4	Anti-fungal	Clotrimazole.
5	Antibiotics	Ciprofloxacin,Norfloxacin, Amoxicillin, Cefadroxil
6	Anti-microbial	Povidone iodine, Thimerosal.
7	Vitamin supplements	Vitamin A, Vitamin E, Vitamin B complex.
8	Others	DaburChyawanprash , Hajmola

BENEFITS OF SELF MEDICATION [7, 8, 9]

Expected health benefit from self medication depends on perceived effectiveness of self medication. In a world of scarce government and in many countries scarce individual resources, responsible self medication should be a cornerstone of healthcare provision and health policy. Responsible self medication can:

1. Help to prevent and treat symptoms and ailments that do not require a doctor.
2. Reduce the pressure on medical services where health care personnel are insufficient.
3. Increase the availability of health care to populations living in rural or remote areas.
4. Enable patients to control their own chronic conditions.
5. Reduce the burden of governments due to health expenditure linked to the treatment of minor health conditions.

Appropriate self-medication can cure diseases, saving time and money which would be spent on visiting doctors and even it can sometimes save the patient's life in acute conditions.

RISKS OF SELF MEDICATION[7, 8,9,10]

Self medication is associated with risks such as misdiagnosis, use of excessive drug dosage, prolonged duration of use, drug interactions and polypharmacy. Inappropriate self-medication can create a lot of problems for patients and even society. For example, self-medication with antibiotics has potential to produce harmful effects on society such as antibiotics resistance and other example is taking more of a painkiller than recommended to treat a headache. Reports have proven that Paracetamol, an antipyretic and analgesic in large doses can cause liver failure.

Inappropriate self-treatment can cause a lot of personal problems (from headache to osteoporosis, cancers and even death) and difficulties in society.

ROLE OF PHARMACIST IN SELF MEDICATION[4, 12]

The pharmacist is an adviser to the public on everyday health care and is a key figure in the supply and delivery of medicines to the consumer. He is a partner of the manufacturer of non-prescription medicines. Both share the common goals of service of high quality for the patient and encouragement of the rational use of medicines. The pharmacist in his professional capacity and in direct contact with patients is competent to provide sound advice on the medicines he supplies, Productselling. Along with these roles pharmacist have following functions

Pharmacist As a communicator[4]

- The pharmacist should initiate dialogue with the patient (and the patient's physician, when necessary) to obtain a sufficiently detailed medication history;
- To address the condition of the patient appropriately the pharmacist must askthe patientkey questions and should give information to him or her regarding how to take the medicines and how to deal with safety issues.
- The pharmacist must be prepared and adequately equipped to perform a proper screening for specific conditions and diseases, without interfering with the prescriber's authority;
- The pharmacist must provide objective information about medicines, able to use and interpret additional sources of information to fulfill the needs of the patient, able to help the patient undertake appropriate self-medication or, when necessary, refer the patient for medical advice.

Pharmacist as a trainer and supervisor.[4]

Pharmacist to be encouraged to participate in continuing professional development activities like as continuing education programs to ensure quality service with update knowledge.Often the pharmacist is

assisted by non-pharmacist staff, so it mustbe ensured that the services provided by these non-pharmacists assistants are according to the established standards of practice. To ensure this pharmacist must developprotocols for referral to the pharmacist, protocols for community health workers involved in handling and distribution of medicines. The pharmacist must give the training to non-pharmacist assistants and must supervise their work.

Pharmacist as a collaborator[4,12]

It is important thatthe pharmacistsmust develop collaborative relationships with other health care professionals, national professional associations, pharmaceutical industries, governments, patients and the general public.

Pharmacistas a health promoter[4, 12]

As one of the member of the health-care team, the pharmacist must participate in health screenings to find out the health problems and which at risk in the community;should participate in health promotion campaigns to improve the awareness of health problems and disease preventions, advice and help individuals to make informed health choices.So the Pharmacists should help the patients and coach them in using the right or even the best medication andalso, they should warn them not to use or suggesting non-OTC drugs without consulting a doctor for appropriate self medication.

CONCLUSION

We find lot of over the counter drugs all over the world, why because the correct self-medication with proper advice by pharmacist is beneficial for governments, health authorities and patients. But inappropriate self medicationwithout proper knowledge and advice can cause number of health problems (sometimes it may leads to death also) and difficulties in society (like development of resistance to antibiotics).I conclude that people, who are using it, should have sufficient knowledge about its dose, time of intake, side effect on over dose can improve safety. However, the pharmacist can play a key role in helping people to make informed choices about self-care, and in providing and interpreting the information available. This requires a greater focus on illness management and health maintenance, rather than on product selling. WHO and governments should pay special attention to this self-medication and try to change people's attitude toward it to avoid serious effects in society.

REFERENCES

1. ShadiSarahroodi, Self-medication: Risks and Benefits. Inter.J. of Pharmacology, 2012.8: 58-59.
2. Farhan H F, Alia AMohamedEltayeb, MayadahHMohamed Ahmed, AsmaNoureldaimMohamed, Shayoub M. E.Self-Medication practice among health science students in international university of Africa (IUA), Khartoum, Sudan, World Journal of Pharmaceutical Research, Apr 2016, 5(5); 61-69.
3. Bushra Ali Sherazi et al Prevalence and Measure of Self Medication: A Review. J. Pharm. Sci. & Res. Vol.4 (3), 2012, 1774-1778.
4. The Role of the Pharmacist in Self-Care and Self-Medicatio https://apps.who.int/medicinedocs/pdf/whozip32e/whozip32e.pdf
5. KandavalliSridevi, MVenkataSubbaiah, M. Surekha, JHarini, DSujana, ARaviSankar **,**Assessment of Self Medication Practices Among Community People**,** IOSR Journal of Dental and Medical Sciences, Volume 16, Issue 5 Ver. I (May. 2017), PP 75-82.
6. PratibhaNand, Roop K. Khar. 'Over the Counter Drugs' In:Hospital&Clinical Pharmacy. Birla publications, 6th edition, 2007,pg :230-234.
7. Vidyavati SD, Sneha A, Kamarudin J, Katti SM ,Self Medication - Reasons, Risks and Benefits ,International J. of Healthcare and Biomedical Research, Volume: 04, Issue: 04, July 2016, 21-24
8. FDA's OTC Active Ingredients list 2010, Available From:https://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf.
9. Ruiz ME .Risks of self-medication practices. Curr Drug Saf 2010 Oct; 5(4):315-23.
10. Hughes CM, et al., Benefits and risks of self-medication. Drug Saf. 2001; 24(14) 1027-1037.
11. Alamgir Khan, Salahuddin Khan, Syed Asif Abbas , Manzoor Khan, Health Complications Associated with Self-Medication; J Phy Fit Treatment & Sports, Volume 1 Issue 4 - January 2018.
12. Sonam Jain et al. Concept of Self Medication.Inter. J. of Pharmaceutical & Biological Archives 2011; 2(3):831-836.

SP-31

PHARMACOKINETIC ESTIMATION OF CURCUMIN HAVING HEPATOPROTECTIVE ACTIVITY

K. KATHIRESAN*, P. PANDIAN

Associate Professor, Department of Pharmacy, Faculty of Engineering and Technology, Annamalai University, Annamalai Nagar 608002, Tamil Nadu, India.
***E-mail: dr.kathiresan123@rediffmail.com**

ABSTRACT
Liver plays a major role in both physiological and biochemical functions of the body.Many disorders like hepatitis B, C, alcoholic liver disease, liver cirrhosis, hepatic failure etc., may damage the liver that due to affect variety of biochemical functions of the body. In clinical studies, drugs are the major reason to induce these disorders. The clinicians may report many drug induced hepato-toxicities and its awareness. Curcumin, the active ingredient of turmeric that may help to prevent many disorders related to cardio-vascular system, diabetes and also for various cancers. Curcumin may exhibit an effective antioxidant and anti-inflammatory property. They also have some hepatoprotective activity. The aim of the study is to estimate the hepatoprotective activity of curcumin on certain hepatotoxic agents like paracetamol, alcohol, lindane, heavy metals by improving its bioavailability through certain pharmacokinetic parameters.

Keywords: Curcumin, Hepatoprotective, Bioavailability, Antioxidant,Anti-inflammatory.

INTRODUCTION

Curcumin (diferuloylmethane) may be a yellow pigment isolated from the dried rhizomes of turmeric L (turmeric).In Asian countries, turmeric is largely used as a dietary spice[1]. A wide range of biological activities has been attributed to curcumin; however the interpretation of its experimental biological benefits into clinical trials is difficult because of the low bioavailability of this pigment when administered orally, observed in both rodents and humans, which is explained by its poor absorption because of the low solubility in water, limited tissue distribution, and rapid rate of metabolism in liver and intestine followed by the rapid excretion from the body[2]. So the low bioavailability of curcumin appears as a serious barrier to succeed in its adequate circulating levels associated with desirable pharmacodynamic actions, hindering its clinical approval as a therapeutic agent for several diseases.Attempting to overcome this situation[3], several vehicles and associations with curcumin have been tested, such as curcumin-loaded nanoparticles, complexing with phospholipids, micro emulsifying, and association with drug bio enhancers, for example Piperine, among others.

CURCUMIN METABOLISM
Indirect evidence suggests that curcumin is metabolized in the intestinal tract. Curcumin glucuronide was identified in intestinal and hepatic microsomes, and curcumin sulfate, tetra hydro curcumin, and hex hydro curcumin were found as curcumin metabolites in intestinal and hepatic cytosol from humans and rats4.

PHYTOPHARMACOLOGY OF TURMERIC
Turmeric has several therapeutic and pharmacologic activities. The following is the most vital rolephytopharmacology and therapeutic properties of turmeric.

1. ANTIOXIDANT ACTIVITY
Curcumin has been shown be a powerful scavenger of oxygen free radicals. Its antioxidant activity is comparable to vitamins C and E. It can protect lipids or hemoglobin from oxidation. It can significantly inhibit the generation of reactive oxygen species (ROS) such as H2O2, superoxide anions and nitrite radical generation by activated macrophages5. Its derivatives, bisdemethoxycurcumin and desmethoxycurcumin also have antioxidant activities.

2. CARDIOVASCULAR AND ANTI-DIABETIC EFFECTS

Turmeric exerts cardio-protective effects mainly by antioxidant activity, lowering lipid peroxidation, antidiabetic activity and inhibiting platelet aggregation6. A study of 18 atherosclerotic rabbits given 1.6-3.2 mg/kg/day of turmeric extract demonstrated decreased susceptibility of LDL to lipid peroxidation, additionally to lower plasma cholesterol and triglyceride levels. Turmeric effect on cholesterol levels may be due to decreased cholesterol uptake in the intestines and increased conversion of cholesterol to bile acids in the liver.

3. INFLAMMATORY AND EDEMATIC DISORDERS

Curcumin is a potent anti-inflammatory with specific lipoxygenase- and COX-2- inhibiting properties. In vitro and in vivo studies have demonstrated its effects at decreasing both acute and chronicinflammation7. Curcumin has inhibited edema at doses between 50 and 200 mg/kg, in mice. A 50% reduction in edema was achieved with a dose of 48 mg/kg body weight, with curcumin nearly as effective as cortisone and phenylbutazone at similar doses.

4. GASTROINTESTINAL EFFECTS

Turmeric exerts several protective effects on the alimentary canal . Turmeric also inhibits ulcer formation caused by stress, alcohol, Indomethacin, reserpine, pyloric ligation, increasing gastric wall mucus in rats subjected to thosegastrointestinal insults. It also inhibits intestinal spasm and increases bicarbonate, gastrin, secretin and pancreatic enzyme secretion8. An open, phase II clinical trial performed on 25 patients with endoscopic ally-diagnosed peptic ulcer, given 600 mg powdered turmeric five times daily, showed completely healed in 48 percent of patients. No adverse reactions or blood abnormalities were recorded. Curcumin reduced mucosal injury in mice with experimentally induced colitis. Ten days before induction of colitis, with 1, 4, 6-trinitrobenzene sulfonic acid, administration of fifty, mg/kg curcumin resulted during a significant reduction of diarrhea, neutrophil infiltration and lipid peroxidation in colonic tissue. Also all indicators inflammation was reduced and the symptoms improved.

5. ANTI-CANCER EFFECT

Numerous animal studies have explored turmeric influence on the carcinogenesis. Several studies have demonstrated that curcumin is able to inhibit carcinogenesis at three stages: angiogenesis, tumor promotion, and tumor growth9. In two studies of colon and prostate cancer, curcumin was shown to inhibit cell proliferation and tumor growth. Turmeric and curcumin are also able to suppress the activity of several common mutagens and carcinogens. The ant carcinogenic effects of turmeric and curcumin have been related to direct antioxidant and free-radical scavenging effects, as well as their ability to indirectly increase glutathione levels, thereby aiding in hepatic detoxification of mutagens and carcinogens, and inhibiting nitrosamine formation. Curcumin has also been shown to inhibit the mutagenic induction effect of UV rays.

6. ANTIMICROBIAL ACTIVITY

Turmeric has been shown to inhibit the growth of a variety of bacteria, pathogenic fungi, and parasites10. A study of chicks infected with Eimear maxima demonstrated that diets supplemented with 1% turmeric resulted during a reduction in intestinal lesion and improved weight gain. In another animal study, topically application of turmeric oil inhibited dermatophytes and pathogenic fungi in guinea pigs at 7 days post-turmeric application. Curcumin has also been found to possess moderate activity against Plasmodium falciparum and Leis mania major organisms.

HEPATOPROTECTIVE AND RENOPROTECTIVE EFFECTS OF TURMERIC

Turmeric has been shown to have Reno protective and hepatoprotective properties similar to silymarin11. Animal studies have demonstrated Reno protective and hepatoprotective effects of turmeric from a spreadof hepatotoxic insults. The hepatoprotective and Reno protective effects of turmeric are mainly thanks toits antioxidant properties, also as its ability to decrease the formation of pro-inflammatory cytokines. Turmeric and curcumin have also reversed fatty changes, biliary hyperplasia and necrosis induced by afflation production. Sodium curcuminate, a salt of curcumin, also exerts choler etic effects by increasing biliary excretion of bile salts, cholesterol, and bilirubin, also as increasing bile solubility, therefore, possibly preventing and treating cholelithiasis.

CONCLUSION

Turmeric is the unique source of various types of chemical compounds, which are responsible for a variety of activities12. Although, a lot of experiments have been done on turmeric, however, more investigations are needed to exploit other therapeutic utility to combat diseases. A drug development programme should be undertaken to develop modern drugs. Although crude extracts from leaves or rhizomes of the plant have medicinal applications, modern drugs are oftendeveloped after extensive investigation of its pharmacotherapeutics, bioactivity, mechanism of action, and toxicities, after proper standardization and clinical trials. As the global scenario is now changing towards the use of non-toxic plant products having traditional medicinal use, development of modern drugs from C. longa13 should be emphasized for the control of various diseases. Further evaluation needs to be carried out on C. longa in order to explore the concealed areas and their practical clinical applications, which can be used for the welfare of mankind.

REFERENCES

1. Ammon HP, Anazodo MI, Safayhi H, Dhawan BN, Srimal RC. "Curcumin: A potent inhibitor of leukotriene B4 formation in rat peritoneal polymorphonuclear neutrophils (PMNL)". Planta Med 1992;58:226.
2. Ammon HP, Wahl MA. "Pharmacology of Curcuma longa". Planta Med 1991;57:1-7.
3. Bernard GT, Esteban P, Christopher JS. "Turmerones: Isolation from turmeric and their structure determination". Chem Commun 1982;6:363.
4. Khajehdehi P. "Turmeric: Reemerging of a neglected Asian traditional remedy". J Nephropathol 2012; 1(1): 17-22.
5. Rao CV, Rivenson A, Simi B, Reddy BS. "Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally occurring plant phenolic compound". Cancer Res 1995;55:259-66.
6. Perkins S,Verschoyle RD, Hill K, Parveen I, Threadgill MD, Sharma RA, "Chemo preventive efficacy and pharmacokinetics of curcumin in the min/+ mouse, a model of familial adenomatous polyposis". Cancer Epidemiol Biomarkers Prev 2002;11:535-40.
7. Kwon Y, Magnuson BA. "Age-related differential responses to curcumin-induced apoptosis during the initiation of colon cancer in rats". Food Chem Toxicol 2009;47:377-85.
8. Zeng Y, Qiu F, Takahashi K, Liang J, Qu G, Yao X. "New sesquiterpenes and calebin derivatives from Curcuma longa". Chem Pharm Bull (Tokyo) 2007;55:940-3.
9.Dujic J, Kippenberger S, Ramirez-Bosca A, DiazAlperi J, Bereiter-Hahn J, Kaufmann R, et al. "Curcumin in combination with visible light inhibits tumor growth in a xenograft tumor model". Int J Cancer 2009;124:1422-8.
10. Huang MT, Lou YR, Xie JG, Ma W, Lu YP, Yen P, "Effect of dietary curcumin and dibenzoylmethane on formation of 7,12-dimethylbenz a anthracene-induced mammary tumors and lymphomas/leukemias in Sencar mice". Carcinogenesis 1998;19:1697-700.
11. Rabiei Z, Rafieian-kopaei M, Heidarian E, Saghaei E, Mokhtari S. "Effects of Zizyphus jujube extract on memory and learning impairment induced by bilateral electric lesions of the nucleus basalis of meynert in rat". Neurochem Res 2014;39(2):353-60.
12. Rabiei Z, Rafieian-Kopaei M, Mokhtari S, Alibabaei Z, Shahrani M. "The effect of pretreatment with different doses of Lavandula officinalis ethanolic extract on memory, learning and nociception". Biomed Aging Pathol 2014; 4(1):71-6.
13. Shpitz B, Giladi N, Sagiv E, Lev-Ari S, Liberman E, Kazanov D, "Celecoxib and curcumin additively inhibit the growth of colorectal cancer in a rat model". Digestion 2006;74:140-4.

SP-32

RECENT ADVANCES IN CANCER THERAPY

SHAIK AMINABEE[1]*, ATMAKURI LAKSHMANA RAO[1],ALIMUNNISA[1], SHABANA BEGUM[1], V. BHAVYA NAGA VANI[1]

[1]Department of Pharmacology, V. V. Institute of Pharmaceutical Sciences, GudlavalleruIndia

ABSTRACT

As per studies in 2015, about 90.5 million people had cancer. About 14.1 million new cases occur a year (not including skin cancer other than melanoma). It caused about 8.8 million deaths (15.7% of deaths). The most common types of cancer in males are lung cancer, prostate cancer, colorectal cancer and stomach cancer. The major cancer treatments are surgery and Radiotherapy which is being replaced by the other therapies like Virotherpay, Robot assisted therapy, Cancer therapy involved targeting proteins DNA double strand break repair, Liquid biopsies, Antiangiogenics, Targeted specific alterations.

Keywords: Cancer therapy, Chemotherapy, targeted therapy, virotherapy, antiangiogenics.

INTRODUCTION

Cancer is an important health problem in developed countries where is the second cause of death mainly associated with ageing of the population and lifestyle. Early diagnosis, universal access to health care and developments in these therapies has resulted in a significant improvement of cancer survival, being estimated that up to two thirds of cancer will be eventually cured with striking differences among tumors.
Recent advances in cancer therapy are given below:

1. Surgery, radiotherpay and endocrine therapy are old bur effective anticancer therapies

Surgery is most effective in treatment of localized primary tumor and associated regional lymphatics. When used as a single treatment surgery cures more patients than any other individual form of cancer therapy because surgery operates by zero-order kinetics, in which 100% of excised cells are killed. Both processes are complementary. Surgery is playing an increasing role in specific clinical situation such as colorectal liver metastasis.

2. Molecular Alterations Targetting Specific

For almost a century, systemic therapy of cancer has been dominated by the use of cytotoxic chemotherapeutics. Most of these drugs are DNA-damaging agents that are designed to kill or inhibit rapidly dividing cells. They are often administered in single doses or short courses of therapy at the highest doses possible without any life-threatening levels of toxicity, called "Maximum Tolerated Dose" (MTD). The high doses of these MTD chemotherapy schedules require an extended treatment-free period to permit recovery of normal host cells[1].

3. Targeting the non-tumor cell: Antiangiogenic strategies

Angiogenesis, that is the construction of new vessels from the pre-existing vasculature, is a crucial event not only in physiological but also pathological conditions. In particular, tumor expansion is dependent on angiogenesis because tumor cells demand oxygen and nutrients to overcome hypoxia and starvation. Following tumor progression, cancer cells metastasis to the distant organs through this angiogenic vasculature[2].

4. Virotherapy

Virotherapy is another concept involving the use of oncolytic viruses that grow selectively in tumor cells to treat cancer. First, viruses, unlike drugs, respond to absent molecular targets such as the lack of interferon (IFN) or tumor supressor pathways[3].

5. Cancer therapy targeting proteins involved in DNA double-strand break repair

Poly-adenosine-diphosphate-ribose (PAR) polymerase (PARP) is a key player in this process. PARP transfers PAR chains covalently to itself and to acceptor proteins in the vicinity of the lesion upon detection

of SSBs. This facilitates the repair of SSBs. DNA helix-distorting base lesions (more complex), induced by UV light are thereby repaired by nucleotide excision repair (NER). Another damage disturbing the helical structure of DNA is represented by base mismatches4.

6. Robot assisted surgery

Till today Operations are main for cancer treatment. The main moto is to remove as much of the cancer cells as possible. Doctor inserts tools and cameras into small cuts in the body in case of robotic surgery. Doctor console and looks into a viewfinder while working the robotic arms with hand and foot controls.

7. Cancer therapy based on dual mode gold nanoparticles

The modalities use light e.g., laser irradiation in an extracorporeal or intravenous mode to activate photosensitizer agents with selectivity in the target tissue. Photothermal therapy (PTT) is a minimally invasive technique for cancer treatment which uses laser-activated photoabsorbers to convert photon energy into heat sufficient to induce cells destruction via apoptosis, necroptosis and/or necrosis[5].

8. Verteporfin-loaded lipid nanoparticles improve ovarian cancer photodynamic therapy in-vitro and in-vivo

Advanced ovarian cancer is the most lethal gynecological cancer, with a high rate of chemoresistance and relapse. Photodynamic therapy offers new prospects for ovarian cancer treatment, but current photosensitizers lack tumor specificity, resulting in low efficacy and significant side-effects[6]. In the present work, the clinically approved photosensitizer verteporfin was encapsulated within nanostructured lipid carriers (NLC) for targeted photodynamic therapy of ovarian cancer.

9. Biologically targeted magnetic hyperthermia

Hyperthermia, the mild elevation of temperature to 40–43◦C, can induce cancer cell death and enhance the effects of radiotherapy and chemotherapy. However, achievement of its full potential as a clinically relevant treatment modality has been restricted by its inability to effectively and preferentially heat malignant cells. The limited spatial resolution may be circumvented by the intravenous administration of cancer-targeting magnetic nanoparticles that accumulate in the tumor, followed by the application of an alternating magnetic field to raise the temperature of the nanoparticles located in the tumor tissue[7].

10. DNA damage detection and repair pathways: Inhibitors of checkpoint kinases in cancer therapy

Insights from cell cycle research have led to the hypothesis that tumors may be selectivity sensitized to DNA-damaging agents, resulting in improved antitumor activity and a wider therapeutic margin. The theory relies primarily on the observation that the majority of tumors are deficient in the G1-DNA damage checkpoint pathway, resulting in reliance on S and G2 phase checkpoints for DNA repair and cell survival[8].

11. P53 research and cancer treatment

TP53, encoding p53, is one of the most famous tumor suppressor genes. The majority of human cancers demonstrate the inactivation of the p53 pathway. Mutant p53 not only, no longer, functions as a tumor suppressor but can also exert tumor promoting effects. The basic function of p53 is to respond to cellular stress[9].

12. Immunoliposome-based cancer therapy

Immunoliposomes have been regarded as an attractive drug targeting vehicle for cancer treatment. In the present review, we focus on recent advances in the design of immunoliposomes incorporating a variety of chemotherapeutics that simultaneously exhibit specific target-cell interactions and stimuli-sensitivity[10].

13.Cancer *prevention vaccines*

Vaccines targeted against hpv (human papilloma virus), which is responsible for 99.9% of cervical cancers, are now available in the market. There are many strains of hpv virus, which cause the cancer.

14. Subcellular targeted cancer therapy based on functional materials

Recently, diverse functional materials that take subcellular structures as therapeutic targets are playing increasingly important roles in cancer therapy. Here, particular emphasis is placed on four kinds of therapies, including chemotherapy, gene therapy, photodynamic therapy (PDT), and hyperthermal therapy,

which are the most widely used approaches for killing cancer cells by the specific destruction of subcellular organelles[11].

15. Liquid biopsies

When a patient has a suspicious lump that might mean cancer, their physician usually performs a tissue biopsy, where they take a sample of the lump and examine cells under the microscope to determine if they're potentially cancerous[12].

CONCLUSION

A good number of novel drugs are awaiting for clinical development. Meanwhile, novel approaches such as the antiangiogenic therapy and virotherapy are reaching the clinical development.As highlighted before newly described cancer biomarkers hardly ever bear the "critical for growth" character that ideal targets need.On top, the dynamic addaptive capability of tumor biology establishes the need for serial determinations, along treatment, of those aspects of cell biology that remain critical for the cancer cell at each time pointRobot-assisted surgery is expensive but can reduce blood loss during the procedure and pain afterwar. Cancer therapy based on dual mode gold nanoparticles various types of NPs, like gold NPs (AuNPs), designed to act both as radiosensitizers and photothermal sensitizing agents due to their unique optical and electrical properties i.e., functioning in dual mode. For ovarian cancer treatment, photodynamic therapy offers new prospects. But due to lack of tumor specificity in current photosensitizers, there is low efficacy and considerable side-effects. The clinically approved photosensitizer verteporfin was encapsulated within nanostructured lipid carriers (NLC) for targeted photodynamic therapy of ovarian cancer in the present work.

REFERENCES

1. Urruticoechea R, AlemanyJ, Balart A, Villanueva F and Capella G. Recent advances in cancer therapy: An overview. Current Pharmaceutical Design. 2010;16(1):3-10.
2. Hodgson D and Collins M. Implications of recent advances in radiotherapy techniques and technology. Nursing Times. 2017;113(3):28-30.
3. Emma Bolderson, Derek J, Richard, Bin-Bing S, and Zhou. Recent Advances in Cancer Therapy Targeting Proteins Involvedin DNA Double-Strand Break Repair. Clinical Cancer Research. 2009;5(2):6314-6320.
4. Oouchida K, Leiri S, Kemmotsu H and Tomikawa M. Robotic surgery for Cancer treatment. Japanese Journal of Cancer and Chemotherapy. 2012;39(1):1-7
5. Ellas Spyratou, Mersini P, Alexandros G, GeorgakilasIand Lembit Sihver. Recent Advances in Cancer Therapy Based on Dual Mode Gold Nanoparticles. Clinical Cancer Research. 2017;14(1):14-21.
6. Thierry Michy, Thibault Massias, Claire Bernard, Laetitia Vanwonterghem, Maxime Henry, Mélanie Guidetti and Guy Royal. Verteporfin-Loaded Lipid Nanoparticles Improve Ovarian Cancer Photodynamic Therapy *In-Vitro* and *In-Vivo.* Cancers. 2019;11(11):21-32.
7. David Chang, May Lim, Jeroen A, Goos, Ruirui Qiao, Yun Yee and Friederike. Biologically Targeted Magneti Hyperthermia: Potential and Limitations. Frontiers in Pharmacology. 2018;9(2):831-836.
8. Susan Ashwell and Sonya Zabludoff. DNA Damage Detection and Repair Pathways_Recent Advanceswith Inhibitors of Checkpoint Kinases in Cancer Therapy. Clinical Cancer Research. 2008;14(13);1-14.
9. Kazufumi Suzuki and Hisahiro Matsubara. Recent Advances in p53 Research and Cancer Treatment. Journal of Biomedicine and Biotechnology. 2011;9(7):54-56.
10. Seyedeh Hoda, Alavizadeh, Fatemeh Soltani and Mohammad Ramezani. Recent Advances in Immunoliposome-Based Cancer Therapy, Current Pharmacology Reports. 2016;2(1):129-141.
11. Anne Szarewski. HPV Vaccination and Cervical Cancer. Current Oncology Reports. 2012;14(2):559-567.
12. Wei-Hai Chen,Guo-Feng Luo and Xian-Zheng Zhang. Recent Advances in Subcellular Targeted Cancer Therapy Based on Functional Materials. Wiley Online Library. 2018.

SP-33

A REVIEW OF APPROACHES IN DEVELOPMENT OF ECO RBC

MOHAMMED JUNAID JAVED*, DR NARAYANA, DR A SRINIVAS RAO, DR A KISHORE BABU
Department of Pharmacy Practice, Bhaskar Pharmacy College, Hyderabad- 500075, India.
Email : junaidaj14@gmail.com

ABSTRACT: The entire system of blood collection, distribution and transfusion revolves around ABO Blood groups. Elimination of this need of classification can be done by enzymatic conversion of A, B, AB to O group which can revolutionise blood banking. Current transfusion problems such as blood supply shortage, blood borne diseases, and emergencytransfusions can be solved by this technique.The antigens of A,B,AB differ only in the terminal monosaccharide N-acetyl galactosamine for group A, galactose for group B and no additional saccharide for group O. A possibility was observed that clipping these saccharides would convert them to H antigen.α-galactosidase reacted with RBC survived normally *in vivo*. Transfusions carried out in clinical trials showed no rejection or haemolysis. A slight rise in anti-b titrewas observed with no explanation.30 times more effective enzymes are available which can directly be injected into blood bag. This technique holds tremendous promise and further research can ensure development of this technology into reality.

KEY WORDS: ECO RBC, Transfusions, Enzymes, Blood, Galactosidas

INTRODUCTION

According to WHO, India suffers from an annual deficit of two million blood units, as only 1% of Indian population donate blood each year. In 2016, the Ministry of Health and Family Welfare reported a donation of 10.9 million units against a requirement of 12 million units. There is a shortage of 30,00,000 blood units every year. one road accident victim can require up to 100 units of blood. Blood group O being universal donor is most requested by hospitals. It can be transfused to A, B and AB recipients. By seeing the great demand of O blood group, scientists discovered different methods of blood conversion and making all blood type universal donor. Enzyme conversion of A,B,AB group was proposed more than 30 years ago as it was a promising approach to achieve the goal of producing universal donor RBC to improve blood supply and enhance safety of clinical transfusion.

VARIOUS APPROACHES TO UPGRADE ENZYMATIC CONVERSION OF RBC

Previous approaches involved masking the antigen on RBC with PEG but survival rate *in vitro*was not considerable as it was immunogenic and induced production of antibodies in rabbits (Garratty, 2004)[1]. an alternate approach was enzymatic conversion of A & B antigen to universally accepted O blood group which was done by Goldstein *et al* (1982).This would eliminate shortage of blood supply. The major distinction between the A, B, AB&O lies with additional α1-3- linked sugar. A cells have α1-3- linked N acetyl galactosamine and B has α1-3- galactose, O cells have no sugar and AB has a mix of both chains. Study was carried out at American Society of Hematology where Goldstein used glycolytic enzyme to alter sugars present on RBC surface.

α galactosidase from Sanos coffee beans(*coffee canephora*)was extracted and purified .RBC treated with α galactosidase maintained structural integrity and functionality *in vitro.* Cells did not haemagglutination. Treatment of RBC with α galactosidase resulted in conversion of B cells to O cells .clinical trials were done in which patients were given 2 units of ECORBC. No antibody responses post transfusion of ECORBC were recorded. Anti B titers monitored in all O group recipients remained unchanged post transfusionand no evidence of overt hemoglobinuria was observed. However this enzyme suffered low turnover rates and required copious amount of enzyme (1-2 gm/unit B cell).another challenge was maintaining pH 5.5 while carrying out conversion reactions which rendered the approach impractical[2,8].

Direct evolution approaches were employed to broaden specificity but challenges of creating fully universal enzyme and formation of GlcNac terminated non H type antigen caused this approach to be abandonedlater on bacterial libraries were screened for conversion of tetra saccharidesubstrates. Enzymes isolated from marine microbes*pseudoalteromonas* for B group and*Arenibacterlatericius*for A group with apparent neutral pHbut was not efficient in RBC conversion. [3,4]

By 2004 American Society of Biochemistry isolated an endo β galactosidase from *clostridium perfringens* and named it E-Abase, it could efficiently cleave tri saccharide of both blood groups but suffered very narrow subtype specificity due to which further research was not continued[5].
Now there was need to identify enzymes that naturally worked better incremental improvement in the previously identified enzymes was not considered useful. Humanmicro biome was investigated, metagenomic approach was used to screen useful activity.Gut bacteria normally help us to digest complex carbohydrates and sometimes digest glycan on surface of intestinal cells for nutrition. Theseglycan were similar to glycan present on RBC. Hence these enzymes were studied by Peter Rahfeld in September 2019,he reported a bacterium named *flavinofactorplauti* found in human gut micro biome isolated from fecal samples. Itcould convert A to universal O type blood group of same Rh type. The enzyme was N-acetyl galactosamine deacetylase which had high activity in whole blood and specificity buffer.it worked well at neutral pH and room temperature which proved to be promising the enzyme stayed in touch with RBC for longer time and it could clip of the whole antigen present[6].

In 2015 scientists from china Hong Wei Gao,Hai Long Zhuo tried to convert A,B RBC to ECO RBC by combined treatment with α-galactosidase extracted from *B.Fragilis* and α-acetylgalactosamide extracted from *emeningospecticum*. Agglutination tests and FACS results showed efficient conversion.α-galactoside c DNA was cloned by RT-PCR from Catimor coffee beans .Recombinant enzyme were purified using ion exchange chromatography, purity was higher than 96%.RBC treated with it maintained membrane integrity, normal cell deformation & morphology.no coagulation between ECO RBC and any human blood group occurred. Normal function for a period of 21 days at 4^0C was observed in monoammonium phosphate nutrient solution. This study suggest that ECORBC could be transfused to patients safely &efficiently. These ECO RBC were transferred to A blood group gibbon's, no harmful effect on donors RBC structure &function nor transfusion rejection was observed this encouraged further research in this particular field.
2014 another research was carried out in rat models with aim to track survival of RBC in circulation.enzyme α-glycosidase isolated from *bacteriodsfragalis* .the results showed successful survival of RBC in blood stream even after 35 days, rat RBC did not react with anti A and anti B. Treatment with α galactosidase enzyme had no effect on survival kinetics of RBC. Data from this study supported use of enzymatic conversion technology into clinical practices[7].

Finally clinical trials were carried out at department of pathology and medicine Massachusetts,USA. Aim was to study safety and efficiency of eco RBC in transfusion dependent paitents.21 patients were given ECORBC, no adverse events occurred.it resulted in appropriate Hb increment and RBC survived.1 patient developedweak DAT without hemolysis. However after 2 weeks 5 out of 19 patients had increase in anti B titers. The biochemical nature of antigen still reactive with human anti A anti B on ECORBC has not been fully clarified but studies suggest that minor amount of residual antigens could be a major cause, currently data from an extended multi Centre autologous study in healthy volunteers are being evaluated and results are under way.

CONCLUSION

Serological incompatibilities are unclear with ECORBC and removal of A epitopes strategies should be developed, better understanding of immunogenic responses and cross matching compatibilities in plasma are pivotal in this aim. The relevance and practical consequences of remaining cross match reactivity is one of the main foci for future work. None the less it's fair to say that over all research program has produced very strong and convincing data supporting ECO RBC production which holds tremendous promise in current transfusion problems such as blood supply shortage, blood borne diseases, emergency blood transfusions and safety in transfusions in near future.

REFERENCES

1. Garratty, G. {2004} progress in modulating the RBC membrane to produce transfusable universal/stealth donor RBCs. *Transfusion Medicine Reviews.*
2. Goldstein, j., Siviglia, G., Hurst, R., Lenny, L. and Reich, L. {1982} Group B erythrocytes enzymatically converted to group O survive normally in A,B, and O individuals. *Science.*
3. Bakunina, I.Y., Sova, V.V, Nedashkovskaya, O.I, Kuhlmann, R.A., Likhosherstov, L.M., Martynova, M.D., Mihailov, V.V. and elyakova, L.A. {1998} Alpha-galactosidase of the marine bacterium *Pseudoalteromonas* s.p. KMM. *Biochemistry {Mosc}.*
4. Bakunina, I.Y., Kuhlmann, R.A., likhosherstov, L.M., Martynova, M.D., Nedashkovskaya, O.I, Mikhailov, V.V. and Elyakova, L.A. {2002} Alpha-N-acetylgalactosaminidase from marine bacterium *Arenibacter Latericius KMM* Removing blood type specificity of A erythrocytes. *Biochemistry {Mosc}.*
5. Roma Jamal, Ananya, Aisha Kamal, Adnan Ahmad , Farhan Ahmad, Recent approach in conversion of universal blood group by enzymatic method , Review Article.
6. Peter Rahfeld, Lyann Sim, Stephen G. Withers , An enzymatic pathway in the human gut microbiome that converts A to universal O type blood. *Nature Microbiology {2019}* .
7. Li SB, Gao HW, Ji SP, Wang YL, Xu LJ, Bao GO, Tian SG, Yu CY, Tan YX, Gong F, The effect of treatment with α glycosidases from Bacteroides fragilis on the survival of rat erythrocytes in the circulation {2014}.
8. Leslie L. Lenny, Rosa Hurst , Jack Goldstein, Lennette J. Benjamin, and Robert L. Jones, Single unit transfusions of RBC enzymatically converted from group B to group O to A and O Normal volunteers. {1991}.

SP-34

ASSOCIATING GENETIC VARIATION WITH VARIATION IN DRUG RESPONSE

ARSHIYA JAHAN*, NISHATH, NARAYANA, DR.A.SRINIVASA RAO, DR A.KISHORE BABU
Department of Pharmacy Practice, Bhaskar Pharmacy College, Hyderabad- 500075, India.

ABSTRACT

Pharmacogenetics is the science which interprets the role of genetic variation in varied drug response. Genes play significant role in drug response. Even though the sequencing of more than 1000 genes has been progressed the implementation of the study into clinical practice is not yet done due to certain obstacles like ethical issues, unavailable dose recombination and absence of completely curable phenotypes. This review highlights the complex processes involving drug response when provided separately or in combination. It addresses genetic contributors to drug efficacy and toxicity. Adverse effects of drug due to single gene mutation, environmental factors and wrong disease determination have been discussed. Modulation of protein function or gene expression due to DNA variants, effects of genes in drug metabolizing enzymes and combinatorial pharmacogenetics is interpreted. Heritable drug related genes and genes which are responsible for diseases related to heredity has addressed. Study of drug related genes and the effect of drugs on non-drug related genes and transferring the strategies into personalized medicine is the main objective and to overcome the issues in pharmacogenetics.

KEY WORDS: Pharmacogenetics, Drug response, Gene mutation, Protein function, Drug metabolizing enzymes.

INTRODUCTION

The pharmacogenetics came into picture in 1931 when sir Archibard garrod wrote a book INBORN FACTORS IN DISEASES[4]. The present strategies of treatment of diseases cannot overcome the toxic effects and sometimes no response to drugs[7].Therefore linking of our present terminologies with genes can be crucial[5].Every individual have unique genes which alter the drug response[9].The study of pharmacogenetics revolve around genome wide association studies and human genome project[1,3].Genes related to metabolism of drugs play a pivotal role in genetic studies[6,7].The complete research on common and rare variants can be useful in discovering of new drugs with respect to pharmacogenetics[8].The main of discussion is the presence of wide variations in the genome and its relation with therapeutic response and how to implement the available knowledge of drug related genes in to personalized clinical treatment[3].The main objective of making future drugs should be pharmacogenetics which can reduce the adverse effects of present day drugs and can increase the positive response of drugs to great extent[8].Tractability of pharmacogenetics is more than that of disease predisposition[10].

METHODOLOGY

GENOME WIDE ASSOCIATION STUDIES

GWAS can be conducted on the basis of population and family. These studies have related hundreds and thousands of single base pair nucleotides .3600 SNPS have been identified by National human research institute for common variants[1].Only high prioritized genes are included in the study which might exclude other rare and structural variants that play a pivotal role in pharmacological responses[3].GWAS have pictured the rareness of phenotypes for better drug response[5].

HUMAN GENOME PROJECT

The human genome has provided us recently with great deal of knowledge on the sequences that comprises the human genetic makeup. We are still in the process of structuring and deciphering $3*10^9$ base pairs of human genome which might help in taking pharmacogenetics forward[2].

DISCUSSION

Metabolism of drugs

If the metabolism is fast levels of drugs in the blood never become enough for the effective action of drug (presence of CYP2D6 gene can metabolize morphine quick and CYP2D6 is also responsible of metabolism of nearly 60 drugs and slow metabolism leads to accumulation of drugs in the body which can cause toxic effects)[4].Drug metabolizing genes are usually sensitive[3].The epithelium of intestine and liver contain the most abundant member of CYP family i.e. CYP3A and these enzymes play a significant role in the metabolism of more than half of the therapeutic drugs[7].

FEW IMPORTANT EXAMPLES

Antimalarial drugs such as chloroquine and primaquine destroyed RBCs and caused haemolytic anaemia in American soldiers due to deficiency of GLUCOSE-6-PHOSPHATE DEHYDROGENASE enzyme.Low levels of N-ACETYL TRANSFERASE in the genome of few individuals significantly affected the response of antituberculosis drug like IONIAZID.The genetic lack of BUTYL CHOLINE ESTERASE in certain individuals have developed life threatening disorder when SUCCINYL CHOLINE injection was given in combination with anaesthetic[4,6].The cytochrome p_{450} are the major gene family that contributes to oxidative metabolism of drugs. The cytochromes $P_{450}CYPD_{6,}$ CYP_2C_9, CYP_3A_4 and $CYPC_{i9}$ have key role as they are encoded by different genes each[10].

FAMILY HISTORY

The ancestory play an important role in identifying clinically important phenotypes, for example N-ACETYLATION variable showed different IONIAZID excretion in dizygotic and monozygotic twins.1236 FDA approved genes are affected by functional variants which depends on ancestory[5].

COMBINATORIAL PHARMACOGENETICS

Study of multiple gene combinations like warfarin and clopidogrel, suggests how the cross examination of very large clinical datasets for individual polymorphisms in multiple individual genes in combination can be useful in figuring out the role of these variants in pharmacological drug response[5].

ENDOPHENOTYPE

It basically, should include population illness based genes, heritable genes, active illness manifestation and co-segregation of genes with family[3].

SNP TRAIT ASSOCIATIONS

Figure -1 SNP STUDIES[1]

IMPORTANT POINTS OF DISCUSSION

- Single genome studies have lead to genome wide association studies[3].
- Linkage analysis described the genetic variations that contribute to rare diseases like Huntington syndrome and common diseases like heart and cancer[1].
- If single gene variants only are included in the study than several side effects can occur for instance

- There are no such exact biomarkers at present which can predict the reasons behind which group of patients respond positively, which type of individuals are non responders and which experience severe adverse effects[8].

ADVANTAGES OF PHARMACOGENETICS

- Potential cost saving approach by increasing intended drug response and decreasing the toxic effects[3].
- Complete picture of individual[3].
- Determination of causative single nucleotide polymorphism[3].
- Avoiding side effects and increasing efficacy[8].

DISADVANTAGES OF PHARMACIGENETICS

- Whole genetic makeup of individual cannot be studied due to fluctuations in genes according to age[4].
- Determining genetic variations responsible for drug actions is time taking process[8].
- Large volumes of sample are required for genetic testing[3].
- Ethical issues like impact on equity and access to drugs[8].
- And complete cost for the genetic studies is not yet predicted[8].

PHARMACOGENETICS -THE FUTURE MEDICINE

PRESENT APPROACH

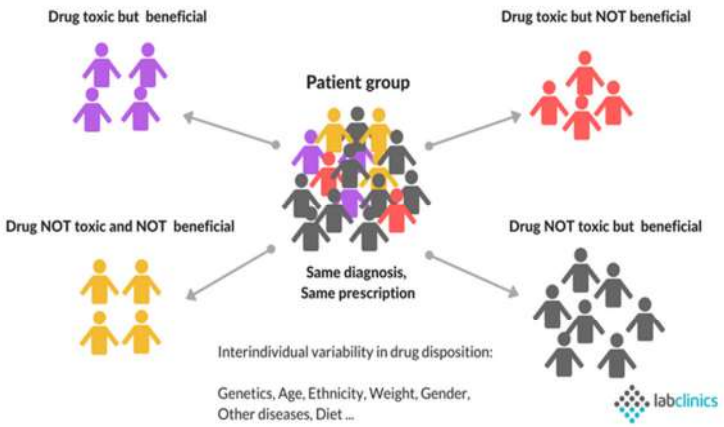
- ✓ Average dose 'one size fits for all' but not perfectly
- ✓ Maximum side effects
- ✓ Sometimes toxic response
- ✓ Treatment based on disease determination
- ✓ Very expensive.

FUTURE APPRAOCH

- ✓ unique gene of individual –unique dose
- ✓ minimum side effects
- ✓ reducing severe effects
- ✓ treatment based on genotype
- ✓ less expensive

RESULTS

The current approach of clinical practices can be changed by our advanced studies made in genetic research by certain genetic engineering techniques which are successful in determination of genetic variants that can alter our drug responses.



Figue -1: Drug responses[5]

CONCLUSION:

Through this review we conclude that there association of genetic variation with variation in drug response.

REFERENCES

1) Michelle chang, Lin He, and Lei Cai (An overview of genome wide association studies).
2) Taramelli R, Etal.eurj cancer.2004(The human genome project and discovery of genetic determinants)
3) Ashraf G. Madian, Heather E. Wheeler, Richard Baker Jones and M. Eileen Dolan (Relating human genetic variation to variation in drug responses).
4) W. Kalow-2006, Department of Pharmacology, University of Toronto (Pharmacogenetics and Pharmacogenemics, Origin, Status and the hope for personalized medicine)
5) Dan M. Roden, MD; Russell A. Wilke, MD, PhD; Heyo K. Kroemer, PhD; C. Michael Stein, MD (Pharmacogenomics, The Genetics of Variable Drug Responses**).**
6) Eichelbaum M, et al. Clin Exp Pharmacol Physiol. 1996 Oct-Nov (Influence of Pharmacogenetics on drug disposition and response).
7) BS Shastri Dept of Biological Science, Oakland University USA.(pharmacogenetics and the concept of individualized medicine)
8) Rajiv Saini, Santosh Saini, RS Sugandha (Pharmacogenetics, the future medicine).
9) Charlotta P.I. Schärfe, Roman Tremmel, Matthias Schwab, Oliver Kohlbacher ,Debora S. Marks (Genetic variation in human drug-related genes
10) British Medical Bulletin(2017)(Pharmacogenetics, a general review on progress to date)

SP-35

ASSESSMENT OF RATIONAL USE OF ANTIBIOTICS IN ORTHOPEDIC PROCEDURES

DR. SHUBHAM BABU GUPTA[1], DR. PANKAJ WADHWA[2]

1.Faculty in MallaReddy Institute of Pharmaceutical Sciences,Hyderabad, Telengana, India.
2.School of Pharmaceutical Sciences, Lovely ProfessionalUniversity,Punjab,India
Mail.id-shubhamrockzz93@gmail.com

ABSTRACT:
Appropriate use of antimicrobial agents is vitally important from clinical perspectives and is essential if the usefulness of antibiotic is to be preserved and the further spread of resistance is to be limited. Antibiotics are one of the pillars of modern medical care and play a major role both in prophylaxis and treatment of infectious diseases. However, their misuse is a worldwide problem with the extent of the problem being greater in the developing countries.To assess rational use of antibiotics for prophylaxis and treatment among patients in orthopedic department who undergone surgery. A prospective and observational study was conducted on 130 medical records of orthopedic patients who undergone surgery. Stratified random sampling technique was used for this study. Data was collected by using a pre-tested structured questionnaire from September 2019 to December 2019 and then analyzed using statistical package for social science (SPSS) version 20.0 software.Out of 130 patients who undergone surgery 70 patients were male and 60 patients were females. Based on age group classification 30 – 40 years of age 50 (38.46%) was mostly affected. The most common diagnosis was ORIF procedures accounts for (43.07%) followed by total hip replacement (20%) of cases. The most frequently prescribed antibiotics class was cephalosporins (76.43%), followed by Penicillin's (15.51%) for prophylaxis Cefuroxime (67.40%)followed by amikacin (14.91%) and for treatment Cefuroxime (58.17%)followed by amikacin (14.90%) .Themost commonlyused antibiotics combination for prophylaxis is Ceftriazone + Amikacin+ Cefuroxime (20.68%) and treatment includes Ceftriaxone+ Cefuroxime +clavulanic acid (34.04%). It was found that 50% of cases for prophylaxis was inappropriate where as 73.07 % of treatment antibiotics failed to adhere to guidelines.Generally, this study indicated that orthopedic patients underwent surgical procedures by majority of patients were ORIF and Total hip replacement. Mostly prescribed antibiotics in the patients who undergone major surgery were cefuroxime followed by Amikacin. In general, this study result indicated some level of inappropriateness which high light need forintervention.

Keywords: Antibiotics; Surgery; Resistance; Prophylaxis; Treatment.

1. INTRODUCTION

Antibiotics are one of the pillars of modern medical care and plays major role both in prophylaxis and treatment of infectious diseases. The issues of their availability, selection, and proper use are of critical importance to the global community. Antibiotic misuses are however, a worldwide problem with the extent of the problem being greater in the developing countries through their purchase (without prescription) in local pharmacies and drug stores, and through inappropriate prescribing habits and an over-Zealous desire to treat severe infections[1]. Prophylactic antibiotics are widely used in surgical procedures and account for substantial antibiotic use in many hospitals. The purpose of SAP is to reduce the prevalence of postoperative wound infection (about 5% of surgical cases overall) at or around the surgical site. By preventing surgical site infections, prophylactic antimicrobial agents have the potential to decrease patient morbidity and hospitalization costs for many surgical procedures that pose significant risk of infection. However, the benefits of prophylaxis are controversial; prophylaxis is not justified for some surgical procedures (e.g., urologic operations in patients with sterile urine). Consequently, the inappropriate or indiscriminate use of prophylactic antibiotics can increase the risk of drug toxicity**,** selection of resistant organisms**,** and costs [2]. Serious morbidity and mortalities are associated with post-operative wound infections. They have an enormous impact on patient's quality of life and contribute substantially to the financial cost of patient care. The use of preoperative antibiotics has become an essential component of the standard of care in virtually all

surgical procedures, and has resulted in a reduced risk of the post-operative infection when sound and appropriate principles of prophylaxis are applied [3]. In 1960s it was reported that pathogens are present during surgery regardless of how aseptic the surgery might appear. Moreover, the correlation between prophylactic antibiotics and post-operative would infections were demonstrated. Therefore, antibiotic prophylaxis now used in almost all surgical procedures, the preoperativeadministration of antibiotics because it can act at the time when the wound is potentially contaminated that the use of antibiotics after 3-4 hours of a bacterial incision is unsuitable [4].Previous studies have demonstrated that antibiotics must be active against major anticipated pathogens and must have reached sufficient concentration in the tissue or body fluids at risk by the time of bacterial challenges. If prophylactic therapy is to be maximally effective in reducing the infection rate of potentially contaminated surgery. The need for continuing antibiotic prophylaxis of operation, however, has been uncertain.[5]Different studies had indicated that there is high proportion of inappropriate antibiotics use for patients who admitted at surgical ward and these studies have also suggested reasons for inappropriateness as an excessive duration of treatment, incorrect timing of administration, inadequate antibacterial spectrum of the drugs used, and unnecessary combination of two antibiotics [6-9]. The increase resistance fate of many important pathogens to currently most available antibiotic has been recognized as an important and potentially life threatening problem. This problem is promoted in part by irrational antibiotics prescribing is behavior and usage. Medically in appropriate, in effective and economically inefficient use of pharmaceuticals is common health care systems problem throughout the world especially in the developing comities [10-16.] This study aimed at identifying inappropriate use of antibiotics for prophylaxis and treatment at surgical ward which serve as a baseline data for health authority and policy maker and it helps in developing strategies or essential drug list guideline for rational use of drugs at the hospital level as well as for improvement of hospital service.

METHODOLOGY:

Study setting: The study was conducted in Tertiary care Hospital.It has a different department and wards like outpatient department (OPD), medical wards, gynecology and Orthopedic ward, pediatrics ward and surgical ward. It delivers diversified health services and clinics including the emergency services, eye clinic, dental clinic, mother and child health (MCH), psychiatry clinic, laboratory, radiology, pharmacy, physiotherapy and follow up of chronic disease. The study was conducted from September2019 to December 2019.

Study design: Prospective and Observational study on assessment of drug use was conducted using patients' medical card. Source population: All medical records of patients, who undergone major surgery from September 2019 to December 2019.

Eligibility criteria

Inclusion criteria: The study includes the randomly selected clean and contaminated surgeries Patients undergoing orthopedic surgery, patients of all ages and either gender.

Exclusion criteria: Patients with confirmed microbiological testing indicative of infections diabetic patients, minor incisions and pregnancy cases were excluded from study.

Sample size: The minimum statistically required sample size was calculated by using the following formula.

$$N = \frac{Z^2 P (1-P)}{D^2}$$

Where:

N=Sample size required

P=Prevalence rate of antibiotics

Z=The standard normal Confidence at interval of 95% =1.96

D=The margin of sampling error tolerated

Sampling technique:

Stratified random sampling method was conducted to select more representative sample. During study period, to calculate sample allocated to each stratum proportionate allocation was used. Then a simple random sampling was done depending on the type of allocation.

Proportionate allocation:
Where:
ni is sample size of the ith stratum
Ni is population size of the ith stratum

Study variables
Independent variables:
Age, Sex, Diagnosis, residence, number of antibiotics used, duration of hospital stay.

Dependent variables:
Appropriateness of antibiotic use.

Data collection procedure:
Relevant information about each patient like demographic factors, type of diagnosis, medication history (antibiotics used for prophylaxis and treatment including other medication used), other comorbid conditions, durations of hospitalization stay and condition of discharge were recorded using well-structured data collection format through reviewing medical records of patients. Other supplementary information was obtained
from register. Appropriateness of antibiotic use was checked by using the W.H.O Standard Guidelines for general hospitals [17.]

Data quality control:
To maximize accuracy of this study, data collection format was validated with its objective and developed enough to assess the objective of the study. This data collection format was pre-tested on 5% of patient's cards from the same source population before starting actual data collection. Patient's cards which used for pre-testing is not used in study[18.] In addition, regular checkup for completeness and consistency of the data was made on daily basis. To check the consistency, data were entered in two different SPSS programs (double method).

Data analysis and presentation:
Data entry and analysis was carried out using statistical package for social science (SPSS) version 20.0. Descriptive statistics were used for statistical analysis. The result was analyzed and presented using tables and graphs. Data available was interpreted and discussed with the results of similar studies.

Ethical consideration:
Ethical clearance was obtained from Lovely Professional University, Ethical Review Committee. A formal letter was written to the Tertiary Hospitals in order to get permission to conduct the study and Official permission was granted fromthe hospital administration. To ensure confidentiality, name and other identifiers of patients and prescribers were not recorded on the data abstraction formats.

RESULT

Socio-demographic characteristics:
A total of 130 patients' medical records that undergone Orthopedic surgery and took antibiotics drugs were analyzed. The majority of the patients were between 30-40 years, 50 (38.46%) and 41-50 years, 25 (19.23). But inappropriateness of antibiotics used for treatment was higher in age between 51-60 years and above 60 years. And also the prevalence of inappropriateness is higher in antibiotics used for prophylaxis (39.47%) and then for treatment (57.89%) (Table 1).

Therapeutic indication:
Out of 130 patients who undergone orthopedic surgery at, Tertiary Hospitals from September 2019 to December 2019. The most frequently diagnosed disease for which antibiotic indicated were ORIF 56 (43.07%) followed by Total Hip replacement 26 (20%) (Table 2).

Total antibiotics used for surgical prophylaxis and treatment:
As shown below in the Table 3, the most commonly prescribed antibiotics for patients who undergone orthopedic surgery for both Prophylaxis and Treatment Were Cefuroxime 67.40% and 58.17% respectively, followed by Amikacin 14.91% and 14.90% (Table 3).

Prescribed antibiotic drug regimens (single/multiple drugs) for prophylaxis and treatments:
130 patients were treated with different regiment of antibiotics drugs. The numbers of patients who were treated with one antibiotics regimen for prophylaxis were 76 (58.91%) and for the treatment were 13 (9.15%) and Two antibiotics regimen was found to be 30 (23.25%) and 95 (66.90%), Three antibiotic regimen 19 (14.72%) and 20 (14.08%) and Four antibiotic regimen was observed as 4 (3.10%) and 14 (9.85%) respectively.

S.No.	Socio-demographic characteristics	Frequency N (%)	Inappropriateness of antibiotic used	
			For ProphylaxisN (%)	For Treatment N (%)
1	Age group (year)			
	30-40	50 (38.46)	25 (38.46)	39 (41.05)
	41-50	25 (19.23)	19 (29.23)	24 (25.26)
	51-60	38 (29.23)	15 (23.07)	22 (23.15)
	>60	17(13.07)	06 (9.23)	10 (10.52)
	Total	130 (100)	65 (100)	95 (100)
2	Sex			
	Male	70 (53.84)	42 (64.61)	55 (57.89)
	Female	60 (46.15)	23 (35.38)	40 (42.10)
N- is the number of patient				

Table1:Socio-demographiccharacteristicscrosstabulationwithinappropriateness of antibiotic used for patients who undergone orthopedic surgery at surgical ward of Tertiary care Hospitals from September 2019 to December 2019.

Diagnosis	Frequency	Percentage (%)
ORIF	56	43.07
Total Hip Replacement	26	20
Wound Debriment	13	10
Arthroplasty	10	7.69
Implants	09	6.92
CRIF	10	7.69
TKR	04	3.07
Skin grafting	02	1.53
Total	130	100

Table 2: Types of diagnosis of orthopedic surgery at Tertiary care Hospitals from September 2019 to December 2019.

Drug name	Prophylaxis	Treatment
	No of patients N (%)	No of patients N (%)
Cefuroxime	122 (67.40)	121 (58.17)
Amikacin	27 (14.91)	31 (14.90)
Metronidazole	10 (5.52)	14 (6.73)
Amoxicillin/ potassium clavulanate	04 (2.20)	04 (1.92)
Ceftriaxone	05 (2.76)	02 (0.96)
Cefoperazonesalbactum	03 (1.65)	02 (0.96)

Cefuroxime/ clavulanic acid	02 (1.10)	28 (13.46)
Levofloxacin	02 (1.10)	01 (0.48)
Linezolid	02 (1.10)	03 (1.44)
Cefotaxime	01 (0.55)	00 (00)
Total	178 (100%)	206(100%)

Table 3: Total antibiotics used for surgical prophylaxis and treatment at for a patient who undergone orthopedic surgery September 2019 to December 2019.

From antibiotic combinations, combinations of two drugs prescribed for prophylaxis were 68 (29.80%),and for the treatment were 130 (57.00%) patients (Figure 1).

Appropriateness of antibiotics

Appropriateantibioticsusedforprophylaxiswere65(50%)and appropriate antibiotics used for treatmentwhere 35 (26.92) (Table 4).

Single prescribed antibiotics

As shown below in Table 5, the most frequently prescribed antibiotics are ceftriaxone; for prophylaxis 98.75% (N=158) and for treatment 96.5% (N=83) (Table 6).

Combination of antibiotics used for prophylaxis and treatment.

According to the result in Table 6 below, antibiotics combination regimenusedforprophylaxiswasCeftriaxone+ Amikacin+ Cefuroxime(20.68%), Metronidazole+Ampicillin+ Cefuroxime (17.24%), Ceftazidine + Amikacin (17.24%), Flouroquinoloes + Amikacin (17.24%),
For treatment; Ceftrixone + Cefuroxime (34.04%), Ceftrixone + Metronidazole+ Amikacin (23.40%), Levofloxacin + Metronidazole+ Amikacin(6.38%)(Table6).

Class of antibiotic prescribed

This study indicate that most prescribed antibiotic classes were cephalosporin 133(76.43%), followed by Penicillin's 27(15.51%) for prophylaxis and cephalosporin 157 (76.21%), and penicillin's 31(15.04%)fortreatmentrespectively(Table7).Presenceofothercomorbidconditionsandothermedications taken,durationofhospitalstayandconditionofdischarge.

As shown in the table below, 36.4% patients had other comorbid conditions and 32.9% patient have taken other medication in addition to antibiotics. Duration of Hospital stay was <10 days for 76.3%.

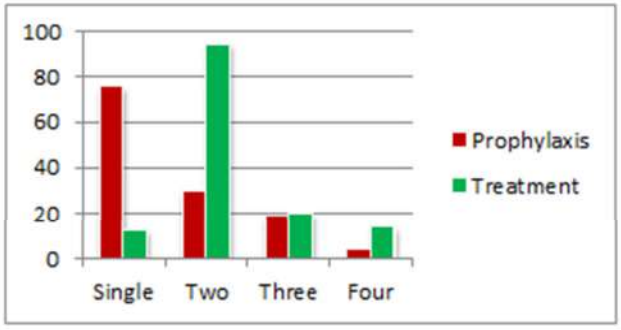


Fig: 1Showing the antibiotic regimen used for prophylaxis and treatment in orthopedic procedures

Reason for use	Appropriateness		Frequency	Percentage (%)
Prophylaxis	Appropriate		65	50.00
	Inappropriate	Inappropriate choice	32	24.61
		Unnecessary combination	33	25.38
		Total	65	50.00

Treatment	Appropriate		35	26.92
	Inappropriate	Excessive duration	40	30.76
		Short duration	03	2.30
		Inappropriate choice	09	6.92
		Unnecessary combination	43	33.07
		Total	95	73.07

Table 4: The appropriateness of antibiotic used for patients who undergone orthopedic surgery at Tertiary care Hospitals September 2019 to December 2019.

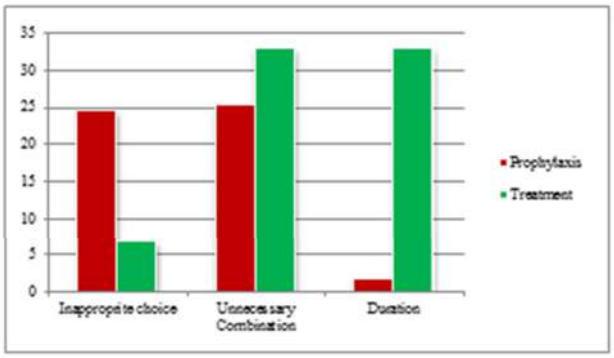


Fig: 2Representing in appropriate antibiotic prescription used for prophylaxis and treatment in orthopedic procedures

Drug name	Prophylaxis No of patients N (%)	Treatment No of patients N (%)	Total N (%)
Ceftriaxone	158 (98.75%)	83(96.5%)	241 (97.97)
Ampicillin	2 (1.25%)	0	2 (0.81)
Nor floxacillin	0	3 (3.5%)	3 (1.22)
Total	160 (100%)	86 (100)	246 (100)

Table 5: Single prescribed antibiotics for patients who undergone orthopedic surgery at Tertiary care Hospitals September 2019 to December 2019.

	Antibiotic combination	Frequency	Percentage (%)
For prophylaxis	Ceftriazone+ Amikacin+ Cefuroxime	06	20.68
	Metronidazole+Amikacin+ Cefuroxime	05	17.24
	Ceftazidine+ Amikacin	05	17.24
	Flouroquinoloes + Amikacin	05	17.24
	Levoflaxacin+Metronidazole+Amikacin	03	10.34
	Flouroquinoloes+Amikacin+metronidazole	02	6.89
	Ceftazidine+Amikacin+ cefixime	01	3.44
	Ceftazidine+ Amikacin+ceftrixone	01	3.44
	Ceftrixone+ cefuroxime	01	3.44
	Total	29	100
For treatment	Ceftriaxone+ Cefuroxime + clavunalic acid	16	34.04
	Ceftriaxone+Metronidazole+Amikacin	11	23.40
	Cefoperazone +cefuroxime/clavinolic acid	06	12.7
	Metronidazole+Amikacin	05	10.6
	Levofloxacin + Metronidazole +Amikacin	03	6.38

	Levoflaxacin+ Amikacin	02	4.25
	Ceftazidine+Amikacin+Cefuroxime	01	1.45
	Total	47	100

Table 6: Combination of antibiotics used for prophylaxis and treatment for patients who undergone orthopedic surgery at Tertiary care HospitalsSeptember2019 to December 2019.

Class of antibiotic	For prophylaxis N (%)	For treatment N (%)	Total (%)
Cephalosporin's	133 (76.43)	157 (76.21)	290 (76.31)
Penicillin's	27 (15.51)	31 (15.04)	58 (15.26)
Nitro imidazole	10 (5.74)	14 (6.79)	24 (6.31)
Oxazolidinone antibiotic	02 (1.14)	03 (1.45)	05 (1.31)
Fluoroquinolones	02 (1.14)	01 (0.48)	03 (0.78)
Total	174(100)	206 (100)	380 (100)

Table 7: Class of antibiotic used for prophylaxis and treatments for patient who undergone orthopedic surgery in Tertiary care Hospitals to September 2019 to December 2019.

DISCUSSION

This study identified that the prevalence of ORIF was found to be (43.07%) followed by Total hip replacement which as observed in 20% of cases. In this study from the prescribed antibiotic drugs Cefuroxime (61.40%) for prophylaxis and (58.17%) for treatment which was different with study done in Navy Hospital, Jakarta, Indonesia 87.8% but, gentamycin use was similar in thesamehospitals3.66%and3.7%respectively[13].Inthisstudy(for treatment) the metronidazole prescribed were (29.58%), cloxacillin (3.66%),ampicillin(5.5%)andgentamycin(3.66%)Whenwecompare with study done in Manipal Teaching Hospital, Pokhara, Nepal [12] the most commonly used individual antibiotics were preparation of ampicillin and cloxacillin (54.64) followed by metronidazole (31.95), ampicillin (18.34) and gentamicin (16.37), which was different but thereisnomoredifferenceincaseofmetronidazole.

In current study the most prescribed antibiotic were Cefuroxime (61.40%), Amikacin (14.91%) and Metronidazole (5.52%) for prophylaxis and 58.17%, 14.90%, 6.73% respectively which was different with study done in Orthopedics and Traumatology Surgical Unit of a Tertiary Care Teaching Hospital in Addis Ababa [22] the most frequently prescribed antimicrobial agent used for prophylaxis was ceftriaxone (70%), cloxacillin(9%)andMetronidazole(7%)wasused.However,ceftriaxone was almost similarly prescribed. The prophylactic antimicrobial regimens included both single as well as combination regimens;single regimens in prophylaxis took the lion's share. In this study the most commonly prescribed regimen among the combination regimens wasCeftriazone +Amikacin + Cefuroxime (20.68%),andmetronidazole+ Amikacin + Cefuroxime (17.24), for treatment it is observed Ceftriaxone + Cefuroxime/ Clavunalic acid (34.04%) followed by Ceftriazone + Amikacin + Metronidazole(23.40%). This study revealed orthopedic surgery for prophylaxis were cephalosporin 133 (76.43%), followed by Penicillin's 27 (15.51%) and Nitro imidazoles 10 (5.74%). Similarly a study done in Medicine surgical inpatient wards of Basaweswara Medical College Hospital [6] the most prescribed antibiotics were 3rdGeneration Cephalosporin's 108 (40.7%), followed by Aminoglycoside 11 (4.2%), Fluoroquinolones 11 (4.2%), and Penicillin 9 (3.4%).

The total number of patients who were treated with monotherapy of antibiotic drugs for prophylaxis were76(58.91%)and for the treatment 13 (9.15%) and from anti-microbial mixes, two medications mix endorsed for prophylaxis were 30 (23.25%), and for the treatment 95 (66.90%) patients and three medications blend for treatment were prescribed19(14.72%), and for four medication routine for prophylaxis 4 (3.10%) and for treatment 14 (9.85%) which was diverse with study done in medication surgicalinpatientwardsofTertiary care hospital share of them are on single anti-infection agents 73 (45.1%), two anti-infection agents 75 (46.3%) and three antimicrobials 14 (8.6%) for prophylaxis. The purpose behind the distinction could be a direct resultof the distinction in study designasthestudydonein Tertiary care hospital was a prospectivestudy.

Restriction of this examination was that the investigation plan we utilized was Prospective and observational investigation which is frequently liable to inclinations (blunders that influence the perceptions of an examination); for instance, in the assortment of dataitisdifficulttoevaluatehowthepatientstooktheirmedicationand if there is any undesirable impact of the medications. Being forthcoming examination alsomadeusnottostudyimportantvariablesincludingeducationlevel, adherence, quiet wellbeing specialist correspondence and supplier and wellbeing framework related variables. Another impediment was the little example size that we have utilized and that this examination included just a single referral emergency clinic of thecountry.

CONCLUSION: For the most part, this investigation demonstrated patients who experienced orthopedic surgeries Mostly recommended antimicrobials in the patients who experienced significant medical procedure were ceftriaxone trailed by Metronidazole. In any case, since ceftriaxone is wide range anti-microbials it has higher opportunity to cause more medication safe microscopic organisms, in this way we suggest the utilization cefazolin which is a first line specialist for prophylaxis for most careful cases. The consequence of this examination showed some degree of impropriety which high light requirement for intercession. The job of clinical drug specialist by examining the endorsing designs was valuable as it assists with surveying if the correct prophylactic treatment and postoperative treatment is being followed in the emergency clinic.

REFERENCES

1.Imranuddin M, Asfin M, Sruthi V, Deshpande S, Reddy MVR, et al. (2015) Cost analysis and prescribing patterns of antibiotics in postoperative surgery patients. Indo Am j pharm 5:2231-6876.
2.Alavi SM, RoozbehF, BehmaneshF, Alavi L (2014) Antibiotics use patterns for surgical prophylaxis site infection in different surgical wards of a teaching hospital in Ahvaz, Iran. Jundishapur J Microbiol 7:e12251.
3.Willems L, Simoen S, Laekeman G (2005) Follow up of antibiotic prophylaxis impact on compliance with guidelines and financial out comes J Hosp infect 60:333-922.
4.Hoonk S, Hay J, YookY (2009) The efficacy of postoperativeprophylacticantibiotics in orthognathic-surgery. Yoncei Med J 50: 55-59.
5. Stone HH, Haney BB, Zaura D, Eard K, Geheber E, et al. (1979) Prophylactic and preventive antibiotic Therapy, timing, duration, and economics. Ann surg 199:691-698.
6.Dinesh R, MoulyaMV, Siddiq A, Bharathi DR (2015) Evaluation of antibiotic usage as surgical prophylaxis in post-operative wards. Amer Jou of PharmTechRes 5:435-443.
7.Anguzu JR, Olila D (2007) Drug sensitivity pattern of bacterial isolates from septic post-operative wound on a regional referral hospital Uganda. Afr Health sci 8:148-154.
8.Martelli A, Mattioli FA (2000) Retrospective study showing the misuse of prophylactic antibiotics in patients undergoing appendectomy and cholecystectomy. Current Thera Res 61:534-539.
9.Sekimuto M, ImmakaY, Evans E, IshizakiT, Fukui T (2004) Practice variation inpreoperativeantibioticuseinjapan.InternJouforQualityinHealthCare16: 367-373.
10.Mohagheghi MA, Mosavi A, Khatemi M (2005) Community based out patent practice of antibiotics use in Tehran. Pharm Epid Drug Saf 14:135-138.
11.Yang SF, Nadimi S, Eggerstedt M, Thorpe E, Pittman A (2016) Antibiotic prophylaxis and postoperative wound infection rates in salvage surgery for head and neck cancer. Head Neck Cancer Res 1:1-5.
12.Kumar NS, VaraprasadCP,Thyag A, Sudarsanan S, Reddy YH (2016) Prescribing pattern of antibiotics in surgical department in a tertiary care teaching hospital. World Jour of Pharma Res 5:1138-1147.
13.Radji M, Aini F, Fauziyah S (2014) Evaluation of antibiotic prophylaxis administration at the orthopedic surgery clinic of a tertiary hospital in Jakarta. Indonesia Asian Pac J Trop Dis 4: 190-193.
14.Rajeshwari, Nagabushan H (2016) Drug utilization study in postoperative patients in obstetrics and gynecology ward of tertiary care hospital. Int J Basic Clin Pharmacol 5: 329-334.
15.Achong MR, Houser BA, Krusky JL (1977) Rational and irrational use of antibiotics in a Canadian teaching hospital. Can med Assoc J 116: 256-259.
16.Chaudhary K, Panka J, Atul J, Arvind MK, Gaurav, et al. (2015) Usage of antimicrobials in post-operative patients in a tertiary care teaching rural hospital in India 2: 328-337.

17.Food, Medicine and Healthcare Administration and Control Authority of Ethiopia Standard Treatment Guidelines for General Hospital (3rd eds.) Ethiopia 2014: 13-20.
18.Testa M, Stillo M, Giacomelli S, Scofonne S, Zotti CM (2015) appropriate use of antimicrobial prophylaxis: un observation study in 21 surgical wards of Public hospital 15: 63.
19.Roy BC, Hanifa A, Alam S, Naher S, Sarkar P (2015) Etiological spectrum of obstructive jaundice in tertiary care. Global Jou of Med Res 4: 1-5.
20.Baktygul K, Marat B, Ashirali Z, Rashid H, Sakamoto J (2011) An assessment of antibiotics prescribed at the secondary health-care level in the Kyrgyz republic 73: 157-168.
21.Soressa U, Mamo A, Hiko D, Prevalence FN (2016) Causes and management outcome of intestinal obstruction in Adama Hospital, Ethiopia. BMC Surgery 16: 1-8.
22.Argaw NA, Shumbash KZ, Asfaw AA, Hawaze S (2017) Assessment of surgical antimicrobial prophylaxis in orthopedics and traumatology surgical unit of a tertiary care teaching hospital in Addis Ababa. BMC Res Notes 10: 2-8.
23.Giri BR, Pant HP, Shankar PR, Sreeramareddy CT, Sen PK (2008) Surgical site infection and antibiotics use pattern in a tertiary care hospital in Nepal. JPMA 58: 148-151.
24.Hogerzeil HV (1995) Promoting rational prescribing: an international perspective. Br J Clin Pharmac 39: 1-6.
25.Fonseca LG, Conterno L (2004) Audit of antibiotic use in a Brazilian University Hospital. Braz J Infect Dis 8: 272-280.

SP-36

DEVELOPMENT AND CHARACTERIZATION OF PENTOXIFYLLINE LIPOSOMAL GEL FORMULATION

NARENDRA PENTU*, SOWJANYA BATTU, KONDE ABBULU

Department of Pharmaceutics, CMR College of Pharmacy, Kandlakoya (V), Medchal
***Email-id:narendrapentu@gmail.com**

ABSTRACT

Pentoxifylline, which have TNF antagonizing effect and anti-proliferative action in the treatment of psoriasis.It has limited bioavailability (20%) due to extensive first pass effect and also shorter half-life. The present study was to prepare and evaluate the feasibility of topical Carbopol gel formulation containing Pentoxifylline encapsulated liposomes. Pentoxifylline loaded liposomes were prepared by thin film hydration technique. The prepared liposomes were characterized for size, shape, lamellarity and entrapment efficiency. The size of vesicles was found to be 5.6 to 7.28 µm with high entrapment efficiency for the formulation containing 9:1 ratio of soya lecithin: cholesterol and 2:1 ratio of chloroform: methanol. Differential scanning calorimetric studies were performed to understand the phase transition behavior. The prepared liposomes incorporated into 1.5% Carbopol gel and the systems were evaluated for drug content, ex-vivo drug release and permeation parameters. The ex-vivo permeation of Pentoxifylline using Wister albino rat skin from liposomal gel was compared with that of plain drug gel. Ex-vivo permeation enhancement assessed from flux, enhancement ratio was significantly higher for liposomal gels compared to plain drug gel formulation. Stability data reveal that the formulations are more stable when stored at 4 ^{0}C. In conclusion, liposomal gel formulation of Pentoxifylline is an affordable drug with TNF antagonizing effect when compared with biologics in the treatment of psoriasis.

Keywords:entoxifylline, liposomes, psoriasis, permeation, Carbopol gel

INTRODUCTION

Carbopol gels are act as pharmaceutical aids. Carbopol gels are anionic hydrogel with good buffering capacity, which may contribute to the maintenance of the desired pH. Transdermal drug delivery have a hurdle i.e. stratum corneum[6]. Several mechanisms could be explained for the ability of liposomes to modulate the diffusion across skin. The fusion of vesicles on the surface of the skin can lead to the establishment of large concentration gradients of the intercalated drug across the skin and hence enhance skin permeation[6]. Pentoxifylline (1-(5'-oxohexyl)-3, 7-dimethylxanthine) is a highly water-soluble methylxanthine derivative with a pKa of 0.28. It is a nonselective phosphodiesterase inhibitor that is commonly used for the treatment of peripheral arterial diseases and intermittent claudication in the lower extremities in humans[9]. It improves peripheral circulation by reducing blood viscosity and increasing erythrocyte flexibility, inhibiting platelet aggregation , and reducing fibrinogen concentration . Further, PTX modulates the concentration of several pro- and anti-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-1-beta (IL-1β), IL-6, and IL-10, in vivoTNFα antagonist. TNFα is a key cytokine in the pathogenesis of psoriasis.Based on this effect, this drug have been successfully used as adjuvant therapy in the treatment of pemphigus vulgaris and resulted in a significant reduction in serum levels of TNF with significantly correlated clinical improvement.Beside their ant TNF effect, both drugs also exert an ant proliferativeeffect on keratinocytes and have been used in the treatment of psoriasis and psoriatic c arthritis with variable results[12]. It has a shorter elimination half-life and extensive first pass effect results in low bioavailability of the drug.Furthermore, oral administration and intravenous injection will cause adverse effects to some extent, including the most common ones of sicchasia, dizziness, headaches, anorexia. Therefore, PTX is better suited to external use for tropical disease[13.]

MATERIALS AND METHODES

Materials

Pentoxifylline was obtained from Sigma labs. Soya lecithin and Cholesterol was obtained by SD fine chemicals. Dialysis membrane [DM-70; molecular weight cutoff (MWCO) 12,000 Da] was purchased from Himedia (Mumbai, Maharashtra, India).All other chemicals used were of analytical or pharmacopoeial grade.

Method

Preparation of Liposomes

Pentoxifylline loaded liposomes were prepared by thin film hydration method. Multilamellar vesicles were formed by using this method. Lecithin, Cholesterol are dissolved in different organic solvents, and the organic solvent was evaporated by using rotary flask evaporator (for 30 min, at 42^0C, 60rpm) using a Rota vapor (Heidolph, Germany) and vacuum pump. The obtained thin film layer was dried overnight in a vacuum oven to ensure complete removal of residual organic solvent. The lipid film was hydrated by phosphate buffer solution pH 7.4 in which the drug Pentoxifylline was pre dissolved (Pentoxifylline 20 mg /mL), and this is maintained at above the gel-to-liquid-crystalline phase transition temperature of the phospholipids[10]. This dissolved layer is subjected to hand shake vigorously for 5min. and followed by heating in round-bottom flask and it was maintained at 40^0C for 60 min, 45rpm to anneal liposomes. The flask was then subjected to intermittent sonication in a bath Sonicator (Citizen, Mumbai) to reduce the vesicle size[1].

Preparation of Carbopol gel

As a base, Carbopol 934 was slowly dispersed into distilled water and allowed to swell for 12 h under normal temperature; Pentoxifylline (200 mg) and enhancer(Propylene glycol, Oleic acid) dissolved in glyceryl alcohol solution like Glycerin were added to the base while stirring. The Carbopol gel was thickened by a few drops of Triethanolamine added to adjust the Carbopol gel to pH 7. Distilled water was added to the gel for a final weight of 20 g.

Incorporation of Liposomes in to the Gel

Liposomes containing Pentoxifylline (separate from the unentrapped drug) were mixed into the 1.5% Carbopol gel with an electric mixer (25rpm, 2min.), the amount of Liposomes of Optimized formulation added into the gel, such that the prepared gel have 2% Pentoxifylline concentration (20 mg drug per 1gm of gel).Plain drug gels (2%w/w) were made under the same conditions. Instead of liposomes, those samples contained free Pentoxifylline were incorporated[11].

Characterization of Pentoxifylline liposomal gel

Physicochemical properties of Pentoxifylline liposomal gel

The liposomes enriched hydro gels were characterized for their physicochemical properties such as colour and pH.

Morphological Evaluation

Liposomal formulation hydrated with phosphate buffer solution and observed under Inverted microscope with 40X magnification. Liposomes were observed for size, lamellarity and shape of vesicular structure. The optimized liposomal formulation observed under optical microscope (SEM) for size and shape of vesicle.

Entrapment efficiency

Entrapment efficiency of liposomes was determined by centrifugation method. Aliquots' (1ml) of Liposomal dispersion were subjected to centrifugation on a laboratory Ultra centrifuge (Remi, India) at 8000 rpm for a period of 30min and the supernatant layer was separated to another centrifugation tube to centrifuge at 8000 rpm for a period of 30min. The clear supernatants were removed carefully to separate non- entrapped Pentoxifylline and absorbance recorded at 274nm on a Shimadzu 1800 UV-Visible spectrophotometer with respect to similarly treated empty liposomes as the blank. Entrapment efficiency was calculated from the difference between the initial amount of Pentoxifylline added and that present in the supernatant and was expressed as percentage of total amount of Pentoxifylline added.Amount of Pentoxifylline in supernatant and sediment gave a total amount of Pentoxifylline in 1ml Liposomal dispersion[5].

The percentage of drug encapsulation EE (%) was calculated by the following equation:

$$\text{Entrapment efficiency (\%)} = \frac{\text{Amount of drug in sediment}}{\text{Total amount of drug}} \times 100$$

In vitro release study

The release studies were carried out in 250 ml beaker containing 100 ml Phosphate buffer. Phosphate buffer pH 7.4 (100 ml) was placed in a 250 ml beaker. The beaker was assembled on a magnetic stirrer and the medium was equilibrated at 37 ± 5^0C. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non-entrapped pentoxifylline, liposome dispersion was filled in the dialysis membrane and other end was closed. The dialysis membrane containing the sample was suspended in the medium. Aliquots were withdrawn (5 ml) at specific intervals, filtered and the apparatus was immediately replenished with same quantity of fresh buffer medium[3].

Ex vivo permeation study

Male albino Wister rats weighing between 180-200 gm used in the study were obtained**.**The animals were housed in separate cages and maintained under controlled condition of temperature and the rats had free access to water and food until they were sacrificed for skin harvesting. The study was conducted with the prior approval of Institutional Animal Ethical Committee, Vaageswari College of Pharmacy.The rats were sacrificed with excess ether inhalation. The abdominal skin was exposed and hair was removed with hair clipper taking extreme precautions not to damage the skin. The rat abdominal skin was isolated, excised and the adhering subcutaneous fat, tissue and capillaries were removed with a pair of scissors. The heat separation technique was adopted to prepare the epidermis which involves soaking of the entire abdominal skin in water at 60°C for 45 seconds. The epidermis was washed with water, wrapped in aluminum foil and stored at -20°C till further use (used within 2 weeks of preparation)[2].

Drug content for liposomal gel formulation

Gel formulations (100mg) was dissolved in methanol and filtered and the volume was made to 100ml with methanol. The resultant solution was suitably diluted with methanol and absorbance was measured at 274nm using Shemadzu 1800 UV Visible Spectrophotometer. Drug content was determined from calibration curve for Pentoxifylline[10].

Skin retention studies

The extent of drug deposited in the epidermal layers (DCS) was determined. After course period of permeation study, the skin was removed from the diffusion cell and washed briefly in methanol for 15s to remove the adhering formulation. The skin was allowed to dry at room temperature for 10 min, chopped into pieces and sonicated for 30min using bath sonicator (Citizen, Mumbai, India) to leach out the drug. The samples were assayed for Pentoxifylline by UV Visible Spectrophotometer. [13]

Stability studies

The formulations stored in glass vials covered with aluminum foil were kept at room temperature and in refrigerator condition for a period of 30 days. At definite time intervals (15and 30 days), samples were withdrawn and hydrated with phosphate buffer solution (pH 7.4) and observed for any sign of morphological changes, Furthermore, the samples were also evaluated for particle size and percent retention of Pentoxifylline. [11]

Permeation data analysis

The cumulative amount of drug permeated (Q) was plotted against time. The steady-state flux (J) was obtained from the slope of linear portion of the cumulative amount permeated per unit area versus time. The effectiveness of penetration enhancers was determined by comparing the flux of PTX in the presence and absence of the enhancer. This was defined as the enhancement factor (EF), which was calculatedusing the following equation: EF = (drug flux of samples containing an enhancer)/ (drug flux of control sample without an enhancer)[7].

Phase transition study

Phase transition study was carried by Differential scanning calorimetry.The thermo tropic properties and phase transition behavior of pure drug, Phosphotidyl Choline, and cholesterol was studied by using differential scanning calorimeter. Thermo grams of both blank and Pentoxifylline liposomal dispersions were recorded individually. Average sample weight of 5± 2mg were heated in hermitically sealed aluminum pan over a temperature range of 20◦C–200◦C under a constant nitrogen gas flow of 30mL/min at a heating rate of 10◦C/min.the scan was recorded and plotted showing heat flow (w/g) on the Y-axis and temperature(^{0}C) on the X-axis.

Fourier Transform IR analysis

The FTIR spectrum of the components of liposomal formulation was recorded in the range of 4000 to 400cm$^{-1.}$ Pentoxifylline, soya lecithin and cholesterol are subjected for FTIR analysis, to study the interaction of the components.

Results and Discussion

Preparation and characterization of liposomes

Liposomes are formed by hydration of lipids with Phosphate buffer medium pH 7.4. Liposomes are prepared by taking different ratios of lipids (soya lecithin and cholesterol) among this 9:1 lipid ratio was selected for forming liposomes with varying concentrations of solvents in which the lipids are dissolved. Solvents have different soluble capacity of lipids and different contact angle with glass in forming thin film in evaporating flask. Solvent mixture chloroform: methanol in 2:1 ratio having the low contact angle with glass so that the surface area of lipid forming was increased i.e. entrapment efficiency also increased.

Microscopy:

Morphology of liposomes studied under compound microscope. The milk white solution of liposomal formulation observed under microscope for size and shape. All batches of liposomes prepared were viewed under microscope after suitable dilution with buffer solution. Vesicles are viewed by microscope with magnification 40X, spherical shape vesicles are observed and calculated the vesicle size. The optimized batch of liposome is observed under Scanning electron microscope for size and shape.SEM analysis of the liposomal formulation was shown that the size of the liposomes formed is 5.51µm and spherical shape of the dispersion.

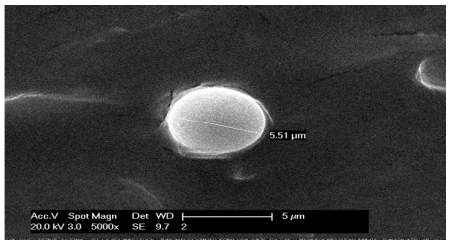


Figure 1:Scanning Electron Microscopy photograph of Optimized Pentoxifylline Liposomes shows spherical shape vesicle[7]

Lamellarity:

Liposomes are prepared by thin film hydration method, by using this technique liposomes are formed in multi lamellar vesicle structure. Lamellarity of Pentoxifylline liposomes were shown by using Boeco NIB-100 inverted microscope (made in Germany).

***Figure 2:*Observation of Pentoxifylline Liposomes under Inverted Microscope Boeco NIB-100[8]**

The cumulative amount release of Pentoxifylline from liposomal formulations was investigated for a period of 8 hours. F1 formulation showed maximum drug release in 8hr period, while other showed less amount of drug release. F1 formulation has correlation coefficient (r = 0.9870) and fallows drug release by peppas model.

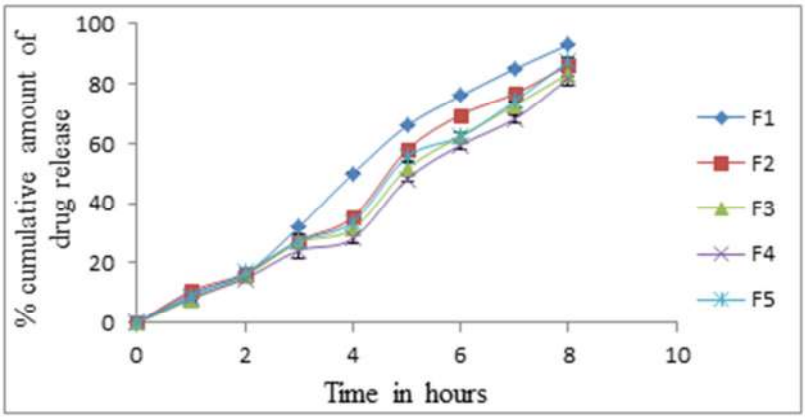


Figure 3:In vitro drug release profiles of Pentoxifylline liposomal formulation.

Ex vivo permeation study

Liposomal formulation incorporated in to the gel formulation and subjected for Transdermal permeability and comparison with the plain drug gel formulations having different permeation enhancer concentrations showed in table.

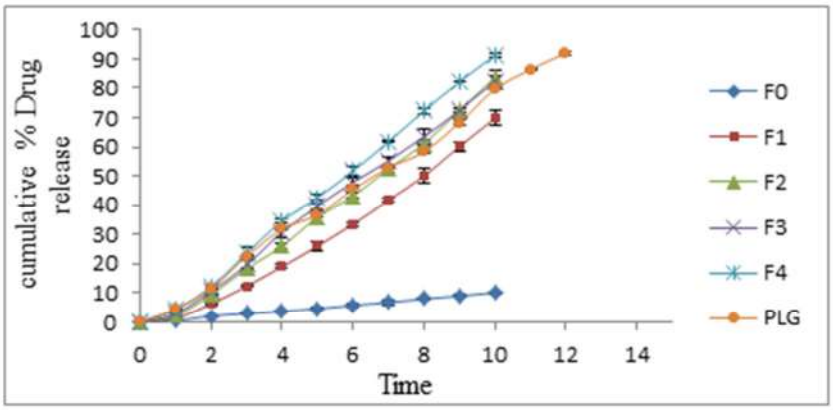


Figure 4:Ex vivo **cumulative drug permeation profiles of Pentoxifylline from various plain drug gel and liposomal gel formulations.**

Skin retention studies

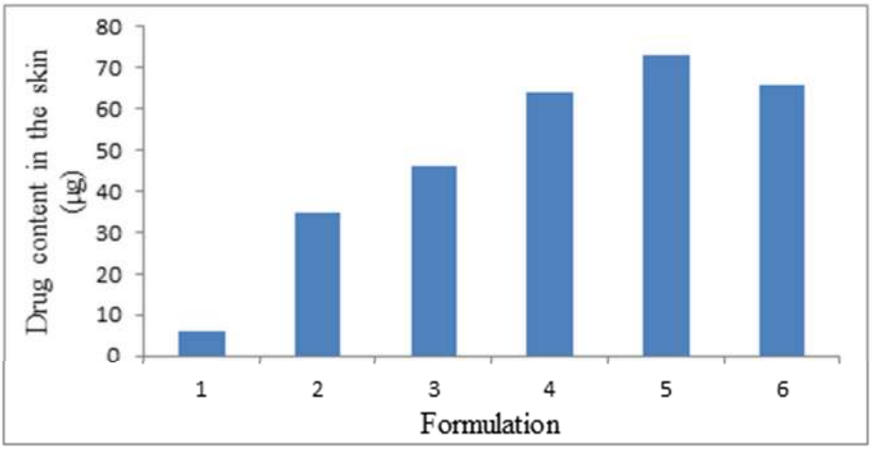


Figure 5:Drug deposited in the rat skin layers following treatment with different liposomal formulations after the time period

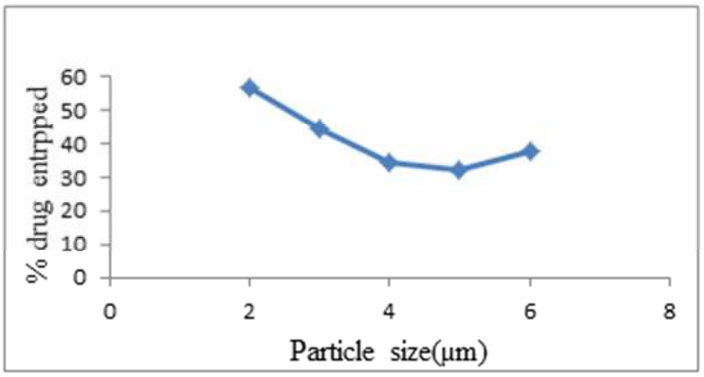


*Figure 6:***Graphical representation of Particle size Vs % Drug entrapment**

STABILITY STUDIES:

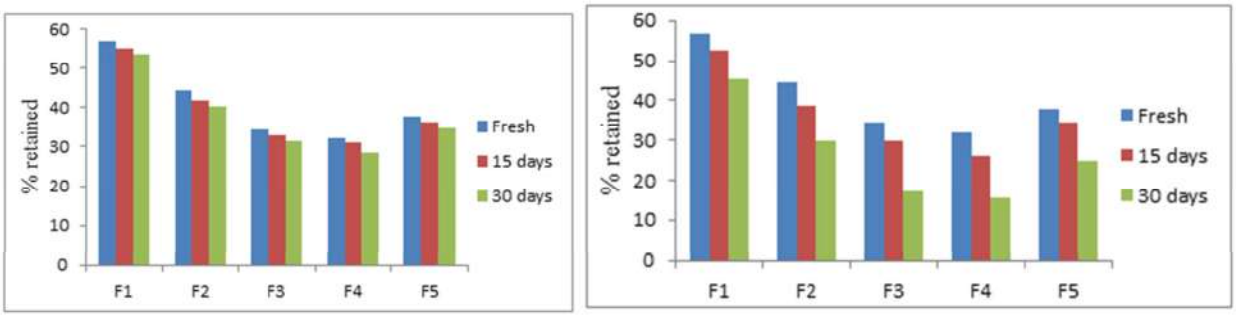


***Figure 7*:Percentage retention of Pentoxifylline liposome formulations upon storage in (A) Refrigerator and (B) Room temperature**

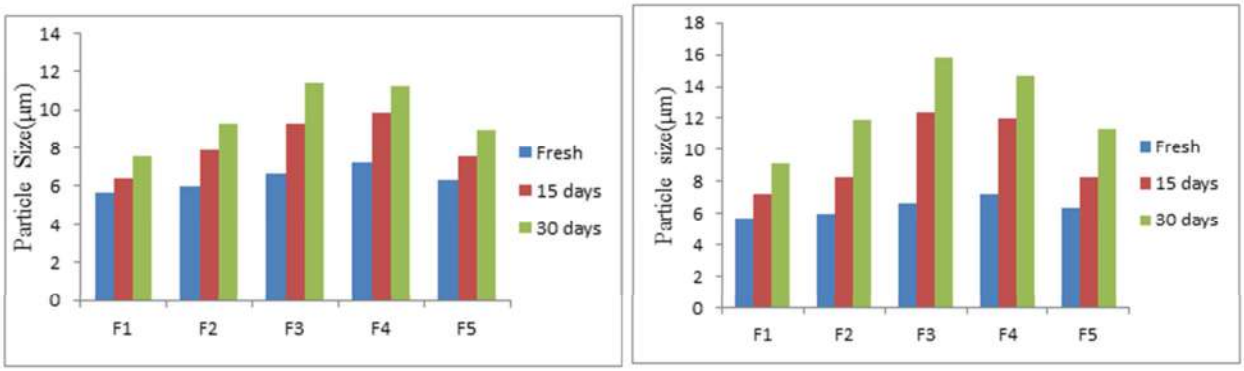


***Figure 8:*Change in size of Pentoxifylline liposomal formulations upon storage in (a) Refrigerator and (b) Room temperature**

***Table 1:*Composition of phosphotidyl choline, cholesterol and solvents for preparation ofliposomes.**

FORMULATION	PC (mg)	Cholesterol (mg)	SOLVENTS		
			CHOLOROFORM	METHANOL	ETHANOL
F1	270	30	2	1	-
F2	270	30	1	2	-
F3	270	30	1	-	3
F4	270	30	3	-	1
F5	270	30	1	-	-

***Table 2:*Pentoxifylline Gel formulation Optimized formula with % to be taken for 20Grams & 2Grams of Gel.**

Chemical	Category	% to be taken	20Gr of Gel	2Gr of Gel
Pentoxifylline	Hemorheologic agent	2	200mg	20mg
Carbapol 934(1.5%)	Gelling agent	60	12Grams	1.2Gr
Glyceryl alcohol		40	7.6Grams	0.75Gr
Propylene Glycol & Oleic acid*	Permeation Enhancer			
Propyl Paraben	Preservative	0.04	8mg	0.8mg
NaOH(0.2M)	Neutralizing agent	1 to 2 drops	1 to 2 drops	1 to 2 drops
Distilled Water		Q.S		
Total			20Grams	2Grams

***Table3:*Formulation chart of Pentoxifylline gel and liposomal gel formulations.**

S.No.	Formulation	F0	F1	F2	F3	F4	F5
1	Drug(mg)	20	20	20	20	20	-
2	Carbapol(934)	1.5%	1.5%	1.5%	1.5%	1.5%	1.5%
3	Propylene Glycol	-	3%	5%	-	-	-
4	Oleic acid	-	-	-	3%	5%	-
5	Pentoxifylline Liposomes(mg)	-	-	-	-	-	20*

***Table 4:*Physical characterization of Pentoxifylline liposomes.**

Formulation	Drug content (mg)	Drug entrapped (mg)	Particle size (µm)±SD	% Drug Entrapped±SD
F1	200	113.68	5.66±0.04	56.84± 0.73
F2	200	88.68	6.86±0.47	44.34±0.57
F3	200	69.10	7.87±0.68	34.55±1.07
F4	200	64.26	8.12±0.25	32.13±1.01
F5	200	75.58	7.28±1.08	37.79±0.83

***Table 5:* Permeation parameters of Pentoxifylline from plain drug gel and liposomal gel formulations across rat skin (*Mean*±SD; n =3).**

Formulations	R^2	Q_{10}(µg)	J(µg/cm^2/hr)	EF
F0	0.997	54.982	1.344	-
F1	0.994	389.32	9.510	7.07
F2	0.999	459.69	11.230	8.35
F3	0.995	631.82	15.44	11.488
F4	0.994	729.59	17.83	13.26
PLG	0.995	646.88*	13.18	-

***amount of Pentoxifylline liposomal gel permeated through the rat skin after 12 hr**

Conclusion

Pentoxifylline Liposomal gel formulation was successfully developed for transdermal drug delivery. The liposomal formulation physical evaluation reveals that, the importance of vesicle composition and the formulation containing Phosphotidyl choline and Cholesterol at a ratio of 9:1 seems to be more stable with desired physicochemical and permeability characteristics. The above study revealed the potential of liposomal gel with improvement in bioavailability of Pentoxifylline and further studies has to be conducted in order to optimize the stability for the efficient transdermal delivery of Pentoxifylline.

ACKNOWLEDGEMENT

Authors are thankful to Vaageswari College of pharmacy, Karimnagar, A.P, India, for providing the facilities to carry out this work.

REFERENCES:

1. Biju S.S, Sushama Talegaonkar, Mishra P.R and Khar R.K. Vesicuar systems: an overview. Indian journal of pharmaceutical sciences.April, 2006; 141-153.
2. Vyas S.P, Khar R.K. Targeted and Controlled Drug Delivery. New Delhi: CBS Publishers & Distributors; 2002.
3. Jain NK. Controlled and Novel Drug Delivery. New Delhi: CBS Publishers & Distributors; 1997.p.101-129.
4. Brandl M, Liposomes as drug carriers: a technological approach. Biotechnology Annual Review 2001; 7: 59-85
5. Raju Jukanti,Sruthi Sheela,Suresh Bandari, Prabhakar r. Veerareddy. Enhanced bioavailability of exemestane via proliposomes based transdermal delivery. Journal of pharmaceutical sciences 2011 Aug; 100(8).
6. Dr. Rakesh P. Patel, Hardik Patel and Ashok H. Baria. Formulation and evaluation of Carbapol gel containing liposomes of ketoconazole (part-II).International Journal of Drug Delivery Technology 2009; 1(2): 41-45.
7. Abdus Samad, Sultana Y, Aqil M. Liposomal Drug Delivery Systems: An Update Review. Current Drug Delivery 2007; 4:297-305.
8. Cristina Dinu Pirvu, Alina Ortan, Mircea Hirjau, Razvan Prisada, Dumitru Lupuleasa, Alexandru Bogdan. Studies Concerning the Optimization of the Pentoxifylline Encapsulation. Romanian Biotechnological Letters 2011; 16(1).
9. Medhat El-Mofty, Mohamed El-Darouti, Hoda Rasheed,Dalia Ahmed Bassiouny, Mona Abdel-Halim, Naglaa Sameh Zaki,Ghada El-Hanafy, Heba El-Hadidi, Omar Azzam, Amany El-Ramly &Marwa Fawzy. Sulfasalazine and pentoxifylline in psoriasis: A possible safe alternative. Journal of Derma tological Treatment. 201 1; 22: 31– 37.
10. Shivhare U.D, Ambulkar D.U, Mathur V.B, Bhusari B.P, Godbole M.D. Formulation And Evaluation Of Pentoxifylline Liposome Formulation.Digest Journal of Nanomaterials and Biostructures.2009;4(4):857-862.
11. Sharma Vijay K, Mishra D N, Sharma A KSrivastava B. Liposomes: Present Prospective and Future Challenges. International journal of current Pharmaceutical review and research 2010 Aug; 1(2).
12. Rang H.P, Dale M.M, Ritter J.M and Flower R.J.Rang and Dale's Pharmacology, Sixth Edition, Philadelphia: Churchill Livingstone Elsevier; 2007.
13. Omid Zargari MD, FAAD. Pentoxifylline: A drug with wide spectrum applications in dermatology. Dermatology Online Journal 2008 November; 14 (11): 2.
14. Alan Ward and Clissold stephen P. Pentoxifylline: A review of its Pharmacodynamic and Pharmacokinetic Properties, and its Therapeutic Efficacy.Drugs 1987; 34:50-97.

SP-37

A REVIEW ON RESTLESS LEGS SYNDROME

[*1]GOSHIKE BHAVITHA, [2]G.JEMIMAH, [3]M.SRI SWETHA, [4]AVSSS GUPTA, [5]JVC SHARMA

JoginpallyB. R. Pharmacy College,Hyderabad- 500075, Telangana, India.
*Corresponding Author Email: bhavithagoshike222@gmail.com,Tel:+91 7013847512

ABSTRACT:
Restless legs syndrome (RLS), a common neurological sensorimotor disorder in western countries, has gained more and more attention in Asian countries. The prevalence of RLS is higher in older people and females. RLS is most commonly related to iron deficiency, pregnancy and uremia. It is a sleep-related movement disorder that involves an almost irresistible urge to move the legs at night. This urge tends to be accompanied by unusual feelings or sensations, called "paresthesia," that occur deep in the legs. These uncomfortable sensations often are described as a burning, tingling, prickling or jittery feeling.The RLS symptoms show a significant circadian rhythm and a close relationship to periodic limb movements (PLMs) in clinical observations.

Keywords:Restless legs syndrome, Iron deficiency, Dopamine, A11 cell group, Augmentation.

INTRODUCTION:

Restless legs syndrome (RLS)/Willis-Ekbom disease (WED), is a neurological sensory-motordisorder that often disturbs sleep.[1] It is characterized by patients as an uncomfortable urge to move their legs in conditions of immobility.[1]Often, the urge to move is accompanied by an unpleasant sensation felt deep inside the affected limb which can be described as sensory disorders of "creeping", "crawling", "burning", "pulling" or "soda bubbling in the veins". As a result, this leads to a patients' symptoms being attributed to other medical conditions, such as peripheral neuropathy, leg cramps, and anxiety.

RISK FACTORS:

There are certain things that may put you in a higher risk category for RLS. But it's uncertain if any of these factors actually cause RLS.

Some of them are:

- **Gender**: Women are twice as likely as men to get RLS.
- **Age**: Although you can get RLS at any age, it's more common and tends to be more severe after middle age.
- **Family history**: You're more likely to have RLS if others in your family have it.
- **Pregnancy**: Some women develop RLS during pregnancy, particularly in the last trimester. This usually resolves within weeks of delivery.
- **Chronic diseases**: Conditions such as peripheral neuropathy, diabetes, and kidney failure, may lead to RLS. Often treating the condition relieves symptoms of RLS.
- **Medications**: Antinausea, antipsychotic, antidepressant, and antihistamine medications may trigger or aggravate symptoms of RLS.
- **Ethnicity**: Anyone can get RLS, but it's more common in people of Northern European descent.

PATHOPHYSIOLOGY:

Even though the majority of cases of primary RLS appear to be idiopathic in orgin, while secondary RLS is encountered in various underlying medical conditions, namely, iron deficiency anaemia, renal failure, and pregnancy

Dopaminergic system dysfunction:

The dopamine agonists and levodopa paves the path to prove that dysfunction of the dopaminergic system may play a role in the pathophysiology of the disease. The role of central dopaminergic system rather than

peripheral is corroborated by the fact that administration of peripheral dopamine antagonists does not decrease the efficacy of a dopamine agonist.[2]

Brain iron metabolism:
Defect in the brain iron metabolism may contribute to the pathogenesis of the disease. Iron is a cofactor for tyrosine hydroxylase. Therefore decrease in iron may affect the availability of dopamine.[3]There is a circadian variation in the activity of tyrosine hydroxylase that may account for the worsening of symptoms in the night. An inverse relationship has been suggested betweensymptom severity and serum ferritin levels finding, which is observed in children as well.

Role of neurotransmitters:
Hypocretin-1 (orexin A) are neurotransmitters produced by the hypothalamus that are essential for normal control of the sleep cycle. They increase arousal and are found to interact with the dopamine system.Inflammation and immune mechanisms:The studies reporting increased prevalence of small intestinal bacterial overgrowth and the case reports of RLS in the presence of infection such as HIV, Streptococcus, mycoplasma, Borrelia and hepatitis C infection and connective tissue diseases such as systemic lupus erythematosus and Sjogren's syndrome. The three gastrointestinal disorders, namely, Crohn's disease, irritable bowel disease and celiac disease are also associated with RLS.

CLINICAL FEATURES:
RLS causes discomfort in the legs that occurs at rest and is relieved by movements. The unpleasant sensations are often described by patients as creeping, crawling, pulling, stretching type of pain, often deep-seated and localised below the knee.Severe symptoms are associated with psychological impairments related to sleep disruption; poor concentration; and difficulties with work, travel and social events.

Figure-**1: OVERVIEW OF RLS**[3]

DIAGNOSIS:
Physical examination may identify secondary causes, such as a peripheral neuropathy or anaemia. Iron studies are important, with a ferritin level <50 µg/L considered significant in the context of RLS symptoms[4]. Polysomnography, useful to evaluate sleep quality (eg. poor sleep initiation or sleep fragmentation from restless legs/periodic limb movements) and the presence of concurrent sleep disorders, such as obstructive sleep apnoea.

MANAGEMENT:
Non Pharmacological Therapy:

- Sleep hygiene should be corrected before all the pharmacological treatment. Sleep deprivation, sleep disturbances and factors that can result in insomnia should all be avoided.
- Assess iron deficiency in all RLS patients. If iron deficiency is established either by a low ferritin (<50 µg/L) or low percentage iron saturation, a cause for iron deficiency should be investigated[5].
- Reduce or abstain from caffeine, nicotine and alcohol.
- Consider ceasing medications that may exacerbate RLS such as antidepressants, in particular selective serotonin reuptake inhibitors (SSRIs) or the serotonin norepinephrine reuptake inhibitors (SNRIs), neuroleptics, dopamine blocking agents (eg. anti-emetics), andsedating antihistamines.

Pharmacological Therapy:

Table-1: Commonly used drugs for RLS[3]

Dose	Initial dose	Usage daily dose range	Common adverse effects
Dopamine precursors			
Levodopa (+carbidopa or benserazide)	50mg	100-200mg	Nausea,vomiting,orthostatic, hypotension,hallucination,augmentationofsymptoms,insomnia
Dopamine agonists			As for levodopa plus nasal congestion and fluid retention
Ropinirole	0.25mg	0.5-3.0mg	
pramipexole	0.125mg	0.5-1.5mg	
Pergolide	0.025mg	0.5mg	Also risk of valvular heart disease and retroperitoneal or pleuroplumonary fibrosis
Non dopaminergic medications			
Gabapentin	300mg	600-2400mg	Sedation,dizziness,fatigue,somnolence,ataxia,sedation,tolerance
Clonazepam	0.25mg	0.25-2.0mg	Sedation,tolerance
Oxycodone	2.5mg	2.5-30.0mg	Constipation,sedation,nausea,vomiting,dependence

Benzodiazepines: Clonazepam is one of the first fewearlier drug that was considered in the treatmentof RLS[6].
Anticonvulsants: Gabapentin was effective in the treatment of mild-to-moderate RLS.
Opiods: Indication of opioids is as second-line agents in patients with augmentation, non-responders to dopamine therapy and in refractory RLS.
Others: Carbamazepine was effective, side effects like sedation and liver function abnormalities prevented it from being used for therapy of RLS. Iron supplementation was shown to be effective in the treatment of RLS patients who had low serum ferritin.

RLS in pregnancy
The prevalence of RLS in pregnancy, as is evident from the epidemiological data, is around 11–27%. The aetiology is most likely due to altered iron metabolism during pregnancy, thereby supporting the concept of iron deficiency as the cause of RLS. The aim of treatment in pregnancy is to provide symptomatic relief and improve the quality of life[7].

Pharmacological therapy in pregnancy: Dopaminergic agents: The use of pramipexole in pregnancy with parkinsonism, which did not show any adverse outcome. Levodopa appears to be safe in pregnancy[8].

Opioids: There is evidence supporting the use and safety of opioids in pregnancy**.**
Benzodiazepines: Benzodiazepines are considered to be effective in treating RLS in pregnancy
Anticonvulsants: Carbamazepine is the first anticonvulsant evaluated in the treatment of RLS.
Maternal and foetal problems.

Management of other secondary RLS:
The prevalence of RLS in haemodialysis patient is 6–60%. It appears to be more severe than primary RLS. The prevalence of RLS is highest among patients with parkinsonism. The long-term anti-parkinsonism treatment results in development of RLS. This may be attributed to the wearing-off episodes of levodopa that mimics symptoms of RLS or the augmentation effects of levodopa.

CONCLUSION:
A multidisciplinary approach in which GPs, neurologists, psychiatrists, sleep specialists, and psychologists engage in interactive communication may lead to improved outcomes, especially for patients.: The long-

term treatment of restless legssyndrome/Willis-Ekbom disease: evidence-based guidelines and clinical consensus bestpractice guidance: a report from the who experience augmentation or rebound. Through advancements in the diagnostic criteria, treatment strategies, and updated guidelines, primary care physicians have more resources than ever to diagnose and effectively manage RLS patients. Future advancement in drug therapy guidelines rests on continued research in investigating pharmaceutical long-term therapy.

REFERENCES:

1. Garcia-Borreguero D, Kohnen R, Silber MH, et al.: The long-term treatment of restless legssyndrome/Willis-Ekbom disease: evidence-based guidelines and clinical consensus bestpractice guidance: a report from the International Restless Legs Syndrome Study Group. SleepMed. 2013, 14:675-684.Doi: 10.1016/j.sleep.2013.05.016
2. Cervenka S,Pålhagen SE,Comley RA,et al. Support for dopaminergic hypoactivity in restless legs syndrome: a PET study on D2-receptor binding. *Brain* 2006*;*129*:*2017.Eisensehr I,Wetter TC,Linke R, et aNormal IPT and IBZM SPECT in drug-naive and levodopa-treated idiopathic restless legs syndrome. *Neurology* 2001*;*57*:*1307
3. Earley CJ,Heckler D, Allen RP The treatment of restless legs syndrome with intravenous iron dextran. *Sleep Med* 2004*;*5*:*231–5.
4. Montplaisir J, Boucher S, Nicolas A, et al. Immobilization tests and periodicleg movements in sleep for the diagnosis of restless leg syndrome. Mov Disord1998;13:324–9.
5. *Sun ER Chen CA, Ho G, Earley CJ Allen RP iron and the restless legs syndrome sleep 1998;21:371-7*
6. Garcia-BorregueroDFerini-StrambiL, KohnenR, et al. European guidelines on management of restless legs syndrome: report of a joint task force by the European Federation of Neurological Societies, the European Neurological Society and the European Sleep Research Society. *Eur J Neurol*2012*;*11*:*1385–96.
7. ManconiM, GovoniV, De VitoA, et al.Pregnancy as a risk factor for restless legs syndrome. *Sleep Med*2004*;*5*:*305–8
8. MucchiutM, BelgradoE, CutuliD, et al Pramipexole-treated Parkinson's disease during pregnancy. *MovDisord.*2004*;*19*:*1114–15.

SP-38

REVIEW ARTICLE ON MEDICATED LOLLIPOPS

[1]*M. PRAVALLIKA JADAV,[2*]B. ARUNKUMAR,[3] SWARUPA ARVAPALLI

Department of Pharmaceutics, JoginpallyB. R. Pharmacy College, Hyderabad- 500075, Telangana, India.Email: pravalikam7@gmail.com

ABSTRACT

The conventional dosage forms liketablets, capsules, syrups are inconvenient for pediatric, geriatric and bedridden patient because of difficult to swallow tablets, capsules or unpleasant taste of drug, resulting in poor patient compliance. The medicated lollipops are flavored medicated dosage form intended to be sucked and held in the mouth or pharynx containing one or more medicaments usually in the sweetened base. As a result, the demand for developing new technologies has been increasing day by day. Medicated lollipop is designed to improve patient compliance, acceptability, increase in bioavailability, reduction in gastric irritation, bypass of first pass metabolism and increase in onset of action. The lollipops were prepared by heating and congealing method using polymer. Lollipops are available in a number of colors and flavors, particularly fruit flavors.

KEY WORDS:**Medicated lollipop, flavored medicated dosage form, heating and congealing method.**

1. INTRODUCTION

Lollipops are solid dosage forms, containing medicament in a sweetened & flavored base, intended to dissolve slowly in the mouth. Lollipops are mainly contained sweetening agent flavoring agent, coloring agent, opacifiers& stabilizing agent. Lollipops are the flavored medicated dosage forms intended to be sucked and held in the mouth or pharynx containing one or more medicaments usually in the sweetened base. Lollipop are intended to relieve oropharyngeal symptoms, which are commonly caused by local infections and also for systemic effect provided the drug is well absorbed through the buccal linings or when it is swallowed. Lollipop are used for patients who cannot swallow solid oral dosage forms as well as for medications designed to be released slowly to yield a constant level of drug in the oral cavity orto bathe the throat tissues in a solution of the Drugs often incorporated into Lollipop include analgesics, anesthetics, antimicrobials, antidepressants, antiseptics, antitussives, aromatics, astringents, corticosteroids, decongestants, and demulcents. However, this is by no means an exhaustive list as many other drugs may lend themselves to delivery by a Lollipop. As well, both single andmulti-ingredient Lollipops can be compounded, depending on the particular patient's needs[1]. oral administration is the most popular route due to ease of ingestion, pain avoidance and most patient compliance. Traditional tablets and capsules are inconvenient for pediatricpatients because of difficult to swallow it or unpleasant taste of liquid dosage forms. Since from past decade, there has been an increased demand for more patient-friendly and compliant dosage forms. As a result, the demand for developing new technologies has been increasing day by day[2].

1.1. General consideration for designing medicated lollipop[3]:-

Since the development cost of a new chemical entity is very high, the pharmaceutical companies are now focusing on the development of new drug delivery systems for existing drug with animproved efficacy and bioavailability together with reduced dosing frequency to minimize side effects Typically, oral candidacies takes the form of an adherent white, curd like, circumscribed plaque anywhere within the oral cavity. There are many drugs dosage forms like lozenges, tablets, inhalers, and syrups, are in markets for the treatment of the same. These preparations are commonly used for the purpose of local effect or systemic effect". New drug design to this area always benefit for the patient, physician and drug industry. There are several dosage forms like in the market; there is a need for more dosage forms which acts effectively and locally as well as systematically.

ADVANTAGES OF MEDICATED LOLLIPOPS[4] :

 Having formulas that are easy to change and can be patient specific.

 It has a pleasant taste and it extends the time that a quantity of drug remains in the oral cavity to elicit a therapeutic effect also, pharmacist can prepare lollipops extemporaneously with minimal equipment and time.
 Lollipops can be given to those patients who have difficulty in swallowing.
 It extends the time of drug in the oral cavity to elicit a specific effect.
 Easy to prepare with minimum amount of equipment and time
 Do not require water intake for administration. Technique is non-invasive, as is the case with parenteral.

DISADVANTAGES OF MEDICATED LOLLIPOPS[4]:

 Heat labile drugs cannot be used in this formulation because of the high temperatures required for preparation.
 Drugs having minimum bitter taste are suitable.

1.2 Medicaments Required for Preparation of Medicated Lollipops[4]:-

Drugs which are required for the preparation of medicated lollipops belong to one of the following categories:

Figure-1: **Medicaments Required for Preparation of Medicated Lollipops[4]**

2.Types of Lollipops[6] :-

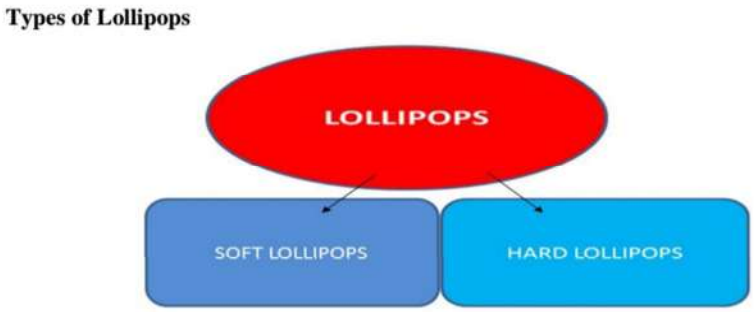


Figure-2:Types of Lollipops[6]

2.1. Hard Lollipops[7]:

Hard lollipops are the solid syrups of sugar. These are prepared by firstly heating sugars and other ingredients together and then pouring the mixture into a mould of desired shape. Hard lollipops are similar to hard candies but the difference is that hard lollipop contains a stick upon which the candy base sticks/adheres. In fact, formulas of many hard lollipops are the modifications of hard candies formulas. Low moisture content is needed to prepare this kind of dosage form and usually have a moisture content of 0.5%-1.5%. So boiling the sugar mixture is needed to evaporate the water during the compound process. Hard candy lollipops are mixtures of sugar and other carbohydrates in an amorphous (or) glassy condition. Hard

lollipops should not disintegrate immediately but instead provide a slow, uniform dissolution (or) erosion over 30 minutes.

2.1.1. Manufacturing of Hard Lollipops

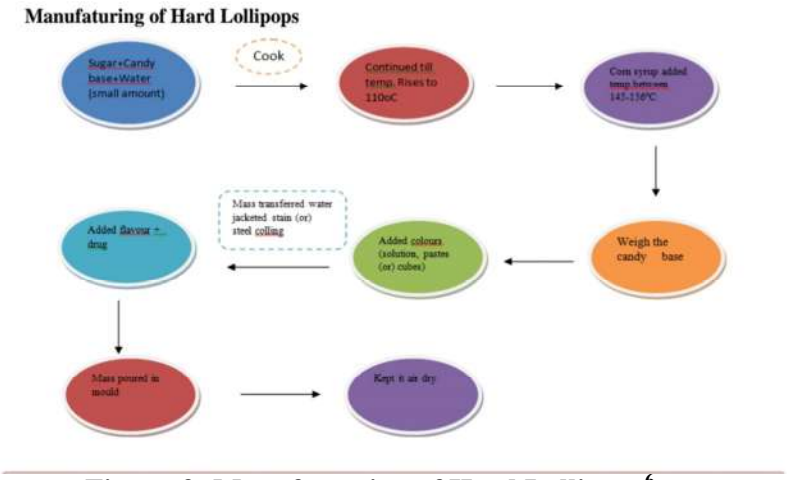


Figure-3 :Manufacturing of Hard Lollipops[6]

2.2. Soft Lollipops[6]

Soft lollipops have become popular because of ease with they can be extemporaneously prepared and their applicability to a wide variety of drugs. The base usually consists of a mixture of various PEGs, acacia (or) similar materials glycerol gelatin (or) an acacia: sucrose base. These lollipops may be coloured andflavoured and they can be either slowly dissolved in the mouth (or) chewed, depending on the intended effect of the incorporated drug

3.SOME MARKETED PRODUCTS[5]:-

3.1. Rainbow Swirl Lollipops:

Attractive to the eye, rainbow swirl lollipops come in exciting flavor combinations like and strawberry and champagne, tropical mix, and fruit salad. They are ideal as party ornaments and come in many different sizes. Swirl lollipops in two-flavor combinations, for example, strawberry, are also quite popular.

3.2. Yummy Earth Vitamin C Lollipops:

Yummy Earth Vitamin C Lollipops are very tasty, and they have the advantage of offering a great nutritious value. When kids are not getting enough Vitamin C from their regular diet, these fun, organic lollipops can be a great supplement.

4.CONLUSION[3]

Medicated lollipop is an novel approach for increasing pediatric elegance. Medicated lollipop will be ideal dosage forms for pediatric patients. These will have additional advantagesof patient compliance, convenience andcomfortless for efficient treatment including low dose, immediate onset of action, reduced dosage regimen and economic. This will offer betterinnovative dosage form. Medicated lollipop enjoy an important position in pharmacy and will continue to remain at the same in future.

5.ACKNOWLEDGEMENTS

I would like to express my deep gratitude to SwarupaArvapalli madam for her valuable and constructive suggestions during the review work.

I would like to express my sincere gratitude towards our beloved principal Dr. J.V.C. Sharma and our vice principal Mr. A.V.S.S.S. Gupta, JoginpallyB.R. Pharmacy College, Hyderabad, for supporting and encouraging me in this regard with great ease and precision.

6.REFERENCES

1. Prathap, Dr.Subhranshu Panda and A. Anusha,Department of Pharmaceutics,Vikas College of Pharmacy, Vissannapeta, Formulation and Evaluation of the SuckableChewable Medicated Lollipops of Venlafaxine Hcl World Journal of PharmaceuticalResearch Volume 5, Issue 01, 568-579**.**
2. Gupta VR, Halakatti PK, Lakshmi MN. Mouth dissolving tablets – an innovative
3. Suchitapundir,AbhayMurari Lal Verma,GRD Institute of Management AndTechnology,Department of Pharmacy,Rajpur Road, Dehraduan.Review Article onLozenges,Journal der PharmazieForschung Vol-2:No-1: 1-10: 2014.
4.PerumallaJagadeesh*, PadmasettyJyothi, G. Nethra Vani and S. Dasthagiri Departmentof Pharmaceutics, Sri Lakshmi Venkateswara Institute of Pharmaceutical Sciences,Proddatur. Review of Medicated Lollipops, WJPR Volume 6, Issue 3, 399-405.
5.www.ebay.com
6.Harsh Mohan (2000), *Text Book of Pathology – The Oral Cavity and Salivary Glands*, 4th Edition, pp. 494-496, Jaypee Brothers, Medical Publishers (P) Ltd., New Delhi.
7. Jain N K (2005), Controlled and Novel Drug Delivery, Chapter 16-Mucoadhesive Drug

SP-39

SYNTHESIS AND CYTOTOXIC EVALUATION OF SOME 1-(6-METHYL-4-PHENYL-2-OXO-1,2,3,4- TETRAHYDROPYRIMIDINYL)-3(ARYL)-2-PROPENE-1-ONE DERIVATIVES

RASAPELLY RAMESH KUMAR[1]*, N. KANNAPPAN[2] AND J. DEVILAL[3]

*[1]*Department of Pharmaceutical Chemistry, MLR Institute of Pharmacy, Dundigal,Hyderabad, Telangana, India.*
[2]Department of Pharmaceutical Chemistry Annamalai University, Chidambaram, Tamilnaadu, India.
[3]Department of Pharmaceutical Chemistry, Geethams School of Pharmacy, Hyderabad, Telangana, India.

ABSTRACT

Condensation of 5-acetyl-4-phenyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine with different aromatic aldehydes led to the formation of the new chalcones (3a-3m) in high yields.. The synthesized compounds were purified by column chromatography. The chemical structures of the title compounds were elucidated by IR, NMR and Mass spectral data and elemental analyses. The cytotoxicity screening in different human cancer cell lines (A-549 lung and HT-29 colon) by the MTT assay showed that 3a and 3kare active compounds.

Keywords: Cytotoxicactivity; chalcone; Biginelli reaction and dihydropyrimidinones.

INTRODUCTION

Chalcones (1,3-diaryl-2-propen-1-ones) are diverse group of compounds which can be obtained as well as synthesized from natural sources[1]. Natural and synthetic chalcones have shown broad spectrum of biological activities such as anti-inflammatory[2], antifungal[3,4], antibacterial[5], antimalarial[6] and anticancer[7].

Pyrimidine nucleus is a pharmacophoric scaffold and represents a class of heterocyclic compounds with a wide range of biological applications. Many of them are widely used as anticonvulsants[8], and analgesics[9]. Some compounds containing pyrimidine moiety were reported to possess anti-inflammatory[10], antimicrobial[11], and antitumor activities[12–14]. Recent development of anticancer agents involves structural modification of chalcones to improve their bioavailability and to study the role of various substituents on phenyl ring[15]. Dihydropyrimidines (DHPMs) comprise of a pyrimidine scaffold having a resemblance with the structure of the nucleic acids has a great importance in new drug discovery.

The aim of the present study was to synthesize 1-(6-methyl-4-phenyl-2-oxo-1,2,3,4-tetrahydropyrimidinyl)-3(aryl)-2-propene-1- one (3a-3m)derivatives as depicted in Scheme-1 and evaluated for cytotoxic activity against two human cell lines; A-549 lung and HT-29 colon using the MTT assay.

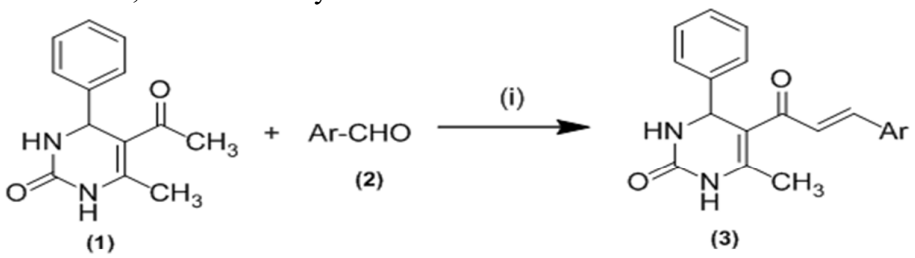
MATERIALS AND METHODS

Chemistry

Melting points were recorded in open capillaries on LABINDIA melting point apparatus (MEPA MP08050204) and were uncorrected. IR spectra were recorded on Perkin Elmer FTIR Spectrometer (Spectrum RXI) using KBr pellet technique. ^{1}H-NMR spectra were recorded on Bruker Advance II 400 MHz spectrometer in $CDCl_3$ using TMS as internal standard. Mass spectra (ESI) were recorded on Waters Micromass Q-TOF Micro and elemental analyses were performed using Thermo EA 2110 series elemental analyser. All chemicals used were of analytical grade and commercially available from E. Merck, Mumbai. Solvents were used without further purification. Silica gel (100–200 mesh; E. Merck, Mumbai) was used for column chromatography. All the reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F254 (mesh) (E. Merck, Mumbai) and spots were visualized under UV light (254 nm).

General procedure for the synthesis of the 1-(6-methyl-4-phenyl-2-oxo-1,2,3,4- tetrahydropyrimidinyl)-3(aryl)-2-propene-1- one (3a-3m):

5-acetyl-4-phenyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine**(1)** have been prepared by reported procedure[17]. A mixture of compound 1 (0.1mol) and aromatic aldehyde **2** (0.1mol) were dissolved in ethanol (30 ml), to the clear reaction mixture 10% NaOH was added drop wise the reaction mixture was stirred at room temperature over night. The reaction mixture was monitored by TLC using ethylacetate: hexane (20%). After completion of reaction neutralized with dilute hydrochloric acid the precipitated product is filtered, washed with water, dried and recrystalised from absolute ethanol



Reaction conditions: (i) absolute ethanol, 10% NaOH, Room Temperature, 12-15hrs.

Scheme 1: Synthesis of dihydropyrimidine chalcones (3a-3m)

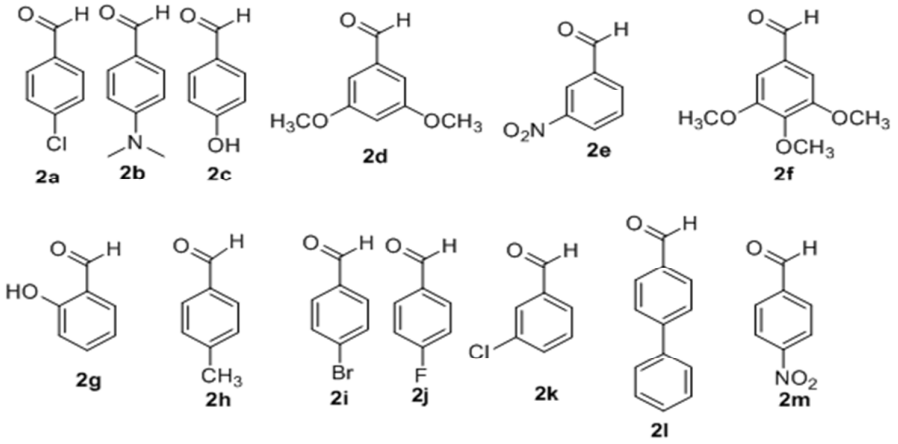


Figure 1: Various aldehyde used in the present study (2a-2m)

*1-(6-methyl-4-phenyl-2-oxo-1,2,3,4-tetrahydropyrimidinyl)-3(4-chlorophenyl)-2-propene-1-one.**(3a):***

IR (KBr) cm^{-1}: 3348(NH), 1628(C=O), 1571(C=C), 1123(C-Cl). ^1H NMR ($CDCl_3$ 400MHz): δ 2.29(s,3H,- CH_3), 5.49(s,1H,H of pyrimidine ring), 6.54(d,1H,*J*=17.6Hz,-CH=CH-), 6.70(d,1H,*J*=7.2Hz,-CH=CH-), 7.22(m,5H,Ar-H), 7.46(d,2H, *J* =8.8Hz), 7.70(d,2H, *J* =8.8 Hz), 7.89(s,1H,-NH), 9.26(s,1H,-NH). Elemental analysis for $C_{20}H_{17}N_2O_2Cl$: calculated: C, 68.08; H, 4.85; N, 7.94; found: C, 68.18; H, 4.82; N, 7.95.

*1-(6-methyl-4-phenyl-2-oxo1,2,3,4-tetrahydropyrimidinyl)-3(4-dimethylaminophenyl)-2-propene-1- one. **(3b):***

IR (KBr) cm^{-1}: 3321(NH), 1642(C=O), 1629(C=C), ^1H NMR ($CDCl_3$ 400MHz): δ 2.22(s,3H,- CH_3), 3.43(s,6H,-N(CH_3)$_2$), 5.19(s,1H,H of pyrimidine ring), 6.24(d,1H,*J* =6.8Hz,-CH=CH-), 6.44(d,1H, *J* = 17.6Hz,-CH=CH-), 8.15(m,5H,Ar-H), 8.43(d,2H, *J* =8.4Hz), 8.76(d,2H, *J* =8.4Hz), 8.33(s,1H,-NH), 9.95(s,1H,-NH). Elemental analysis for $C_{22}H_{22}N_2O_4$: calculated: C, 73.10; H, 6.41; N, 11.62; found: C, 73.13; H, 6.37; N, 11.63.

*1-(6-methyl-4-phenyl-2-oxo-1,2,3,4-tetrahydropyrimidinyl)-3(4-hydroxyphenyl)-2-propene-1- one.**(3c):***

IR (KBr) cm^{-1}: 3526(OH), 3305(NH), 1617(C=O), 1575(C=C).^1H NMR ($CDCl_3$ 400MHz) : δ 2.30(s,3H,- CH_3), 5.19(s,1H,H of pyrimidine ring), 6.32(d,1H, *J* =8.8Hz,-CH=CH-), 6.53(d,1H, *J* =19.2Hz,-CH=CH-), 7.17(m,5H,Ar-H), 7.53 (d,2H, *J*=8.4Hz), 7.67(d,2H, *J*=8.4Hz) 8.77(s,1H,-NH), 10.16(s,1H,-NH). 10.35(s,1H, -OH). Elemental analysis for $C_{20}H_{18}N_2O_3$: calculated: C, 71.84; H, 5.42; N, 8.38; found: C, 71.85; H, 5.38; N, 8.38.

*1-(6-methyl-4-phenyl-2-oxo-1,2,3,4-tetrahydropyrimidinyl)-3(3,5-dimethoxyphenyl)-2-propene-1-one.**(3d):***

IR (KBr) cm^{-1}:3327(NH), 1673(C=O), 1469(C=C) ,11939C-O-C). ^1H NMR ($CDCl_3$ 400MHz): δ 2.35(s,3H,- CH_3), 3.91(s,3H,OCH_3), 3.94(s,3H,OCH_3), 5.21(s,1H,H of pyrimidine ring), 6.91 (d,1H, *J*=8.8Hz,-CH=CH-), 7.24 (d,1H, *J*=17.2Hz,-CH=CH-), 7.32(m,8H,Ar-H), Elemental analysis for $C_{22}H_{22}N_2O_4$: calculated: C, 69.83;H, 5.85;N, 7.40; found: C, 69.84; H, 5.82; N, 7.40.

*1-(6-methyl-4-phenyl-2-oxo-1,2,3,4-tetrahydropyrimidinyl)-3(3-nitrophenyl)-2-propene-1-one.**(3e):***

IR (KBr) cm^{-1}: 3356(NH), 1652(C=O), 1584(C=C). ^1H NMR ($CDCl_3$ 400MHz): δ 2.53(s,3H,- CH_3), 4.92(s,1H,H of pyrimidine ring), 6.75(d,1H, *J*=17.6Hz,-CH=CH-), 6.85(d,1H, *J*=7.6Hz,-CH=CH-), 7.12(m,5H,Ar-H), 7.27(m,3H,Ar-H), 7.46(s,1H,-NH), 8.02(s,1H,-NH). Elemental analysis for $C_{20}H_{17}N_3O_4$: calculated: C, 66.11; H, 4.71; N, 11.56; found: C, 66.11; H, 4.68; N, 11.57.

*1-(6-methyl-4-phenyl-2oxo-1,2,3,4-tetrahydropyrimidinyl)-3(3,4,5-trimethoxyphenyl)-2-propene-1-one.**(3f):***

IR (KBr) cm^{-1}: 3327(NH), 1633(C=O), 1592(C=C),1230(C-O-C): ^1H NMR ($CDCl_3$ 400MHz) : δ 2.29(s,3H,- CH_3), 3.61(s,3H,-OCH_3), 3.91(s,6H,-OCH_3), 5.19(s,1H,H of pyrimidine ring), 6.24(d,1H, *J*=5.6Hz,-CH=CH-), 6.45(d,1H, *J* =16.8Hz,-CH=CH-),7.95(m,7H,Ar-H), 8.53(s,1H,-NH), 8.76(s,1H,-NH). Elemental analysis for $C_{23}H_{24}N_2O5$: calculated: C, 67.63; H, 5.92; N, 6.86; found: C, 67.64; H, 5.88; N, 6.86.

*1-(6-methyl,4-phenyl-2-oxo-1,2,3,4-tetrahydropyrimidinyl)-3(2-hydroxyphenyl)-2-propene-1-one. **(3g):***

IR (KBr) cm^{-1}: 3436(OH), 3320(NH), 1720(C=O), 1623(C=C).^1H NMR ($CDCl_3$ 400MHz): δ 2.35(s,3H,- CH_3), 5.42(s,1H,H of pyrimidine ring), 6.46(d,1H, *J* =8.8Hz,-CH=CH-), 6.62(d,1H, *J* =19.2Hz,-CH=CH-), 7.16(m,9H,Ar-H), 8.92(s,1H,-NH), 9.13(s,1H,-NH). 9.38(S,1H,-OH). Elemental analysis for $C_{20}H_{18}N_2O_3$: calculated: C, 71.84; H, 5.42; N, 8.38; found: C, 71.85; H, 5.38; N, 8.38.

*1-(6-methyl-4-phenyl-2-oxo-1,2,3,4-tetrahydropyrimidinyl)-3(4-methylphenyl)-2-propene- 1-one.**(3h):***

IR (KBr) cm^{-1}: 3328(NH), 1618(C=O), 1522(C=C), ^1H NMR ($CDCl_3$ 400MHz) : δ 2.33(s,3H,- CH_3), 2.77(s,3H,-CH_3), 5.18(s,1H,H of pyrimidine ring), 6.33(d,1H, *J*=7.6Hz,-CH=CH-), 6.52(d,1H, *J*=16.4Hz,-CH=CH-), 7.18(m,5H,Ar-H), 7.56(d,2H,J=6Hz), 7.67(d,2H,J=6Hz) 8.77(s,1H,-NH), 10.16(s,1H,-NH). Elemental analysis for $C_{21}H_{20}N_2O_2$: calculated: C, 75.88; H, 6.06;N, 8.43; found: C, 75.90; H, 6.02; N, 8.43.

*1-(6-methyl-4-phenyl-2-oxo-1,2,3,4-tetrahydropyrimidinyl)-3(4-bromophenyl)-2-propene-1-one.**(3i):***

IR (KBr) cm^{-1}: 3328(NH), 1684(C=O), 1575(C=C). ^1H NMR ($CDCl_3$ 400MHz) : δ 2.28(s,3H,-CH_3), 5.41(s,1H,H of pyrimidine ring), 6.83(d,1H, *J* =7.2Hz,-CH=CH-), 7.32(d,1H, *J* =16.5Hz,-CH=CH-), 8.12(m,9H,Ar-H), 7.83(s,1H,-NH), 8.92(s,1H,-NH). Elemental analysis for $C_{20}H_{17}N_2O_2Br$: calculated: C, 60.55;H, 4.31;N, 7.06; found: C, 60.60; H, 4.29; N, 7.07.

*1-(6-methyl-4-phenyl-1,2,3,4-dihydropyrimidinyl)-3(4-fluorophenyl)-2-propene-1-one.**(3j):***

IR (KBr) cm^{-1}. 3329(NH), 1635(C=O), 1529(C=C), 1192(C-F). ^1H NMR ($CDCl_3$ 400MHz) : δ 2.39(s,3H,- CH_3), 5.25(s,1H,H of pyrimidine ring), 6.68(d,1H,J=8.8Hz,-CH=CH), 7.12(m,5H,Ar-H), 7.25(d,1H, *J* =16Hz,-CH=CH-),7.53(d,2H, *J* =8.8Hz), 7.72(d,2H,J=8.8 Hz), 8.01(s,1H,-NH), 9.74(s,1H,-NH). Elemental analysis for $C_{20}H_{17}N_2O_2F$: calculated: C, 71.42; H, 5.09; N, 8.33; found: C, 71.64; H, 5.07; N, 8.35.

*1-(6-methyl-4-phenyl-2-oxo-1,2,3,4-tetrahydropyrimidinyl)-3(3-chlorophenyl)-2-propene-1-one.**(3k):***

IR (KBr) cm^{-1}: 3356(NH), 1674(C=O), 1469(C=C). ^1H NMR ($CDCl_3$ 400MHz) : δ 2.42(s,3H,-CH_3),5.31(s,1H,H of pyrimidine ring), 6.54(d,1H,J=16.4Hz,-CH=CH-), 6.91(d,1H, *J* =8.4Hz,-CH=CH-),7.32(m,6H,Ar-H), 7.53(m,3H Ar-H), 7.63(s,1H,-NH), 8.12(s,1H,-NH). Elemental analysis for $C_{20}H_{17}N_2O_2Cl$: calculated: C, 68.08; H, 4.85; N, 7.94; found: C, 68.18; H, 4.82; N, 7.95.

*1-(6-methyl-4-phenyl-2-oxo-1,2,3,4-tetrahydropyrimidinyl)-3(phenyl)-2-propene-1-one.**(3l):***

IR (KBr) cm^{-1} :3320(NH), 1652(C=O), 1460(C=C). ^1H NMR ($CDCl_3$ 400MHz) : δ 2.34(s,3H,- CH_3), 5.56(s,1H,H of pyrimidine ring), 6.16(d,1H, *J* =9.2Hz,-CH=CH-), 6.51(d,1H, *J* =17.2Hz,-CH=CH-), 7.00(m,10H,Ar-H),7.80(s,1H,-NH), 8.90 (s,1H,-NH). Elemental analysis for $C_{20}H_{18}N_2O_2$: calculated: C, 75.45; H, 5.69;N, 8.80; found: C, 75.47; H, 5.66; N, 8.80.

*1-(6-methyl-4-phenyl-2-oxo-1,2,3,4-tetrahydropyrimidinyl)-3(4-nitrophenyl)-2-propene-1-one.**(3m):***
IR (KBr) cm^{-1}: 3332(NH), 1790(C=O), 1671(C=C). ^1H NMR ($CDCl_3$ 400MHz) : δ 2.35(s,3H,- CH_3), 5.48(s,1H,H of pyrimidine ring), 6.97(d,1H, *J* =7.2Hz,-CH=CH-), 7.19(d,1H, *J* =16.5Hz,-CH=CH-), 7.68(m,9H,Ar-H), 8.17(s,1H,-NH), 8.91(s,1H,-NH). Elemental analysis for $C_{20}H_{17}N_3O_4$: calculated: C, 66.11; H, 4.71; N, 11.56; found: C, 66.11; H, 4.68; N, 11.57.

Cytotoxicity studies

In order to assess the *in vitro* cytotoxicity potential of these compounds, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on A-549 and HT-29 cell lines was performed. Exponentially growing cell lines were harvested from 25 cm^2. Tissue culture flasks and a stock cell suspension (1×10^5 cell/mL) were prepared. A 96-well flat-bottom tissue culture plate was seeded with 1×10^4 cells in 0.1 ml of MEM and DMEM mediums supplemented with 10% FBS and allowed to attach for 24 h. Test compounds were prepared just prior to the experiment in 0.5% DMSO and serially diluted with medium to get the working stock of 500, 250, 125 and 62.5µg/mL solution. After 24 h of incubation, cells were treated with 20 µL of test solutions from the respective top stocks, 80µL of fresh medium was added, and the cells were incubated for 48 h. The cells in the control group received only the medium containing 0.5% DMSO. Each treatment was performed in triplicates. After the treatment, the drug containing media was removed and washed with 200µL of phosphate buffered saline (PBS). To each well of the 96-well plate, 100µL of MTT reagent (stock, 1 mg/mL in PBS) was added and incubated for 4 h at 37°C. After 4 h of incubation, the plate was inverted on tissue paper to remove the MTT reagent. To solubilize formazan crystals in the wells, 100µL of 100% DMSO was added to each well. The optical density was measured by an enzyme-linked immunosorbent assay plate reader at 540 nm[16].

Results and discussion

Chemistry

As illustrated Scheme-1 the 1-(6-methyl-4-phenyl-2-oxo-1,2,3,4-tetrahydropyrimidinyl)-3(aryl)-2-propene-1- ones have been synthesized. The mechanism of Claisen–Schmidt condensation between 5-acetyl-4-phenyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine (1) and appropriate aromatic aldehydes (Fig.1) in presence of alcoholic basic medium results in the formation of different chalcones (3a–3m). The chemical structures were confirmed through physical and spectral data. The ^1H NMR spectrum 3a showed signal δ 6.719 (*J*=7.2 Hz) and δ 6.592 (*J*=17.6Hz), the aromatic protons are shown in multiplets around 7.22-7.78. The LC-MS showed its molecular ion peak at 353 (M+H), the higher magnitude of coupling constants (*J* value) for both protons indicate *trans* configuration, based on the above spectral data the structure of the compound 3a was confirmed as 1-(6-methyl,4-phenyl-2-oxo-1,2,3,4-tetrahydropyrimidinyl)-3(4-chlorophenyl)-2-propene-1-one.

Cytotoxic activity

The data obtained from *in vitro* cytotoxicity screening suggest that compounds (3a-3m)exhibited milb to moderate activity. In addition, it was found that 3a showed potent against the A-549 cell line with IC_{50} value of 118 µg/mL and HT-29 cell line with IC_{50} value of 126 µg/mL, and could be due to presence of chloro group at 4th position on aromatic ring-B of chalcone. To determine the SAR, functionality modification was carried out on the aromatic ring, while different groups were introduced at the side chain of DHPMs. The possibility to change the position of chlorine from para to meta in the phenyl ring was then explored (3a and 3k). Interestingly, this modification proved detrimental, Compounds bearing electron withdrawing groups (3e, 3i, 3j and 3m) on the aromatic core showed improved cytotoxic activity, where as electron releasing groups could have decrease the cytotoxic activity (3b, 3c, 3d, 3f, 3g and 3l). Therefore, such compounds would represent a useful cytotoxic agent and deserves further investigation and derivatization.

The results obtained from *in vitro* screening of test compounds are summarized in **Table 1.**

Table 1: IC_{50} of compounds 3a-m by MTT assay IC_{50}[a] (µg/mL)

Compound code	A-549	HT-29
3a	121	127
3b	238	294
3c	229	251
3d	275	-
3e	192	232
3f	267	352
3g	235	192
3h	227	224
3i	191	222
3j	185	242
3k	159	191
3l	311	364
3m	195	187

[a]50% inhibitory concentration.A-549, human lung carcinoma; HT-29, human colon carcinoma.

CONCLUSION

In summary a series of new 1-(4-phenyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidinyl)-3-(aryl)-2-propene-1-ones were synthesized, characterized and evaluated for *invitro* cytotoxic activity by MTT assay. Among all the derivatives tested, compound **3a** exhibited promising cytotoxic activity might be due to the presence of chlorine group. Hence, the reported results are expected to contribute toward deeper insight into structure-activity relationship and could be helpful in further designing dihydropyrimidinones as potential cytotoxic agents.

ACKNOWLEDGEMENTS

The authors grateful to Management of MLR Institute of Pharmacy, Dundigal, Hyderabad, India, for providing laboratory facilities for research work. We would also like to thank Dr.K.G.Bhat, Maratha Mandal's NGH institute of Dental sciences and research center, Belgaum, Karnakata, India for helping us for cytotoxicity results.

REFERENCES

1. Avila, H.P., Smânia Ede, F., Monache, F.D., Smânia Jr, A. 2008. Structure-activity relationship of antibacterial chalcones. Bioorg Med Chem. 15; 16(22): 9790-4. doi: 10.1016/j.bmc.2008.09.064.
2. Yang, H.M., Shin, H.R., Cho, S.H., Bang, S.C., Song, G.Y., Ju, J.H. 2007. Structural requirement of chalcones for the inhibitory activity of interleukin-5. Bioorg Med Chem. 15: 104–11.
3. Laura Svetaz.,Alejandro Tapia.,Silvia, López, N.,Ricardo., Furlán L.E,Elisa Petenatti., Rosanna Pioli.,Guillermo Schmeda-Hirschmann., Susana, A. Zacchino 2004. Antifungal Chalcones and New Caffeic Acid Esters from Zuccagnia punctata Acting against Soybean Infecting Fungi.J Agric. Food. Chem. 52: 3297–00.
4. Sortino, M., Delgado, P., Juarez, S., Quiroga, J., Abonia, R., Insuasty, B. 2007. Synthesis and antifungal activity of (Z)-5-arylidenerhodanines. Bioorg. Med. Chem.15: 484–94.
5. José Domínguez N., Caritza León., Juan Rodrigues., Neira Gamboa de Domínguez., Jiri Gut., Philip, J., Rosenthal. 2005. Synthesis and Evaluation of New Antimalarial Phenylurenyl Chalcone Derivatives. J. Med. Chem. 48: 3654–58. doi: 10.1021/jm058208o
6. Alain Valla, A., Valla, B., Cartier, D., Guillou, R.L., Labia, R., Florent, L. 2006. New syntheses and potential antimalarial activities of new 'retinoid-like chalcones'. Eur J Med Chem 41:142–146
7. Seo, W.D., Ryu, Y.B., Curtis-Long, M.J., Lee, C.W., Ryu, H.W., Jang, K.C., Park K.H. 2010. Evaluation of anti-pigmentary effect of synthetic sulfonylamino chalcone.Eur J Med Chem. 45(5):2010-7. doi: 10.1016/j.ejmech.2010.01.049.

8. Easmon, J., Purstinger, G., Heinisch, G., Roth, T., Fiebig, H.H., Holzer, W.. 2001. Synthesis, cytotoxicity, and antitumor activity of copper(II) and iron(II) complexes of (4)N-azabicyclo[3.2.2]nonane thiosemicarbazones derived from acyl diazines.J. Med. Chem.44: 2164–71.
9. Dai, Y., Hartandi, K., Ji, Z., Ahmed, A.A., et al. 2007. Discovery of N-(4-(3-amino-1H-indazol-4-yl)phenyl)-N'-(2-fluoro-5-methylphenyl)urea (ABT-869), a 3-aminoindazole-based orally active multitargeted receptor tyrosine kinase inhibitor.J. Med. Chem. 50: 1584–97.
10. Jordan, A.M.., Khan, T. H., Malkin H., Osborn, H. M. 2002. Synthesis and analysis of urea and carbamate prodrugs as candidates for melanocyte-directed enzyme prodrug therapy (MDEPT). Bioorg. Med. Chem. 10: 2625–33.
11. Lima, L. M., Barreiro, E. J. 2005. Bioisosterism: a useful strategy for molecular modification and drug design.Curr. Med. Chem. 12: 23–49.
12. Tangeda, S. J., Garlapati, A. 2010. Synthesis of new pyrrolo[2,3-d]pyrimidine derivatives and evaluation of their activities against human colon cancer cell lines. Eur. J. Med. Chem.45: 1453–58.
13. Mugnaini, Petricci, C.E., Botta, M., Corelli, F., Mastromarino P., Giorgi, G. 2007. Synthesis and biological evaluation of 4-alkylamino-6-(2-hydroxyethyl)-2-methylthiopyrimidines as new rubella virus inhibitors.Eur. J. Med. Chem 42: 256–62.
14. Raffa, D., Edler, M. C., Daidone, G., Maggio, B., Merickech, M., Plescia, S. 2004. Synthesis, cytotoxity and inhibitory effects on tubulin polymerization of a new 3-heterocyclo substituted 2-styrylquinazolinones. Eur. J. Med. Chem. 39: 299–04.
15. Prasad, B. D., Sastry, V. G., Kiran, G. 2015. Green chemistry protocol for the synthesis and antimycobacterial activity of multicomponent biginelli condensation catalyzed by surfactant in aqueous media. Pharmacologia. 6:300-06.
16. Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays.J. Immnol. Methods.65 : 55–63.
17. Rasapelly Ramesh Kumar, Kannappan N and Devilal J. 2019. A novel, efficient, cost-effective, and green methodology for biginelli-reaction: soy lecithin-catalyzed synthesis of 4 aryl-1, 2, 3, 4 -tetrahydropyrimidine-2(1h)ones / thiones in water. Int J Pharm Biol Sci; 9 (2): 811-818

SP-40

A REVIEW ON ROLE OF IGF-1 IN OSTEOPOROSIS.

B.DIVYA, V. SHRAVYA, DR.A.V. KISHORE BABU, DR.A.SRINIVASA RAO.

Department of Pharmacy Practice, Bhaskar Pharmacy College, Hyderabad- 500075, India.

ABSTRACT:
Osteoporosis is a progressive metabolic bone disease that decreases bone density with deterioration of bone structure, skeletal weakness leading to fractures with minor or inapparent trauma. A new treatment is introduced to treat osteoporosis i.e. Insulin like growth factor(IGF-1)is a primary mediator for effects of growth hormone.Growth hormone is secreted in the anterior pituitary gland is released into the blood streams and then stimulates the liver to produce IGF-1.IGF-1 then stimulate systemic body growth on every cell of body especially skeletal muscles cartilage bone.IGF-1 can also regulate cellular DNA synthesis.Both GH and IGF-1 administration significantly increase bone resorption and bone formation.It is also stipulated but highly debated that IGF-1 can increase the size of a tumour in cancer patients. It can be taken in the form of tablets or injections.

KEY WORDS: Osteoporosis, GH, IGF-1, Bone resorption, Osteoclasts, Osteoblasts, Bone metabolism, Tumor, Side effects, Dosing.

INTRODUCTION:

One of the vitaltissues of our body is bone, which undergoes continuous resorption by osteoclasts and new bone is formed by osteoblasts[(1)].The growth hormone/insulin-like growth factor I (GH/IGF-I) axis is pivotal for the management of bone formation. GH and IGF-I play a major part in the formation of bone mass in adolescence as well as maintain the bone mass in the adult stage. Osteoporosis and bone-loss disorders are caused due to the lack of GH/IG-1[2,3,4,5].Aging results in a rapid decline in the number of osteoblasts and less osteoblast activity, but osteoclast activity is not changed. Secondary osteoporosis is associated with bone loss which may result from different causes, like Cushing's syndrome, or overuse of glucocorticoids[6,7].

The IGF-1 plays a crucial part in cellular growth, survival, and cell cycle progression, differentiation[8] and IGF-1 level is necessary for the proper building of high bone mass; furthermore, GH protects against ovariectomy-induced bone loss. IGF-1 helps to reduce osteoblast apoptosis and promotes osteoblastogenesis through the phosphoinositide 3-kinase (PI3K) pathway[9].Pre-clinical studies showed that IGF-1-deficient mice developed smallerskeletonswithasignificantdelayinmineralization.In agreementwiththefindingsfrompre-clinical studies, clinical studies showed a positive association between serum IGF-1 levelsandBone-Mass-Densityindifferentracial groups[10,11].Osteoporoticfractures are caused due to low serum levels of IGF[12,13]. This explains us about the importance of IGF-1in bone formation and mineralization.

GH/IGF-1 AXIS:

GH is secreted by the anterior pituitary gland which is a polypeptide hormone. The GH primarily acts in the liver where it stimulates IGF-1 production[14]. The action of GH is mediated by the binding of GH to the trans membrane GH receptor (GHR) which is present on the surface of most cells[15]. The GH has two different actions i.e., dependent and independent mechanism of action, one directly through the GHR and the other inducing IGF-1 secretion by the liver. Circulating IGF-1 is mostly synthetized in the liver, but IGF-1 is expressed in all tissues, suggesting that paracrine/autocrine effects of local IGF-1 may be a major mechanism controlling tissue growth [16].

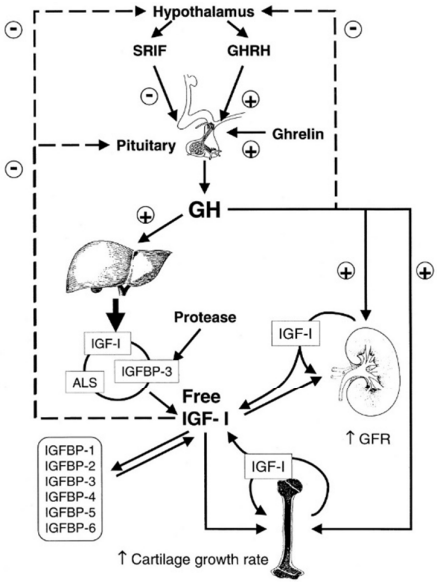


Figure:1 The Stomatotropic Axis.[25]

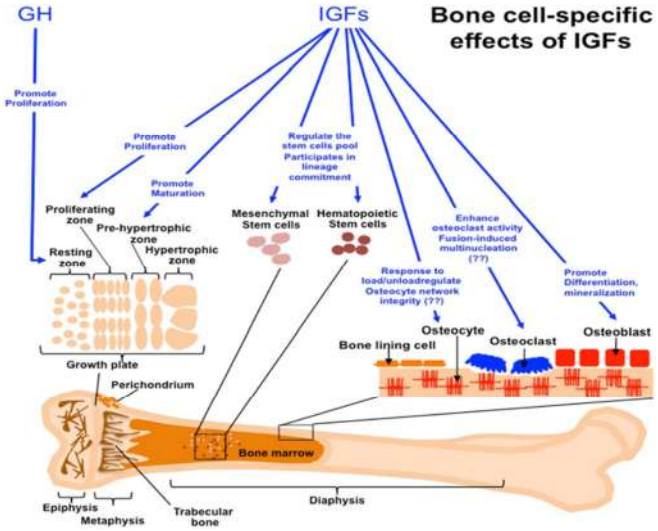


Figure:2 Bone cell-specific effects of IGFs.[26]

Effect of GH/IGF-1 on Osteoblasts and Osteoclasts:

The balance between bone resorption and formation is called as Bone Turnover. The resorption process starts with drafting of osteoclasts followed by the activity of osteoblasts which moves the old bone and replace it with the new matrix. Osteoblastic cells originate from a group of skeletal stem cell, known as skeletal mesenchymal stem cells (MSC). MSC reach bone surfaces from the circulation through vascular channels [17]. Once they have arrived at the bone surface, osteoblastic cells produce bone matrix that becomes mineralized. The old osteoblasts die by apoptosis or become embedded in bone matrix as osteocytes. [18,19,20,21].

Function of IGF-1 on osteoclasts:

Osteoclasts express IGF-1 receptorsand it hasdirecteffectsontheirfunction[22]. Invitro,IGF-1inducesRANK-L(receptoractivatorofnuclear factorκB)synthesisand causesosteoclastogenesis [23,24].

Effect of IGF-1 Administration in Osteoporosis and Bone Metabolism:

Recombinant human IGF-1 (rhIGF-1) has been used for manyyears for the treatment of osteoporosis and in clinical studies on this issue, it resulted in age variation considerably (from 12 to 74 years),which may explain some differences in results. Furthermore,great differences in the dose have been reported (from 0.5to 240 μg/kg/day) and the duration of the therapy has variedfrom 1 to 6 days. Significant bone resorption and formationhas been demonstrated in all studies and no significant sideeffects have been reported.

Cancer Risk Related toGH and IGF-1 Administration:

The GH and IGFs have mitogenic and proliferative propertiesand the potential risk in tumour promotion and progressionhas been suspected.

Side Effects of IGF-1:

It should be well noted that the continuous administration of IGF-1 in high doses has been confirmed to cause hypoglycaemia, but in this case, it is not as severe as that caused by insulin. It is also stipulated but highly debated that IGF-1 can increase the size of a tumour in cancer patients. Even though this factor might be true in patients with existing cancer cases, everyone should be aware that IGF-1 does not cause cancer. In fact, the human body requires IGF-1 to regulate heart functions, brain cell stimulation and to improve the functioning of the nervous system.

Dosing and Injections:

IGF-1: This variant of IGF-1 should be taken daily for 7 days in a week. It's best to take it after a workout. You should dose it at about 20 mcg to 50 mcg. Since IGF-1 has a very short half-life, desensitization will rarely be noticed.
IGF-1 LR3: This variant of IGF-1 should be taken daily for 7 days in a week. You should dose it at about 20 mcg to 50 mcg. Desensitization occurs at around 40 days or approximately 4 weeks.
DES IGF-1: DES IGF-1 should be dosed multiple times in a day, most preferably, before you embark on your training activities. You should target specific sites and muscles with a dose of about 50 mcg to 150 mcg. Since DES IGF-1 has a very short half-life, desensitization can rarely be noticed.

Conclusion:
IGF1 therapy has great effect on bone resorption and bone anabolism thus this therapy is used to treat osteoporosis. measurement of serum IGF1 levels help in early identification of young women at risk of developing osteoporosis and it can be treated by giving IGF1 therapy .androgens and estrogens also interact with IGF1 and helps in optimizing bone mass acquisition.

REFERENCES:

1. Fernández-Tresguerres Hernández-Gil IF, Alobera Gracia MA, del Canto Pingarrón M.BlancoJerez L. Physiological bases of bone regeneration I. Histology and physiology of bone tissue. Med Oral Patol Oral Cir Bucal. 2006;11:47-51.
2. Giustina A, Mazziotti G, Canalis E. Growth hormone, insulin-like growth factors, and the skeleton. Endocr Rev. 2008;29:535-559.
3. Simpson AH, Murray IR. Main differences in osteoporotic fracture models: which should I use? Injury. (2016) 47 (Suppl 1):S15–20. doi: 10.1016/S0020-1383(16)30004-3.
4. Aubin JE, Bonnelye E. Osteoprotegerin and its ligand: a new paradigm for regulation of osteoclastogenesis and bone resorption. Osteoporosis. (2000) 11:905–13. doi: 10.1007/s001980070028
5. Manolagas SC, Jilka RL. Bone marrow, cytokines, and bone remodelling. Emerging insights into the pathophysiology of osteoporosis. N Engl J Med. (1995) 332:305–11. doi: 10.1056/NEJM199502023320506.
6. Ucer S, Iyer S, Kim H-N, Han L, Rutlen C, Allison K, et al. The effects of aging and sex steroid deficiency on the murine skeleton are independent and mechanistically distinct. J Bone Miner Res. (2017) 32:560– 74. doi: 10.1002/jbmr.3014 10. Liu W, Qi M, Konermann A, Zhang L, Jin F, Jin Y. The p53/miR-17/Smurf1 pathway mediates skeletal deformities in an age-related model via inhibiting the function of mesenchymal stem cells. Aging. (2015) 7:205–18. doi: 10.18632/aging.100728.
7. S. Yakar, H. Courtland, and D. Clemmons, "IGF-1 and bone: new discoveries from mouse models," Journal of Bone and MineralResearch,vol.25,no.12,pp.2543–2552,2010.
8. J. C. Fritton, K. B. Emerton, H. Sun et al., "Growth hormone protectsagainstovariectomy-inducedbonelossinstatesoflow circulatingInsulin-likeGrowthFactor(IGF-1),"JournalofBone andMineralResearch,vol.25,no.2,pp.235–246,2010.
9. Cao JJ, Kurimoto P, Boudignon B, et al. Aging impairs IGF-I receptor activation and induces skeletal resistance to IGF-I. J Bone Miner Res 2007;22:1271–9.
10. Langlois JA, Rosen CJ, Visser M, et al. Association between insulin-like growth factor I and bone mineral density in older women and men: the Framingham Heart Study. J Clin Endocrinol Metab 1998;83:4257–62.
11. Zhao HY, Liu JM, Ning G, et al. Relationships between insulin-like growth factor-I (IGF-1) and OPG, RANKL, bone mineral density in healthy Chinese women. Osteoporosis Int 2008;19:221–6.
12. Yamaguchi T, Kanatani M, Yamauchi M, et al. Serum levels of insulinlike growth factor (IGF); IGF-binding proteins-3, -4, and -5; and their relationshipstoboneminеraldensityandtheriskofvertebralfracturesin postmenopausal women. Calcif Tissue Int 2006;78:18–24.
13. Ohlsson C, Mellström D, Carlzon D, et al. Older men with low serum IGF-1 have an increased risk of incident fractures: the MrOS Sweden study. J Bone Miner Res 2011;26:865–72.

14. A. Giustina and J. D. Veldhuis, "Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human," Endocrine Reviews, vol. 19, no. 6, pp. 717–797,1998.
15. M. Ballesteros, K. Leung, R. J. M. Ross, T. P. Iismaa, and K. K. Y. Ho, "Distribution and abundance of messenger ribonucleic acid for growth hormone receptor isoforms in human tissues," Journal of Clinical Endocrinology and Metabolism,vol.85,no.8, pp.2865–2871,2000.
16. S. Yakar, J.-L. Liu, B. Stannard et al., "Normal growth and development in the absence of hepatic insulin-like growth factor-1," Proceedings of the National Academy of Sciences of the United States of America,vol.96,no.13,pp.7324–7329,1999.
17. T. L. Andersen, T. E. Sondergaard, K. E. Skorzynska et al., "A physical mechanism for coupling bone resorption and formation in adult human bone," The American Journal of Pathology, vol.174,no.1,pp.239–247,2009.
18. C. Ohlsson, B. Bengtsson, O. G. P. Isaksson, T. T. Andreassen, and M. C. Slootweg, "Growth hormone and bone,"Endocrine Reviews,vol.19,no.1,pp.55–79,1998.
19. V.Krishnan,H.U. Bryant,andO.A. MacDougald,"Regulation of bone mass by Wnt signalling," Journal of Clinical Investigation, vol.116,no.5,pp.1202–1209,2006.
20. H. Clevers, "Wnt/β-catenin signalling in development and disease,"Cell,vol.127,no.3,pp.469–480,2006.
21. P. Hou, T. Sato, W. Hofstetter, and N. T. Foged, "Identification and characterization of the insulin-like growth factor I receptor in mature rabbit osteoclasts," Journal of Bone and Mineral Research, vol.12, no.4, pp.534–540,1997.
22. T. Niu and C. J. Rosen, "The insulin-like growth factor-I gene and osteoporosis: a critical appraisal," Gene, vol.361, no.1-2, pp. 38–56,2005.
23. G. Mazziotti, A. Bianchi, S. Bonadonna et al., "Prevalence of vertebral fractures in men with acromegaly," Journal of Clinical Endocrinology and Metabolism, vol. 93, no. 12, pp. 4649–4655, 2008.
24. T.L. McCarthy and M. Centrella,"Regulation of IGF activity in bone,"AdvancesinExperimentalMedicineandBiology,vol.343, pp.407–414,1993.

SP-41

"*In-Vivo* evaluation of Antidepressant activity on *Gomphrena serrata* extracts in experimental mice"

DR.PALAKSHA M N[1]* &NANDINI K.N[1], SENTHIL KUMAR G P[2]& TAMIZH MANI T[2]

1.Department of Pharmacology, Bharathi COLLEGE of Pharmacy, Bharathinagara, Mandya-571422.
2. Bharathi College of Pharmacy, Bharathinagara, Mandya-571422

ABSTRACT

The aim of the present study was designed to evaluate the antidepressant activity of "*Gomphrena serrata"* using different animal models. The degree of antidepressant activity was determined by measuring the immobility time in locomotor activity, forced swim test and tail suspension tests. In this study animals Animals treated with all three doses of *Gomphrena serrata* (100,200 and 400mg/kg). Animals treated with all three doses of *Gomphrena serrata* (100, 200 and 400mg/kg) witnessed a increased locomotor activity (*Gomphrena serrata* 400mg/kg shows 142.0±0.17, when compare to control group 100.2±0.73) and a decrease in their immobility times in forced swim test (*Gomphrena serrata* 400mg/kg shows 82.0±0.17, when compare to control group 130.2±0.73) and tail suspension tests *(Gomphrena serrata* 400mg/kg shows 98.78±0.30, when compare to control group 150.92 ± 0.40) which was significant when compared with control. Similarly, animals treated with Imipramine (15mg/kg), as expected showed a significant decrease in the immobility time.

Keywords:*Gomphrena serrata*, locomotor activity, forced swim test, tail suspension test, anti depressant.

Introduction:

Since ancient time naturally occurring plants have played an important role in the discovery of new therapeutic agents. Almost all antibiotics are subjected to the problem of bacterial resistance. Therefore, newer herbal antibacterial compounds from plants to overcome the resistance are under investigation[1]. The plant kingdom still holds many species of plants containing substances of medicinal value which have yet to be discovered and large numbers of plants are constantly being screened for their pharmacological value in addition to the already exploited plants[2].The genus Gomphrena (Fam: Amaranthaceae) comprises approximately 120 species found in the Americas, Australia, and Indo-Malaysia, only a few species are found in forest4. A number of Brazilian Gomphrena species are employed in the treatment of bronchial asthma, diarrhea, and fever, and as an analgesic, tonic, or carminative[3].*Gomphrena serrata L* that belong to family *Amaranthaceae*. It is used as adiuretic, antioxidan[4].Depression is a state of intense sadness and melancholy that has become disruptive to an individual's ability to function socially and complete the daily activities of life. Most people feel depressed and "down" at some point in their life; it is when these feelings are prolonged that clinical depression is suspected[5]. Incidence of depressive mood disorders is rising in the modern stressful society leading to increased risks of self harm or suicide as well as increased mortality from related general medical conditions[5]. As many as 10-15% of individuals with this disorder exhibit suicidal tendency during their life time[6].Mental depression affects a person's mood, thoughts, physical health and behavior. Symptoms of depression include biological and emotional components. Biological symptoms include retardation of thought and action, loss of libido, sleep disturbances and loss of appetite. Emotion symptoms include misery, apathy and pessimism, low self-esteem consisting of feeling of guilt, inadequacy and ugliness, indecisiveness and loss of motivation[7]. Unipolar and bipolar are the two types of depression earlier occurs due to stressful life events and later due to familiar pattern[7]. Patients have symptoms that reflect decrease in brain monoamine with depression neurotransmitters, specifically norepinephrine, serotonin and dopamine[8].Since the depressive disorders are having a huge impact on our lives, it is worth evaluating the alternative forms of medicines which can be used for its treatment. So in this study, an effort was made to investigate the antidepressant effect of *Gomphrena serrata* in experimental animals using different type of models of depression.

Materials and Methods

Experimental animals

Swiss albino mice weighing 18-30 gm, were used for the study. The mice were inbred in the central animal house of the Department of Pharmacology, Bharathi College of Pharmacy, Mandya, under suitable conditions of housing, temperature, ventilation and nutrition were used for antidepressant activity. They were kept in clean dry cages week before the beginning of the experiment to acclimatize with the experimental conditions. The animals were fed with standard pelleted diet (champaka feeds pvt ltd, Bengaluru), distilled water *ad libitum* was maintained at 21^0C-23^0C under a constant 12hrs light and dark cycle. The animal care and experimental protocols were in accordance with CPCSEA /IAEC.

Plant material

The *Gomphrena serrata* belong to family *Amaranthaceae*. were collected from the local area of Mandya and was authenticated was authenticated by Prof. Dr, Gurukar Mathews .Botanist and thecertificate was presented to the departmental library for future reference.

Extraction and preparation of test sample

The whole plant were washed 2 or 3 times with tap water so that it was made free from all dust materials. They were cut into small pieces and dried under shade dried. The dried plants were powdered with the help of mixer grinder and 100g of powder was extracted with ethanolic solvent using Soxhlet extractor.

Acute toxicity:

Acute toxicity studywas carried out as per the OECD guidelines (423)[9].

Preparation and administration of doses

All the doses (A dose of 100mg/kg, 200mg/kg and 400mg/kg body weight) were prepared in distilled water using 0.3% CMC solution as suspending agent and administered orally. In all cases, the concentrations were prepared in 5 ml/100g of body weight. The test substances were administered in a single dose using a gastric intubation tube after fasting for 3 to 4 h.

Observations

Animals were observed initially after dosing at least once during the first 30 min, periodically during the first 24 h. additional observations like changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems and somatomotor activity and behavioral pattern were also done.

Antidepressant Activity

Preparation of animals

The animals were selected in such a way that they were free from illness, injury, disease and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. Only those animals which are healthy having weights 18-30 g were selected and maintained at standard laboratory conditions.

Antidepressant models

Locomotor activity[10]:

The locomotor activity was measured on innate pretreated mice by an actophotometer. Actophotometer functioned on photoelectric cells that were attached in circuit with a counter. When the ray of light dropping on the photocell was cut off by the animal, a count was noted. These cutoffs were calculated for a period of 10 min and the number was used as a degree of the locomotor activity of the animal.

Forced Swim Test[11]

Mice were individually placed into a glass cylinder filled with 15 cm of water for 6 min. As a measure of depression-like behavior, the total duration of immobility and the number of immobility episodes were recorded. Immobility is defined as the absence of movement, unless they are necessary for the animal to stay afloat (head above water).Maintained the temperature of water at 26 ± 1 ^{o}c. At this height of water, animals were not able to support themselves by touching the bottom or the side walls of the chamber with their hind-

paws or tail. Water in the chamber was changed after subjecting each animal to FST because "used water" has been shown to alter the behavior. Each animal showed vigorous movement during initial 2 min period of the test. The duration of immobility was manually recorded during the next 4 min of total 6 min testing period. Mice were considered to be immobile when they ceased struggling and remained floating motionless in water, making only those movements necessary to keep their head above water. Following swimming sessions, the mice were dried with towel and placed in a cylinder heated under 60 W bulb. The animals were dried under heated cylinder for 15 minutes before returning to the home cages.

Groups of six animals were used for present study were

- Group I – Received 0.05ml/10g of 0.3% CMC
- Group II – Received 15 mg/kg Imipramine orally.
- Group III – Received 100 mg/kg Gomphrena serrata orally.
- Group IV – Received 200 mg/kg Gomphrena serrata orally.
- Group V – Received 400mg/kg Gomphrena serrata orally.

Tail Suspension Test: [12]

Animals were moved from their housing colony to laboratory in their own cages and allowed to adopt to the laboratory conditions for 1-2 hr. Each mice was individually suspended to the edge of a table, 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Each animal under test was both acoustically and visually isolated from other animals during the test. Total period of immobility was recorded manually for 6 min. Animal was considered to be immobile when it didn't show any body movement, hung passively and completely motionless. The test was conducted in a dim lighted room and each mice was used only once in the test. The observer, recording the immobility of animals was blind to the drug treatment given to the animals under study.

Results and discussion:

Screening of Antidepressant activity of *Gomphrena serrata*:

Antidepressant models namely Forced Swim Test (FST), Tail Suspension Test (TST) and locomotor activity

Locomotor activity:

The locomotor activity was measured on innate pretreated mice by an actophotometer. Actophotometer functioned on photoelectric cells that were attached in circuit with a counter. When the ray of light dropping on the photocell was cut off by the animal, a count was noted. These cutoffs were calculated for a period of 10 min and the number was used as a degree of the locomotor activity of the animal[13]. In locomotor activity, as per Table1, Imipramine (15 mg/kg) shows a significant increase, locomotor activity and in different doses *Gomphrena serrata* (100, 200 and 400mg/kg) respectively. (Table No. 1).

Forced Swim Test;

In FST, Table No. 2 shows that animals treated with three doses of Gomphrena serrata (100, 200 and 400 mg/kg) showed decrease in their immobility times, which was significant when compared with control. Similarly, animals treated with Imipramine (15 mg/kg), as expected, showed a significant decrease in the immobility time. Animals treated with high dose (400mg/kg) and moderate dose (200 mg/kg) shows more significant decrease in immobility time when compared with low dose (100 mg/kg). (Table 2).

Tail Suspension Test;

Animals treated with three doses *Gomphrena serrata* (100, 200 and 400mg/kg) showed decrease in their immobility times, which was significant when compared with control. Similarly, animals treated with imipramine (15 mg/kg) as expected showed a significant decrease in the immobility time. Animals treated with high dose (400 mg/kg) showed more significant decrease in immobility time when compared with low dose (100 mg/kg). (Table No. 3).

Table No 1. Table No 1. Effect of *Gomphrena serrata*on locomotor activity:

Group No.	Drug Treatment	Dose (mg/kg)	Immobility period
I	Control	0.05 ml/10 g	100.2±0.73
II	Imipramine	15	162.3±0.17***
III	*Gomphrena serrata*	100	108.5±0.30*
IV	*Gomphrena serrata*	200	128.0±0.22***
V	*Gomphrena serrata*	400	142.0±0.17***

Values were mean ± S.E.M. for (n=6) expressed as the time (in sec) of 6 animals each group. Data analysis was performed using Dunnett's test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

Table No 2. Effect of *Gomphrena serrata* on Immobility time in FST:

Group No.	Drug Treatment	Dose (mg/kg)	Immobility period
I	Control	Vehicle	130.2±0.73
II	Standard (Imipramine)	15	62.3±0.17***
III	*Gomphrena serrata*	100	118.5±0.30*
IV	*Gomphrena serrata*	200	99.0±0.22***
V	*Gomphrena serrata*	400	82.0±0.17***

Values were mean ± S.E.M. for (n=6) expressed as the time (in sec) of 6 animals each group. Data analysis was performed using Dunnett's test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

Table No 3. Effect of *Gomphrena serrata* on Immobility time in TST:

Group No.	Drug Treatment	Dose (mg/kg)	Immobility period
I	Control	Vehicle	150.92 ± 0.40
II	Standard (Imipramine)	15	62.23±0.68***
III	*Gomphrena serrata*	100	132.63±0.49*
IV	*Gomphrena serrata*	200	118.98±0.07**
V	*Gomphrena serrata*	400	98.78±0.30***

Values were mean ± S.E.M. for (n=6) expressed as the time (in sec) of 6 animals in each group. Data analysis was performed using Dunnett's test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control

The exact mechanism underlying antidepressant effect is not clear but it may be apparently related to active compounds present in *Gomphrena serrata*. The Phytochemical screening of *Gompherna serrata* leaf extract revealed the presence of alkaloids, steroids, saponins, glycosides etc[4]. These metabolites may be responsible for the antidepressant activity.

Conclusion

The present study was aimed to expose the antidepressant activity of *Gomphrena serrata* in swiss albino mice using locomotor activity, Forced swim test and Tail suspension test respectively. The data obtained was satisfactory in conclusion the present data indicate that the administration of *Gomphrena serrata* to mice has shown significant dose dependant antidepressant activity supporting folk information regarding antidepressant activity of the *Gomphrena serrata*, relatively sub-chronic study may be necessary to arrive at a better picture.Hence further studies would be necessary to evaluate the contribution of active chemical constituents for the observed antidepressant activity as it still remains to be determined which components were responsible for these effects.

ACKNOWLEDGEMENT:

The authors are thankful to **Sri. Madhu G.Madegowda, Chairman of Bharathi education trust,** for providing necessary facilities for this research work. The authors are also thankful to laboratory staffs of Bharathi college of pharmacy, Bharathinagara, Mandya-571422.

REFERENCES:

1. Palaksha MN, Mansoor A and Sanjoy D. Antibacterial activity of garlic extracts on streptomycin resistant *Staphylococcus aureus* and *Eascherichia coli* solely and in synergism with streptomycin. J Natural Sci Biol and Med 2010; 1(1):12-15.
2. Palaksha MN, Ravishankar K and Girija Sastry V. Evaluation of in-vitro antibacterial and anthelmintic activities of *Melochia corchorifolia* plant extracts. Int J Boil Pharm Res 2013; 4(8):577-581.
3. Palaksha MN, Sudheer V, Satyaambika Y, Alekhya V, Srilakshmi Y, NagalakshmiY And SitasrilakshmiY., "Evaluation of Diuretic and *In-Vitro* Anti-Urolithiatic Activities of Ethanolic Leaf Extract *of Gompherna Serrata"*International Journal of Institutional Pharmacy and Life Sciences. 7(2): 2017:74-80.
4. Nandini KN, Palaksha MN, Gnanasekaran D, Senthil kumar GP,Tamizh mani T, "A review of *Gomphrena serrata"*, International Journal of Science and research methodology. 2018, 11(1), 492-503.
5. Gabbard, Glen O. Treatment of Psychiatric Disorders. 3rd ed. Washington, DC: American Psychiatric Publishing; 2000.
6. Maity T, Adhikari A, Bhattacharya K, Biswas S, Debnath PK, Maharana CS. A study on evalution of antidepressant effect of Imipramine adjunct with Aswagandha and Bramhi. Nepal Med Coll J 2011; 13(4): 250-253
7. Gold PW, Goodwin FK and Chrousus GP. Clinical and biochemical manifestations of depression in relation to the neurobiology of stress. Part1. New England Journal of Medicine. 1988;319: 348 – 353.
8. Vimal Kant Sharma, Nagendra Singh Chauhan. Antidepressant activity of Zizyphus Xylopyrus. International Journal of Phytomedicine 2009:12-17.
9. OECD/OCDE guidelines 423 for testing of chemicals, acute toxicity procedure along with the conventional LD50 test and the fixed dose procedure (FDP), OECD test guidelines 423.Adopted 17th December 2001.
10. Vogel HG, editor. Drug Discovery and Evaluation:Pharmacological Assays. New York: Springer; 2008,11-12.
11. Borsini F, Meli A. Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacol 1988; 94:147–60.
12. Thierry B, Steru L, Simon P, Porsolt RD. The tail suspension test: ethical consideration. Psychopharmacol 1986; 90:284-5.
13. Kulkarni S. K. *Handbook of Experimental Pharmacology*. 3rd. New Delhi, India: Vallabh Prakashan; 2005.

SP-42

FORMULATION AND EVALUATION OF ORO-DISPERSIBLE TABLETS OF LORATADINE USING DIRECT COMPRESSION AND SUBLIMATION METHODS

KARTHIK[1], Pallavi Kanagala[1]*, Naseeb Basha Shaik[1], Khoday Namratha[1]

*[1]*Department of Pharmaceutics, G. Pulla Reddy College of Pharmacy, Osmania University, Mehdipatnam, Hyderabad-28, India.E-mail: pallavikanagala@gmail.com*

ABSTRACT

Oro-dispersible tablets (ODT's) have emerged as an alternative to conventional oral dosage forms to improve the patient compliance. Due to problem in swallowing ability with age, the pediatric and geriatric patients complain of difficulty to take conventional solid dosage forms. The ODT's are solid dosage forms that dissolve or disintegrate rapidly in the oral cavity, which results in solution or suspension without the need of water. The objective of this study was to prepare ODT's of loratadine by direct compression and sublimation methods using three different superdisintegrants i.e. Emcosoy, Tapioca starch and Alginic acid in the concentrations 4%, 8% and 12% and different co-processed and multifunctional excipients like MCC sanaq burst, Starlac, Disintequik ODT, Ludipress, Pearlitol flash, F-Melt type C, SmartEx QD-100 and PROSOLV ODT G2. The prepared tablets were evaluated for thickness, hardness, weight variation, friability and drug content, disintegration time, wetting time and water absorption ratio and *In-vitro* dissolution studies. As increasing the concentration of all the three superdisintegrants less disintegration time, less wetting time and high water absorption ratio were observed. Among three superdisintegrants formulations, emcosoy formulations showed excellent disintegration time, wetting time, water absorption ratio and *in-vitro* drug release in both preparation methods. Among co-processed and multifunctional excipients formulations, MCC sanaq burst in direct compression method and Disintequik ODT in sublimation method showed best results.The results indicated that among all the formulations in direct compression method, D10 formulation containing MCC sanaq burst and in sublimation method, S7 formulation containing Disintequik ODT were found to be optimized which showed excellent DT, wetting time, water absorption ratio and *in-vitro* drug release. Stability studies of optimized formulations revealed that formulations were stable.

Keyword: Oro-dispersible tablets, Ludipress, Disintegration time, Wetting time, Emcosoy formulations

INTRODUCTION

Oro-dispersible tablets (ODTs)

The oral route of administration is considered as the most widely accepted route because of its convenience of self-administration, compactness and easy manufacturing. But the most evident drawback of the commonly used oral dosage forms like tablets and capsules is difficulty in swallowing, leading to patients incompliance particularly in case of pediatric and geriatric patients, but it also applies to people who are ill in bed and to those active working patients who are busy or traveling, especially those who have no access to water[1].ODTs are also called as orally disintegrating tablets, quick disintegrating tablets, mouth dissolving tablets, fast disintegrating tablets,fast dissolving tablets, rapid dissolving tablets, porous tablets, and rapid melts. However, of all the above terms, United States pharmacopoeia (USP) approved these dosage forms as orally disintegrating tablets. Recently, European Pharmacooeia has used the term oro-dispersible tablet for tablets that disperses readily and within 3 min in mouth before swallowing [2,3,4].

MATERIALS AND METHODS

Loratadine was obtained as a gift sample from Vasudha Pharma Chem Ltd, MCC sanaq burst was obtained as a gift sample from PharmatransSanaq AG, Starlac was obtained as a gift sample from MEGGLE Excipients & Technology, Disintequik ODT was obtained as a gift sample from Kerry, Ludipress was obtained as a gift sample from BASF India Limited, F-Melt type C was obtained as a gift sample from Fuji Chemical Industries Co., Ltd, SmartEx QD-100 and Ceolus PH-101 were obtained as gift samples from

Arihantinnochem Pvt. Ltd, Pearlitol flash and Pearlitol SD-200 were obtained as gift samples from Roquette, Prosolvodt G2, Emcosoy and Pruv were obtained as gift samples from JRS Pharma, Tapioca starch was obtained as a gift sample from Angel Starch and Food Pvt. Ltd, Alginic acid was obtained as a gift sample from SNAP Natural & Alginate products Pvt. Ltd, Sucralose was obtained as a gift sample from JK Sucralose Inc. and Aerosil-200 Pharma was obtained as a gift sample from Evonik industries.

Formulations of ODTs by direct compression method

In formulations D1 to D9 the superdisintegrants emcosoy, tapioca starch and alginic acid were used in the concentrations of 4%, 8% and 12% and the diluents/ binders Pearlitol SD-200 and CEOLUS PH-101 were used in the ratio 90:10[2]. Formulations D10 to D17 were prepared with marketed co-processed and multifunctional excipients[7].

Preparation of oro-dispersible tablets of loratadine by direct compression method

1. All the ingredients were sieved separately through sieve no.100.
2. Then all the weighed ingredients except sucralose, cherry flavor, aerosil 200 pharma and pruv were mixed in a blender for 15 minutes.
3. Then sucralose, cherry flavor and aerosil 200 pharma were added to the above blend and mixed for 5 minutes.
4. Finally the blend was lubricated using pruv and compressed by using 8mm flat punches.

Formulations of ODTs by sublimation method

From formulations D1 to D9 (4%, 8% and 12% of different superdisintegrants) formulated by direct compression method, the formulations containing 12% of superdisintegrants (D3, D6 and D9) were selected as optimized and hence 12% of superdisintegrants were used for sublimation method. Formulation S1 was prepared without superdisintegrant, formulations S2, S3 and S4 were prepared with 12% of superdisintegrants emcosoy, tapioca starch and alginic acid respectively and formulations S5 to S12 were prepared with marketed co-processed and multifunctional excipients. In all these formulations menthol was used as subliming agent[3].

Preparation of oro-dispersible tablets of loratadine by sublimation method

1. All the ingredients including menthol as subliming agent were sieved separately through sieve no. 100.
2. Then all weighed ingredients except sucralose, cherry flavor, aerosil 200 pharma and pruv were mixed in a blender for 15 minutes.
3. Then sucralose, cherry flavor and aerosil 200 pharma were added to the above blend and mixed for 5 minutes.
4. Finally the blend was lubricated using pruv and compressed by using 8m flat punches.
5. After compression the tablets were heated in an oven at 60 ºC until constant weight was obtained to ensure the complete removal of volatile component.

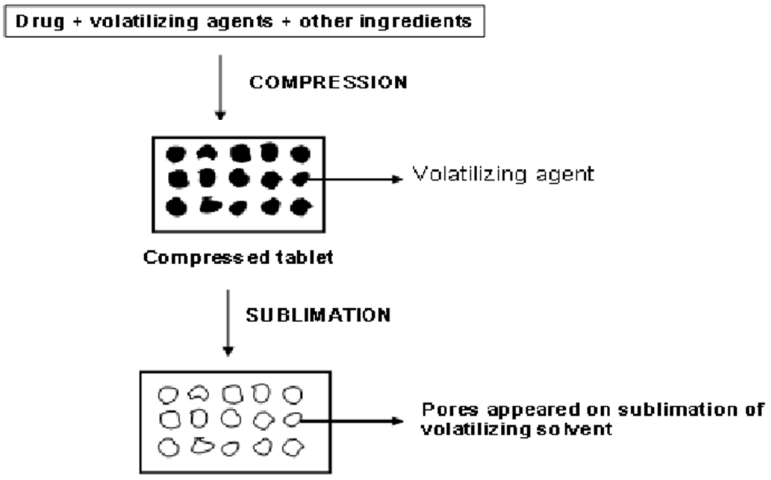


Fig. 1. Steps involved in sublimation method

EVALUATION OF POWDER BLEND:

Precompression parameters like angle of repose, bulk density, tapped density, Compressibility index and Hausner ratio were conducted as per pharmacopoeial methods [5,7,13,14].

Table 1: Formulation of oro-dispersible tablets of loratadine by direct compression method

Ingredients (Weight in Mg.)	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17
Loratadine	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Emcosoy	7.2	14.4	21.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tapioca starch	-	-	-	7.2	14.4	21.6	-	-	-	-	-	-	-	-	-	-	-
Alginic acid	-	-	-	-	-	-	7.2	14.4	21.6	-	-	-	-	-	-	-	-
Pearlitol SD-200	139.59	133.11	126.63	139.59	133.11	126.63	139.59	133.11	126.63	-	-	-	-	-	-	-	-
Ceolus-101	15.51	14.79	14.07	15.51	14.79	14.07	15.51	14.79	14.07	-	-	-	-	-	-	-	-
MCC SANAQ burst	-	-	-	-	-	-	-	-	-	162.3	-	-	-	-	-	-	-
Starlac	-	-	-	-	-	-	-	-	-	-	162.3	-	-	-	-	-	-
DISINTEQUIK ODT	-	-	-	-	-	-	-	-	-	-	-	162.3	-	-	-	-	-
Ludipress	-	-	-	-	-	-	-	-	-	-	-	-	162.3	-	-	-	-
Pearlitol flash	-	-	-	-	-	-	-	-	-	-	-	-	-	163.2	-	-	-
F-MELT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	163.2	-	-
SmartEx QD 100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	163.2	-
PROSOLV ODT G2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	163.2
Sucralose	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	-	-	-	-
Cherry flavor	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Pruv (SSF)	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
Aerosil-200 Pharma	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7
Total in Mg.	180	180	180	180	180	180	180	180	180	180	180	180	180	180	180	180	180

Note: *All quantities are in milligrams; PEARLITOL 200 SD and CEOLUS PH-101 are in the ratio 90:10*

Table 2: Formulation of oro-dispersible tablets of loratadine by sublimation method

Ingredients (Weight in Mg.)	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
Loratadine	10	10	10	10	10	10	10	10	10	10	10	10
Emcosoy	-	21.6	-	-	-	-	-	-	-	-	-	-
Tapioca starch	-	-	21.6	-	-	-	-	-	-	-	-	-
Alginic acid	-	-	-	21.6	-	-	-	-	-	-	-	-
Pearlitol SD-200	146.07	126.63	126.63	126.63	-	-	-	-	-	-	-	-
Ceolus-101	16.23	14.07	14.07	14.07	-	-	-	-	-	-	-	-
MCC SANAQ burst	-	-	-	-	162.3	-	-	-	-	-	-	-
Starlac	-	-	-	-	-	162.3	-	-	-	-	-	-
DISINTEQUIK ODT	-	-	-	-	-	-	162.3	-	-	-	-	-
Ludipress	-	-	-	-	-	-	-	162.3	-	-	-	-
Pearlitol flash	-	-	-	-	-	-	-	-	163.	-	-	-

									2			
F-MELT	-	-	-	-	-	-	-	-	-	163.2	-	-
SmartEx QD 100	-	-	-	-	-	-	-	-	-	-	163.2	-
PROSOLV ODT G2	-	-	-	-	-	-	-	-	-	-	-	163.2
Sucralose	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	-	-	-	-
Cherry flavor	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Pruv (SSF)	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
Aerosil-200 pharma	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7
Menthol	9	9	9	9	9	9	9	9	9	9	9	9
Total wt. in mg.	189	189	189	189	189	189	189	189	189	189	189	189

Note: *All quantities are in milligrams; PEARLITOL 200 SD and CEOLUS PH-101 are in the ratio 90:10*

EVALUATION OF ORO-DISPERSIBLE TABLETS

The prepared tablets were evaluated for various parameters like thickness, hardness, weight variation, friability and drug content, disintegration time, wetting time and water absorption ratio, content uniformity, *In-vitro* dissolution studies and stability studies[5,7,13,14].

RESULTS AND DISCUSSION

Results of Pre-compression evaluation studies of powder blend

Blend ready for compression were subjected to pre-compression parameters like angle of repose, bulk density, tapped density, compressibility Index and Hausner's ratio. The values for angle of repose were found to be in the range of 20.780 to 43.480. The bulk density was varied in the range of 0.472 gm/ml to 0.742 gm/ml, tapped density range between 0.657 gm/ml to 1.021 gm/ml, compressibility index was varied in the range of 18.92% to 29.38% and Hausner ratio in the range of 1.233 to 1.416. These all parameters indicate that most of the formulations had shown good flow and some of them had shown satisfactory flow.

Table 3: Data for evaluation of pre compression parameters of various powder blends.

Formulation	Angle of repose (θ)	Bulk density (g/ml)	Tapped density (g/ml)	Compressibility Index (%)	Hausner Ratio
D1	31.13	0.582	0.786	25.93	1.350
D2	31.98	0.612	0.788	22.33	1.288
D3	32.80	0.543	0.734	26.04	1.352
D4	28.83	0.585	0.796	26.51	1.361
D5	26.66	0.614	0.833	26.34	1.358
D6	28.94	0.611	0.821	25.53	1.343
D7	29.68	0.646	0.813	20.51	1.258
D8	25.47	0.629	0.786	20.00	1.250
D9	26.84	0.627	0.802	21.74	1.278
D10	30.80	0.742	1.021	27.37	1.377
D11	37.07	0.655	0.916	28.42	1.397
D12	27.91	0.651	0.895	27.27	1.375
D13	34.09	0.628	0.883	28.88	1.406
D14	34.08	0.627	0.829	24.35	1.322
D15	24.92	0.694	0.855	18.92	1.233
D16	20.78	0.625	0.833	25.00	1.333
D17	29.81	0.704	0.892	21.11	1.268
S1	27.97	0.538	0.735	26.79	1.366
S2	42.35	0.472	0.657	28.13	1.391
S3	40.77	0.528	0.745	29.12	1.411
S4	23.67	0.543	0.755	28.06	1.390
S5	41.79	0.643	0.889	27.66	1.382

S6	43.48	0.577	0.790	26.92	1.368
S7	38.24	0.548	0.741	26.09	1.353
S8	28.56	0.547	0.774	29.38	1.416
S9	38.66	0.500	0.706	29.18	1.412
S10	23.13	0.595	0.807	26.27	1.356
S11	34.08	0.595	0.783	24.00	1.316
S12	23.83	0.662	0.895	26.09	1.353

Results of Post compression evaluation parameters

Table 4: Physical evaluation of oro-dispersible tablets

For mulations	Wt. variation (mg) (n=20)	Thickness (mm) (n=3)	Hardness (Kg/cm^2) (n=3)	Friability (%) (n=10)	Drug Content (%) (n=3)	Disintegration time (sec) (n=6)	Wetting time (Sec) (n=3)	Water absorption ratio (%) (n=3)
D1	179.35±1.93	2.73±0.012	2.73±0.115	0.856	97.32±1.00	36.00±0.02	53.67±3.79	110.58±4.62
D2	179.60±1.27	2.71±0.017	3.00±0.002	0.430	98.61±1.48	29.25±2.517	52.00±1.73	129.08±8.64
D3	179.80±1.06	2.71±0.006	3.20±0.200	0.281	98.74±2.09	27.00±0.01	51.67±3.51	157.68±3.28
D4	179.20±2.61	2.76±0.010	3.13±0.321	0.433	100.60±0.73	111.50±4.95	103.67±7.09	58.23±1.09
D5	177.40±2.93	2.76±0.017	3.10±0.361	0.439	98.38±1.42	64.00±5.29	98.50±8.19	63.38±5.26
D6	177.95±2.31	2.80±0.006	3.30±0.361	0.578	97.63±1.09	34.50±0.70	41.50±2.12	65.27±2.24
D7	178.85±1.42	2.70±0.000	3.63±0.153	0.580	97.13±1.32	129.50±7.77	150.67±4.04	55.98±2.73
D8	179.85±1.42	2.70±0.002	3.93±0.058	0.564	99.41±0.46	105.00±4.24	139.00±1.41	75.79±2.72
D9	179.70±1.49	2.81±0.002	2.23±0.058	0.565	98.92±0.66	37.50±2.12	67.33±7.64	75.63±3.29
D10	180.30±1.03	2.79±0.017	2.00±0.058	0.576	98.30±1.02	7.00±1.00	6.33±0.58	125.13±3.76
D11	178.80±1.74	2.60±0.015	2.07±0.153	0.290	96.82±1.37	31.00±1.41	39.00±1.41	62.95±2.45
D12	177.80±1.24	2.78±0.015	2.20±0.100	0.850	97.98±1.15	34.50±0.70	50.00±0.02	67.73±4.67
D13	178.35±1.31	2.79±0.002	2.57±0.058	0.146	96.94±1.88	21.00±1.41	63.00±4.24	47.93±1.05
D14	179.95±1.54	1.79±0.017	2.47±0.153	0.436	97.35±0.49	38.50±0.70	47.00±5.20	63.56±3.01
D15	180.00±1.72	2.76±0.015	2.70±0.100	0.434	97.54±1.13	176.00±7.50	178.00±4.95	115.92±1.73
D16	179.95±1.50	2.72±0.015	3.70±0.100	0.143	97.41±0.86	164.00±6.00	129.50±6.78	53.76±0.97
D17	178.50±2.12	2.62±0.002	4.77±0.252	0.433	101.23±1.03	>180.00	>180.00	117.39±6.35
S1	181.45±1.19	2.90±0.015	1.18±0.029	0.901	97.55±0.67	175.00±5.00	>180.00	57.08±3.37
S2	177.27±1.19	2.94±0.010	1.33±0.153	0.730	98.10±0.95	24.50±0.50	38.50±0.71	148.59±3.63
S3	177.53±1.46	2.91±0.023	1.10±0.100	0.863	100.57±0.55	34.50±0.50	34.50±2.21	71.02±0.8
S4	183.40±0.70	2.97±0.010	1.17±0.058	0.531	98.52±0.91	101.00±1.00	134.00±6.49	69.44±3.68
S5	178.00±1.33	2.99±0.017	0.75±0.153	Break	98.25±0.37	4.50±0.50	7.50±0.71	135.05±2.96
S6	180.25±1.33	2.92±0.010	0.77±0.153	Break	97.97±1.10	17.00±1.00	18.00±1.41	53.85±1.55
S7	179.90±1.17	3.08±0.015	1.30±0.002	0.558	97.82±1.01	12.00±1.00	15.00±1.41	63.42±3.27
S8	180.15±1.09	3.20±0.006	1.40±0.200	0.698	96.59±1.54	15.50±0.50	43.00±1.41	45.59±1.88
S9	177.13±1.81	2.93±0.006	1.10±0.058	1.721	98.19±0.95	14.50±0.50	19.00±1.41	60.87±1.39
S10	181.78±1.00	3.15±0.002	2.50±0.173	0.138	97.81±0.74	45.50±0.50	58.00±0.01	90.07±1.53
S11	180.10±0.79	2.71±0.015	1.23±0.115	0.559	101.20±1.06	173.00±3.00	178.00±2.83	64.82±0.02
S12	180.05±1.32	2.99±0.010	1.60±0.200	0.278	99.10±0.82	21.00±1.00	25.50±0.71	113.89±2.36

Note: *All values are expressed in Mean ± S.D*

In- vitro **Dissolution studies**

Table 5: % Drug release profiles of D1 – D12

Time (min)	Cumulative % drug release																
	D1	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10**	**D11**	**D12**	**D13**	**D14**	**D15**	**D16**	**D17**
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	71.19	80.40	102.22	40.49	51.18	74.26	42.09	32.80	101.25	97.44	87.53	60.50	70.64	68.08	39.38	34.77	39.38
4	100.50	101.13		96.81	98.24	100.41	84.38	99.91			101.64	90.65	101.10	100.25	62.90	49.60	44.49
6							99.72					100.19			69.03	62.90	67.50
10															96.65	90.51	97.67

Table 6: % Drug release profiles of S1-S12

Time (min)	Cumulative % drug release											
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
0	0	0	0	0	0	0	0	0	0	0	0	0
2	86.56	100.17	98.85	99.32	99.68	78.40	100.33	92.67	86.32	97.89	60.40	99.98
4	99.98					99.73		101.04	99.63		80.40	
6											83.95	
10											100.00	

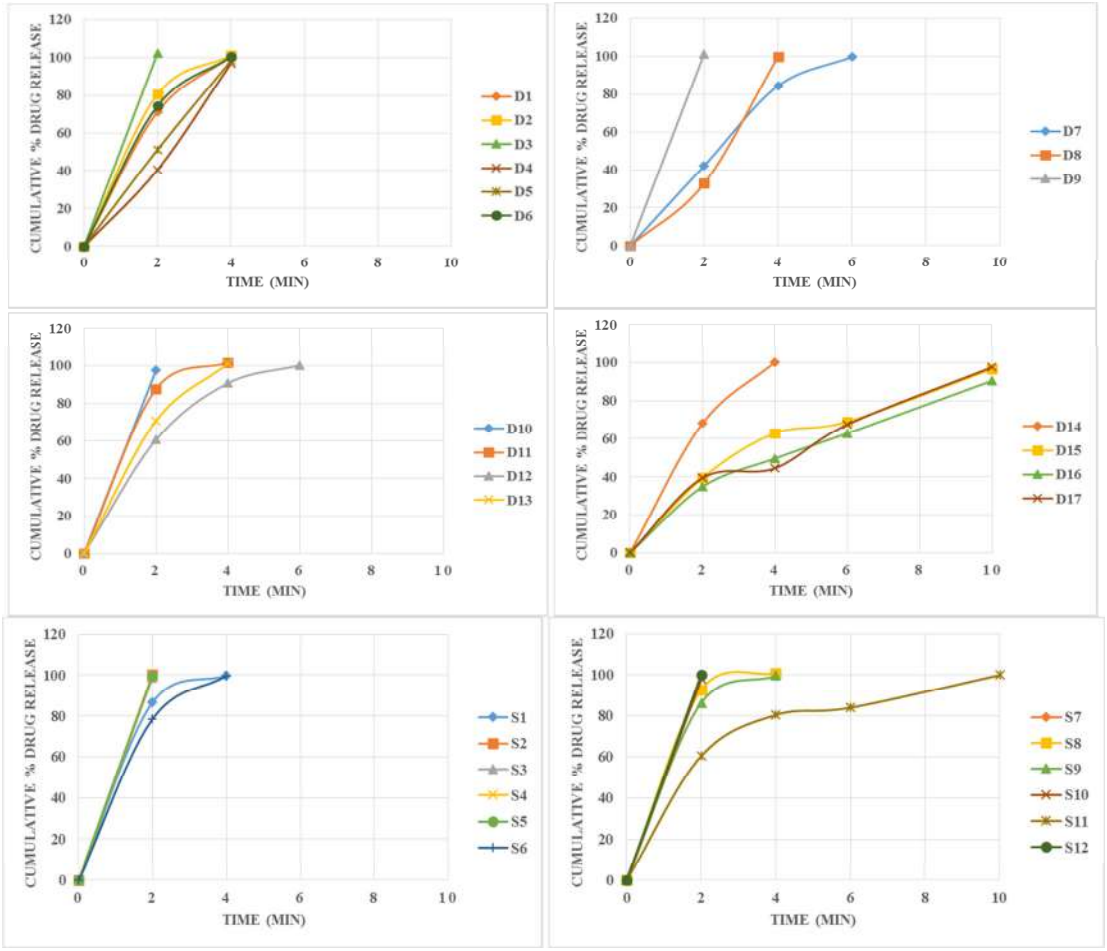


Fig. 2. Comparative drug release profiles of all formulations

In direct compression method the rapid drug dissolution was noticed in formulations D3, D9 and D10 which showed 102.22%, 101.25% and 97.44% of drug release respectively at 2 minutes. In sublimation method the rapid drug dissolution was noticed in formulations S2, S3, S4, S5, S7, S10 and S12 which showed 100.17%, 98.85%, 99.32%, 99.68%, 100.33%, 97.89% and 99.98% respectively at 2 minutes.Most of the formulations of sublimation method showed rapid drug dissolution as compared to direct compression method formulations.

Optimization of ODT tablets

In direct compression method, after comparing all the evaluation parameters of all the formulations, D10 formulation containing Mccsanaq burst showed good results with disintegration time 7 sec , wetting time 6.33 sec, water absorption ratio 125.13 % and 97.44 % drug release in 2 minutes. Hence D10 formulation was optimized in direct compression method.In sublimation method, S5 and S7 showed good disintegration time, wetting time, water absorption ratio and drug release but friability test was failed for S5 formulation. So S7 formulation containing Disintequik ODT which showed disintegration time 12 sec, wetting time 15 sec, water absorption ratio 63.42% and 100.33% drug release in 2 minutes was optimized in sublimation method. Even though these two formulations were optimized most of the other formulations also showed good results[2,3,5,7,9,13,14]

Comparison of optimized batches with marketed formulation

Table 7: Evaluation parameters of optimized batches and marketed formulation

S.NO.	Parameter	Opimtized batch (D10)	Optimized batch (S7)	Marketed formulation
1	Disintegrating time (sec)	7.00±1.00	12.00±1.00	12.67±1.15
2	Wetting time (sec)	6.33±0.58	15.00±1.41	47.67±2.52
3	Water absorption ratio (%)	125.13±3.76	63.42±3.27	145.00±1.41
4	Drug content (%)	98.30±1.02	97.82±1.01	101.66±0.86

Note: *All values are expressed in Mean ± S.D*

Table 8:% Drug release profiles of optimized batches and marketed formulation

Time (min)	Cumulative % drug release		
	D10	S7	Marketed formulation
0	0	0	0
2	97.44	100.33	101.48

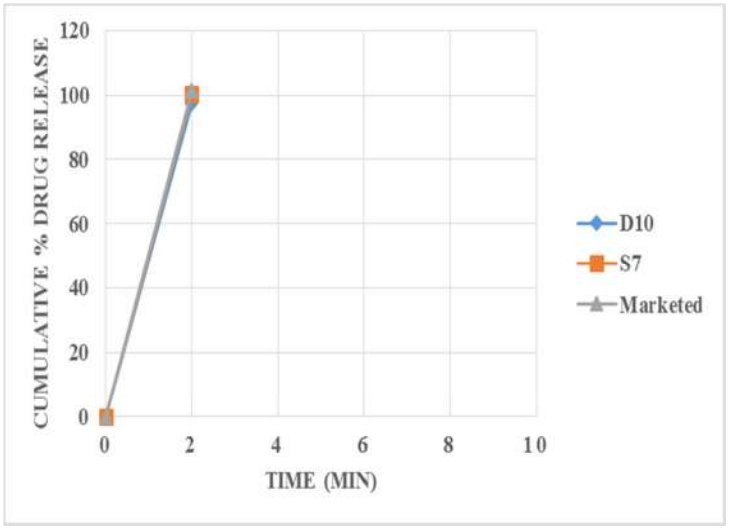


Fig. 2. Comparative drug release profile of D10, S7 and marketed

STABILITY STUDIES

Stability of a drug has been defined as the ability of a particular formulation, in a specific container, to remain within its physical, chemical, therapeutic and toxicological specifications. Optimized formulations were packed in aluminum foils and stored at room temperature for one month and evaluated for all the parameters.

Table 9: Results of stability study of optimized formulations

S.NO.	Parameter	Optimized batch (D10)		Optimized batch (S7)	
		0 month	1 month	0 month	1 month
1	Thickness (mm)	2.79±0.017	2.81±0.010	3.08±0.015	3.09±0.006
2	Hardness (kg/cm^2)	2.00±0.058	2.00±0.10	1.30±0.002	1.25±0.06
3	Disintegrating time (sec)	7.00±1.00	7.67±0.58	12.00±1.00	14.33±0.58
4	Wetting time (sec)	6.33±0.58	7.00±1.00	15.00±1.41	15.67±0.58
5	Water absorption ratio (%)	125.13±3.76	125.82±4.06	63.42±3.27	64.95±2.01
6	Drug content (%)	98.30±1.02	100.12±1.40	97.82±1.01	99.43±0.82

Table 10: % Drug release profiles of optimized batches during stability study

Time (min)	Cumulative % drug release			
	Optimized batch (D10)		Optimized batch (S7)	
	0 month	1 month	0 month	1 month
0	0	0	0	0
2	97.44	99.84	100.33	98.52

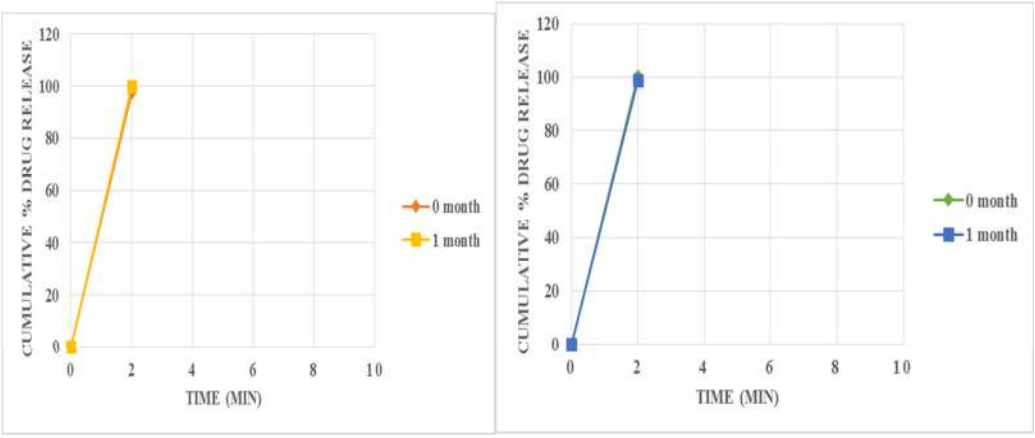


Fig. 3. Comparative drug release profile of D10 & S7 before and after stability study

The results of stability study observed as no change in the physical appearance of the tablets and there were no significant changes in hardness, disintegration time, wetting time, drug content and in vitro drug release. This indicated that the optimized formulations were fairly stable.

CONCLUSIONS

In the present research work, Oro-dispersible tablets of loratadine were prepared by direct compression and sublimation methods by using various superdisintegrants, considering all evaluation parameter results and drug release profiles of all the formulations, the formulation D10 containing Mccsanaq burst in direct compression method and the formulation S7 containing Disintequik ODT in sublimation method were optimized. Among both the methods, the direct compression method is easy, economical and less time consuming method as compared to the sublimation method.

REFERENCES

1. Velmurugan S and Sundar Vinushitha 'Oral Disintegrating Tablets: An Overview', International Journal of Chemical and Pharmaceutical Sciences 2010,,1(2), pp.1-12.
2. Harkare BR, Kulkarni AS, Aloorkar NH and Majumdar SH 'Effect of Mannitol and MCC on Rapidly Disintegrating Tablets', World Journal of Pharmaceutical Research, 2014, 3(6), pp. 1040-1065.
3. Kshirasagar N, Deepika P, Malvey S, Adukondalu D, Pavani S and Reddy MV 'Formulation and Evaluation of Acyclovir Orodispersible Tablets using Sublimation Method', *Journal of General Practise*, 2015, 3(4), pp. 1-5.
4. Leon Lachman, Herbert A. Lieberman, *The Theory and Practice of Industrial Pharmacy*, 4th edition, CBS publishers and distributors, India. 2013, Chapter-8 "Dissolution" pp 209-211.
5. Naseeb Basha Shaik, Gundogiwar Pooja Rani, Latha Kukati and Pallavi Kanagala 'Formulation and Evaluation of Press Coated Tablets of Esomeprazole', World Journal of Pharmaceutical Research, 2018, 7(9), pp. 1711-1741.
6. Pahwa R and Gupta N, 'Superdisintegrants in the Development of Orally Disintegrating Tablets: A Review', *International Journal of Pharmaceutical Sciences and Research*, 2011, 2(11), pp. 2767-2780.
7. Patil J, Kadam C, Vishwajith V and Gopal V,'Formulation, Design and Evaluation of Orally Disintegrating Tablets of Loratadine using Direct Compression Process', *International Journal of Pharma and Bio Sciences*, 2011, 2(2), pp. 389-400.
8. Patro C, Patro SS, Panda BP and Bhanoji ME,'Formulation and Evaluation of Cetirizine HCl Mouth Fast Dissolving Tablets', *Scholar Research Library*, 2011, 3(4), pp. 63-70.

9. Rajitha K, Shravan Y, Adukondalu D, Gannu R and Madhusudan Y, 'Formulation and Evaluation of Orally Disintegrating tablets of Buspirone', *International Journal of Pharmaceutical Sciences and Nanotechnology*, 2009, 1(4), pp. 327-334.
10. Raymond C Rowe, Paul J Sheskey and Marian E Quinn, *Handbook of Pharmaceutixal excipients*, 6th edition, 2009, Pharmaceutical Press, UK.
11. Roy A, 'Orodispersible Tablets: A Review', *Asian Journal of Pharmaceutical and Clinical Research*, 2016, 9(1), pp. 19-26.
12. Velmurugan S and Sundar V, 'Oral Disintegrating Tablets: An Overview', International Journal of Chemical and Pharmaceutical Sciences, 2010, 1(2), pp. 1-12.
13. Naseeb Basha Shaik*, Manasa Toleti, Divija Kanaru and Latha Kukati, 'Solubility Enhancement of Aceclofenac by Solid Dispersion', American Journal of PharmTech Research, 2018, 8(3), pp.107-130.
14. Naseeb Basha Shaik*, Sravani P, Latha Kukati and Mobina Begum 'Formulation and Evaluation of Press Coated Tablets of Mesalamine', Inventi Rapid: PharmTech, 2019, 2019 (3), pp. 1-11.

SP-43

EVALUATION OF ANTI-GOUT ACTIVITY OF *AEGLE MARMELOS* LEAF EXTRACT AGAINST POTASSIUM OXONATE INDUCED GOUT IN MALE SPRAGUE DAWELY RATS

MITTA RAGHAVENDRA[1]*, BORROLA SAI KUMAR[2], DESAM NIKITHA[2], MANDLEM VENKATAKIRAN KUMAR[3], KONDE ABBULU[4]

[1,2,3]*Department of Pharmacology, CMR College of Pharmacy, Kandlakoya (V), Hyderabad-501401, Telangana state, India.*
[4]*Department of Pharmaceutics, CMR College of Pharmacy, Kandlakoya (V), Hyderabad-501401, Telangana state, India.***Email: mittargv@gmail.com**

ABSTRACT
The aqueous extract of *Aegle marmelos* leaves (AEAML) was investigated for its anti-gout activity against potassium oxonate induced gout in male Sprague dawely rats. In this study, total 30 animals were randomly divided into 5 groups of 6 animals in each group. Group I served as normal control, Group II as toxic control, Group III as standard received Allopurinol and Group IV & V as treatment groups (200 and 400 mg/kg, b. wt*p.o*). Blood Parameters such as WBC, Lymphocytre, Esionophils, serum biochemical parameters such as Urea, Uric acid, Total protein and Tissue homogenates for Lipid peroxidation, Catalase and Reduced glutathione levels were estimated.Preliminary phytochemical study of AEAML showed the presence of Alkaloids, Glycosides, Steroids, Flavanoids, Phenols and tannins. Toxic control showed significant ($p<0.001$) increase in the Urea, Uric acid and Total protein content. There was a significant increase in the oxidative stress makers such as lipid peroxidation and decrease in the levels of reduced glutathione and Catalse observed in toxic control ($p<0.001$) when compared to the normal control. In the treatment groups, significant decrease in the blood, serum biochemical parameters and normalized tissue antioxidant levels were observed in a dose dependent manner. The present experimental findings suggested that *Aegle marmelos* leaves have potential anti-gout activity might be due to the presence of flavonoids, phenols and tannins.

Key words:*Aeglemarmelos, Anti-gout, Potassium oxonate, Allopurinol*

INTRODUCTION
Gout is a prevalent disorder of uric acid metabolism that can lead to recurrent episodes of joint inflammation, tissue deposition of uric acid crystals. Pain comes on rapidly, reaching maximum intensity in less than 12 hours. The joint at the bottom of the big toe is affected in most of the cases, and leads to the joint destruction if left untreated. Gout is a disease that develops when uric acid is not properly excreted from blood through urine. It may also results in kidney stones, tophi or urate nephropathy. The risk of developing gout increases as the Serum uric acid level rises [>7 mg/dl][1].*Aegle Marmelos*iscommonly called as Bael, belongs to the Rutaceae family. It is considered as spiritual by Hindus. It is a moderate-sized, slender, deciduous and aromatic tree. It is native to India and is abundantly found in the Himalayan tract, Bengal, Central and South India and in most of the countries of southeastern Asia. The roots of *A.marmelos*are fairly large, woody and often curved[2]. The leaves of *Aegle Marmelos* was proved as antibacterial activity[3], analgesic and anti-inflammatory activity[4], hepatoprotective activity[5], insecticidal activity[6], hypoglycemic and antioxidant activity[7], anxiolytic and antidepressant activity[8], anticonvusant activity[9], antifertility activity[10] and in the treatment of myocardial infarction[11]. The aim of the present study was to evaluate the anti-gout activity of *Aegle Marmelos* leaf extract against potassium oxonate induced gout in male Sprague dawely rats.

MATERIALS AND METHODS

Collection and authentication of plant material
Thefresh leaves of *Aegle Marmelos*collected from the local forest area, Medchal, Medchal district, Telangana in the November 2018. Plant authentication was done by Dr. L. Rasingam, Scientist In-charge of Botanical survey of India, Deccan Regional Centre, Attapur village, Hyderabad.

Preparation of extract

The leaves of *Aegle Marmelos*cleaned with distilled water to remove the dirt and foreign particles and shade dried for four weeks. The leaves were made into coarse powdered and extracted by a successive Soxhlet extraction process using petroleum ether, ethyl acetate, chloroform, ethanol and distilled water. Thereafter, filtered and the filtrates were dried in a rotary evaporator. The suitable plant extract was selected based on the phytochemical analysis.

Phytochemical investigation

The extracts were subjected to qualitative phytochemical evaluation for the identification of phytoconstituents[12].

Experimental animals

Male Sprague dawely rats (36) weighing in between 180-220 g were procured from Sai Thirumala enterprises, Hyderabad, India. Animals were acclimatized to the laboratory environment for 2 weeks. Rat feed was provided with ad libitum. The study was completed as per the guidelines of CPCSEA, Government of India, after IAEC approval (IAEC number: CPCSEA/1657/IAEC/AMRCP/Col-18/69).

Treatment schedule

Group 1: Normal group (Received 5ml/kg b.wt of distilled water, *p.o*for *7* days)
Group 2: Disease control (Potassium oxonate 250 mg/kg/body weight, *i.p* daily for 7 days).
Group 3: Standard drug (Allopurinol 10mg/kg/bodyweight, *p.o*), and simultaneously administered with Potassium oxonate (250 mg/kg/bodyweight, *i.p*), daily for 7 days.
Group 4: Aqueous extract of *Aegle marmelos* (200 mg/kg/body weight, *p.o*), simultaneously administered Potassium oxonate (250 mg/kg/bodyweight, *i.p.*), daily for 7 days.
Group 5: Aqueous extract of *Aegle marmelos* (400 mg/kg/bodyweight, *p.o*), and simultaneously administered Potassium oxonate (250 mg/kg/body weight, *i.p.*), daily for 7 days. After the treatment scheduled, blood was collected by retro orbital method and divided into two parts. First portion for estimation of WBC, lymphocyte and Eosinophil counts using Medonichematology cell counter and the second portion for evaluation of serum parameters such as urea, uric acid, Creatinine and total protein using coral diagnostic kits, India. The kidneys were homogenized and post mitochondrial supernant was used for the estimation of oxidative stress markers such as lipid peroxidation, reduced glutathione and catalase levels [13-15].

Statistical analysis

The values were expressed as Mean ± SEM, n=6 in each group. The statistical analysis was carried out by one way ANOVA followed by Dunnett's test. $^{a}p<0.001$, $^{b}p<0.01$ and $^{c}p<0.05$ Vs Disease control; $^{d}p<0.001$, $^{e}p<0.01$ and $^{f}p<0.05$ Vs Standard Allopurinol. Values were significant at $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical screening of AEAML

Based on the phytochemical analysis, we selected aqueous extract of *Aegle Marmelos* leaves (AEAML) was further studies. The phytochemical analysis of AEAML was found to be flavonoids, phenolics, saponins and triterpenoids as major secondary metabolites.

Effect of AEAML treatment on WBC, lymphocytes and Eosinophils

An intra-peritoneal injection of Potassium oxonate of 250 mg/kg b. wt dose (daily for 7days) has lead to significant rise in levels of WBC, lymphocyte and Eosinophil counts($p<0.05$) in rats of gout control as compared to the normal control. This might be occurred due to inflammation and also represents the chronic inflammation. Increased number of platelets count also noticed in other inflammatory conditions like rheumatoid arthritis, inflammatory bowel disease and nephritis. On activation, platelet secretes a large number of biological active molecules, which are able to induce an inflammatory process[16-18]. Administration of AEAML at doses of 200 and 400 mg/kg b.wt markedly blocked increased WBCand Eosinophil count in a dose dependent manner and in the standard Allopurinol treatment. But moderate effect

was observed in the Lymphocyte count in the treatment as well as standard Allopurinol groups. Effect of AEAMLon WBC, lymphocytes and Eosinophilswere presented as Mean ± SEM in Figure No.1.

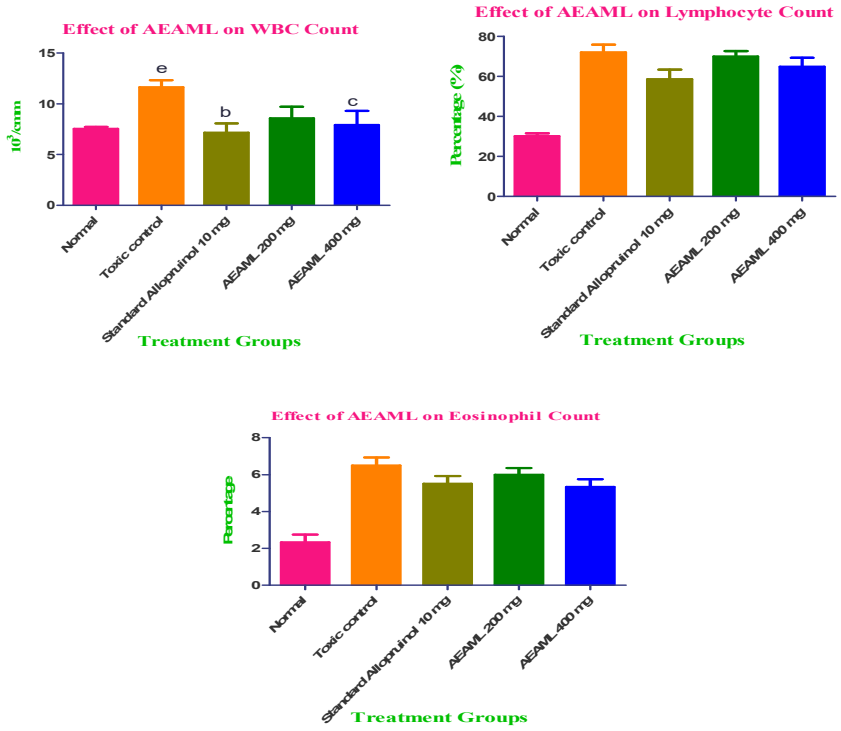
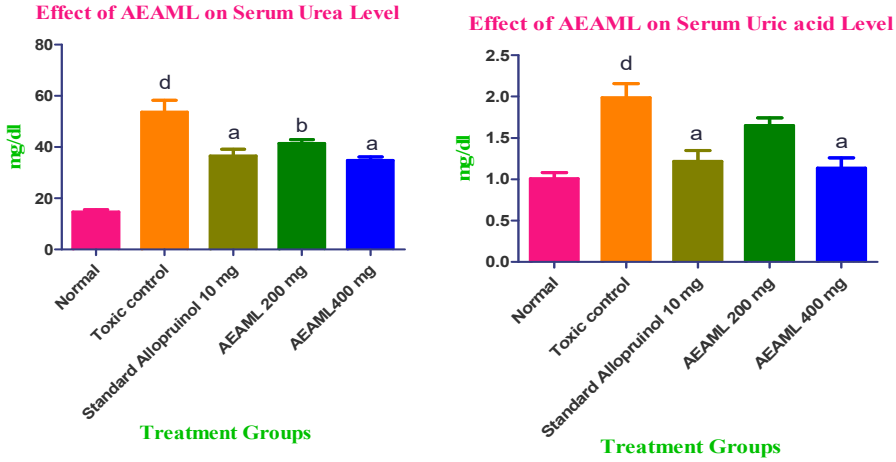


Figure No. 1: Effect of aqueous extract of AEAML on WBC, lymphocytes and Eosinophils.

Effect of AEAML on kidney serum profile

Hyperuricemia is the most important risk factor for the development of gout. Hyperuricemia occurs, as a result, increasing in uric acid production; impair renal uric acid excretion, or a combination of these mechanisms. The development of gout requires three distinct steps: Prolonged hyperuricemia, the formation of monosodium urate monohydrate (MSU) crystals and interaction between MSU crystals, and the inflammatory system[19]. Effect of AEAML on blood serum urea, uric acid, creatinine and total protein were estimated and presented as Mean ± SEM in Figure No.2. There was a significant increase in the serum urea, uric acid and creatinine whereas a decrease in the total protein level in toxic control group **(**$p<0.001$**)** when compared to the normal control. Treatment with AEAML showed significant decrease in serum levels of urea, uric acid and creatinine in a dose dependent manner ($P<0.001$, $P<0.01$and $p<0.05$) and also increased total protein levels observed at a dose of 400 mg/kg b. wt when compared to standard Allopurinol. The present study correlated with the study of Sarvaiya et al., 2015[20].



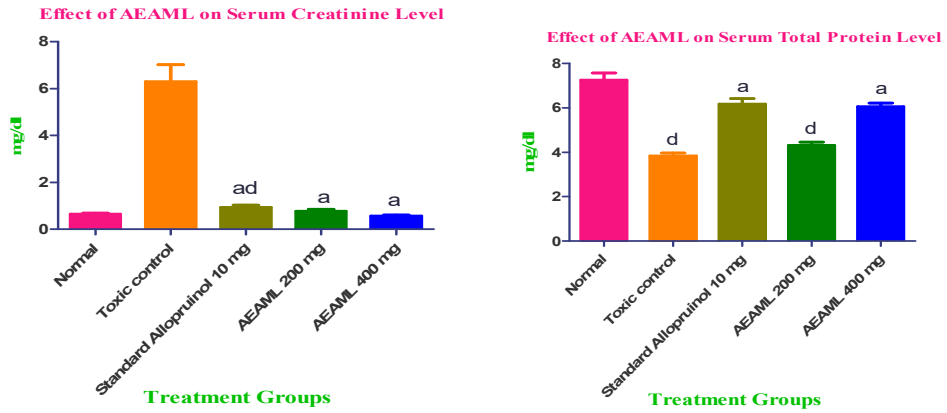
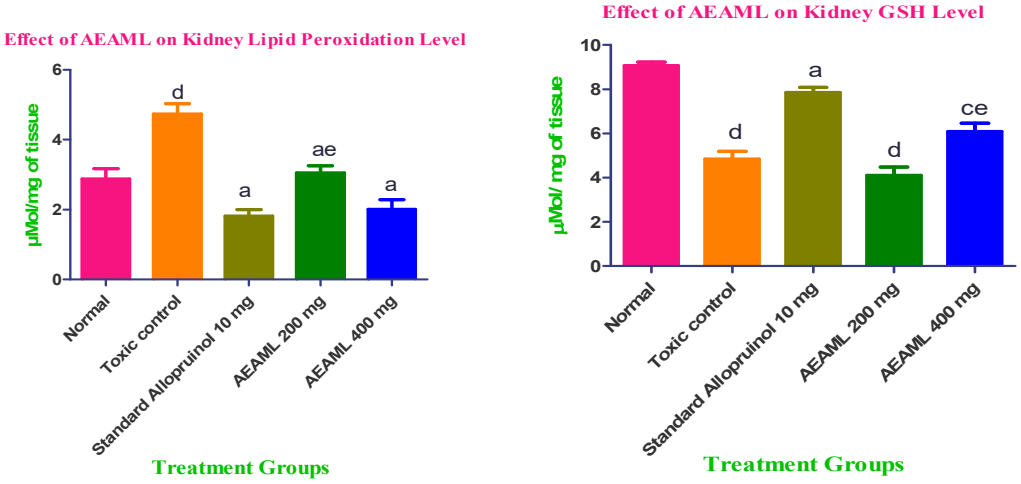


Figure 2: Effect of AEAML on kidney serum profile.

Effect of AEAML treatment on oxidative stress markers in potassium oxonate induced gout

Effect of AEAML on oxidative stress markers such as lipidperoxidation, reduced glutathione and catalase of kidney homogenate were summarized in Figure No.3. There was a significant increase in the lipidperoxidation and decrease in the levels of reduced glutathione and catalase observed in toxic control ($p<0.001$) when compared to the normal control. Treatment with AEAML showed significant recovery from lipidperoxidation and increased levels of reduced glutathione and catalase in a dose dependent manner ($p<0.05$, $p<0.01$ and $p<0.001$) when compared to the Standard Allopurinol control.Flavonoidsare well known anti-oxidants and used as therapeutic agents for diseases mediated by free radical induced oxidative stress. Earlier of studies clearly demonstrated that different chemical classes of flavonoids has been reported as potential inhibitors of xanthine oxidase since, phytochemical screening of the AEAMLrevealed the presence of flavonoids accounting for its antioxidant property, the present activity of the extract may be attributed to the same[21].



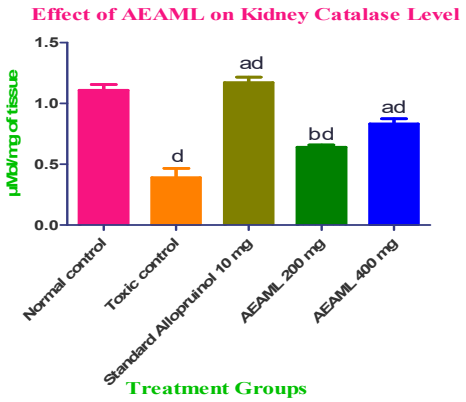


Figure No. 3: Effect of AEAML treatment on oxidative stress markers in potassium oxonate induced gout.

CONCLUSION

The study concluded that aqueous extract *Aegle marmelos* leaves significantly decreased inflammation, serum uric acid levels and oxidative stress in potassium oxonate induced gout. Therefore, leaves of *Aegle Marmelos* could be used for the treatment of gout.

AUTHORS CONTRIBUTION STATEMENT

Dr. M. Raghavendra, Mr. B. Sai Kumar and Miss. D. Nikitha conceptualized and gathered all the results and data by performing research study. Dr. MV Kiran Kumar and Dr. K. Abbulu provided necessary suggestions for designing of work and manuscript.

ACKNOWLEDGMENT

We acknowledge special thanks to Mr. Ch. Gopal Reddy, Secretary and correspondent, CMR College of Pharmacy, Medchal-Malkajgiri district, Telangana State for providing necessary facilities for successful completion of the study.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Ragab G, Elshahaly M, Bardin T. Gout: an old disease in new perspective–a review. Journal of advanced research. 2017 Sep 1;8(5):495-511.
2. Patkar AN, Desai NV, Ranage AA, Kalekar KS. A review on Aegle marmelos: a potential medicinal tree. International Research Journal of Pharmacy. 2012; 3(8):86-91.
3. Rajasekaran C. In vitro evaluation of antibacterial activity of phytochemical extracts from leaves of Aegle marmelos (L.) Corr.(Rutaceae). Ethnobotanical leaflets. 2008; 2008(1):148.
4. Krishnakanth K, Abhilash BG, Neeraja K, HaariJagannadha Rao G, ChakrapaniCheekavolu. Analgesics and anti-inflammatory activity of ethanolic extracts of leaf and fruit pulp of *Aegle Maarmelos* in albino rats. International journal of Basic & Clinical Pharmacology. 2017; 6(7): 1596-1601.
5. Singanan V, Singanan M, Begum H. The hepatoprotective effect of bael leaves (Aegle marmelos) in alcohol induced liver injury in albino rats. International Journal of Science & Technology. 2007;2(2):83-92.
6. Kumar R, Kumar A, Prasal CS, Dubey NK, Samant R. Insecticidal activity Aegle marmelos (L.) Correa essential oil against four stored grain insect pests. International Journal of Food Safety. 2008;10:39-49.

7. Upadhya S, Shanbhag KK, Suneetha G, Balachandra Naidu M, Upadhya S. A study of hypoglycemic and antioxidant activity of Aegle marmelos in alloxan induced diabetic rats. Indian J PhysiolPharmacol. 2004 Jan 1;48(4):476-80.
8. Kothari S, Minda M, Tonpay SD. Anxiolytic and antidepressant activities of methanol extract of Aegle marmelos leaves in mice. Indian J PhysiolPharmacol. 2010;54(4):318-28.
9. Sankari M, Chitra V, Silambujanaki P, Raju D. Anticonvulsant activity of ethanolic extract of Aegle marmelos (leaves) in mice. International Journal of PharmTech Research. 2010;2(1):640-3.
10. Chauhan A, Agarwal M. Reversible changes in the antifertility induced by Aegle marmelos in male albino rats. Systems biology in reproductive medicine. 2008 Jan 1;54(6):240-6.
11. Rajadurai M, Prince PS. Comparative effects of Aegle marmelos extract and alpha-tocopherol on serum lipids, lipid peroxides and cardiac enzyme levels in rats with isoproterenol-induced myocardial infarction. Singapore medical journal. 2005 Feb;46(2):78.
12. Kumar RS, Venkateswar C, Samuel G, Rao SG. Phytochemical screening of some compounds from plant leaf extracts of Holopteleaintegrifolia (Planch.) and Celestrusemarginata (Grah.) used by gondu tribes at Adilabad district, Andhra Pradesh, India. International Journal of Engineering Science Invention 2013; 2: 65-70.
13. Niehaus Jr WG, Samuelsson B. Formation of malonaldehyde from phospholipid arachidonate during microsomal lipid peroxidation. European journal of biochemistry. 1968 Oct;6(1):126-30.
14. Jollow DJ, Mitchell JR, Zampaglione NA, Gillette JR. Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3, 4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology. 1974;11(3):151-69.
15. Greenwald AR. Handbook of methods for oxygen radical research. CRC Press, 1985: 283.
16. Hartwell DW, Wagner DD. New discoveries with mice mutant in endothelial and platelet selectins. Thrombosis and haemostasis. 1999;82(08):850-7.
17. Gasparyan AY, Stavropoulos-Kalinoglou A, Mikhailidis DP, Douglas KM, Kitas GD. Platelet function in rheumatoid arthritis: arthritic and cardiovascular implications. Rheumatology international. 2011 Feb 1;31(2):153-64.
18. Wang F, Wang NS, Yan CG, Li JH, Tang LQ. The significance of platelet activation in rheumatoid arthritis. Clinical rheumatology. 2007 May 1;26(5):768-71.
19. P. Richette and T. Bardin, "Gout," The Lancet, vol. 375, no. 9711, pp. 318–328, 2010.
20. Sarvaiya VN, Sadariya KA, Pancha PG, Thaker AM, Patel AC, Prajapati AS. Evaluation of antigout activity of Phyllanthusemblica fruit extracts on potassium oxonate-induced gout rat model. Veterinary world. 2015 Oct;8(10):1230.
21. Mo SF, Zhou F, Lv YZ, Hu QH, Zhang DM, Kong LD. Hypouricemic action of selected flavonoids in mice: structure–activity relationships. Biological and Pharmaceutical Bulletin. 2007 Aug 1;30(8):1551-6.

SP-44

ASSESSMENT OF ANTI-ULCER ACTIVITY OF *MANILKARA ZAPOTA* LEAF EXTRACT IN EXPERIMENTAL ANIMALS

MITTA RAGHAVENDRA[1*], INAPANURI SUNDAR DEEPTHI[2], DAKE POOJA SRI[2], MANDLEM VENKATA KIRAN KUMAR[3], KONDE ABBULU[4]

[1,2,3]*Department of Pharmacology, CMR College of Pharmacy, Kandlakoya (V), Hyderabad-501401, Telangana state, India.*
[4]*Department of Pharmaceutics, CMR College of Pharmacy, Kandlakoya (V), Hyderabad-501401, Telangana state, India*Email: mittargv@gmail.com

ABSTRACT
In the present study ethanolic extract of *Manilkara zapota* leaves (family: *Sapotaceae)* used to evaluate the anti-ulcer activity by pylorus ligation method in male Wistar albino rats. In the pylorus ligation ulcer model, total 24 animals were randomly divided in 4 groups of 6 animals in each. Group I served as normal control, Group II as standard received Ranitidine and Group III and IV served as low dose (200mg/kg, b. wt*p.o*) and high dose (400mg/kg, b. wt*p.o*) treatment groups. Parameters such as total acidity, free acidity, gastric volume, pH and ulcer index were determined. Preliminary phytochemical study of EEMZL showed the presence of alkaloids, glycosides, steroids, flavanoids, phenols and tannins. Pylorus ligation model showed significant ($p<0.001$) increase in the total acidity, free acidity ulcer index and decrease in the gastric volume and pH. In the treatment, significant decreased in the total acidity, free acidity, ulcer index and increased gastric volume and pH were observed in a dose dependent manner. The present experimental findings suggested that *Manilkara zapota* leaves have potential anti-ulcer activity might be due to the presence of flavanoids, phenols and tannins.

Keywords:*Manilkara zapota,* **Gastric ulcer, Pylorus ligation, Ranitidine**

INTRODUCTION
The use of natural plants for treatment of different diseases is much more common globally due to the significant source of secondary metabolites such as alkaloids, glycosides, steroids, flavonoids, terpenoids, phenols and tannins. Now a day's allopathic medicine came into existence, but these drugs may produce deleterious effects. So the natural plants are considered as most important in the maintenance of health and supervision of diseases[1]. Approximately 10% of global population was severely suffering from Peptic ulcers. The risk of ulcers is more prone in men when compared to the women[2].Medicinal plants and their derived medicines have been used in traditional ways and also for the preparation of semi-synthetic chemical compounds; by this they can be used as synthesis of new fusion. Medicinal plants and their derivatives are habitually using from long established as culture plants like Quinine, Taxol, Vincristine, Colchicine, Morphine, Poopy etc are for cure of various illnesses[3,4]. *Manilkara zapota* (L.). Royen commonly called as Sapodilla, chickoo and Sapota, belongs to family Sapotaceae. Its origin in Mexico and is native to Central America, although it is also widely cultivated in Asian countries. The leaves of *Manilkara zapota* proved as antioxidant[5], anti-diabetic[6], anti-microbial[7], anti-arthritic[8] and anti-inflammatory activities[9]. The aim of the study was to evaluate the anti-ulcer activity of *Manilkara zapota* leaf extract in pylorus ligation induced ulcers in male Wistar albino rats.

MATERIALS AND METHODS

Collection and authentication of plant material
The fresh leaves of *Manilkara Zapota* collected from the local forest area, Medchal, Medchal district, Telangana in the November 2018. Plant authentication was done by Dr. L. Rasingam, Scientist In-charge of Botanical survey of India, Deccan Regional Centre, Attapur village, Hyderabad.

Preparation of extract

The leaves of *Manilkara zapota* cleaned with distilled water to remove the dirt and foreign particles and shade dried for four weeks. The leaves were made into coarse powdered and extracted by a successive Soxhlet extraction process using petroleum ether, ethyl acetate, chloroform, ethanol and distilled water. Thereafter, filtered and the filtrates were dried in a rotary evaporator. The suitable plant extract was selected based on the phytochemical analysis.

Phytochemical investigation

The extracts were subjected to qualitative phytochemical evaluation for the identification of phytoconstituents[10].

Acute toxicity studies

The acute toxicity of selected plant extract was performed according to the OECD guideline 425.

Experimental animals

Male Wistar albino rats (24) weighing in between 200-230 g were procured from Sai Thirumala enterprises, Hyderabad, India. Animals were acclimatized to the laboratory environment for 2 weeks. Rat feed was provided with ad libitum. The study was completed as per the guidelines of CPCSEA, Government of India, after IAEC approval (IAEC number: CPCSEA/1657/IAEC/AMRCP/Col-18/68).

Treatment schedule

Group 1: Normal group (Received 5ml/kg b.wt of distilled water, *p.o* for14 days)
Group 2: Disease control (Received 50 mg/kg/b.wt, *p.o* for 14 days).
Group 3: Ethanolic extract of *Manilkara zapota* leaves (200 mg/kg/b.wt, *p.o*) daily for 14 days.
Group 4: Ethanolic extract of *Manilkara zapota* leaves (400 mg/kg/b.wt, *p.o*) daily for 14 days.

After completion of treatment schedule, rats were fasted 48 hrs with water ad libitum. Then pyloric ligation was done by ligating the pyloric portion of stomach of rats under mild ether anesthesia. Animals were allowed to recover and stabilize in individual cages and were deprived of water during the post-operative period. After 4 hrs of surgery, rats were sacrificed and ulcer scoring was calculated. Gastric juice was collected and gastric secretion studies were performedsuch as total acid level, free acid level, pH and gastric volume[11].

Statistical analysis*:*

The values were expressed as Mean ± SEM, n=6 in each group. The statistical analysis was carried out by one way ANOVA followed by Dunnett's test. $^{a}p<0.001$, $^{b}p<0.01$ and $^{c}p<0.05$ Vs Disease control; $^{d}p<0.001$, $^{e}p<0.01$ and $^{f}p<0.05$ Vs Standard Allopurinol. Values were significant at $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical screening of EEMZL

Based on the phytochemical analysis, we selected ethanolic extract of *Manilkara zapota* leaves (EEMZL) was further studies. The phytochemical analysis of EEMZL was found to be flavonoids, phenolics, saponins and triterpenoids as major secondary metabolites.

Acute toxicity studies

The acute toxicity study of EEMZL revealed that the plant extract was safe in rats at the limit dose of 2000 mg/kg and that the median lethal dose (LD_{50}) of the extract is above 2000mg/kg b. wt (*p.o*), no deaths and no change in general appearance was observed. So that $1/10^{th}$ and $1/5^{th}$ of test dose were fixed at low (200mg/kg) & high (400mg/kg) doses by oral for evaluating antiulcer activity by pyloric ligation model.

In vivo Anti- ulcer activity-Pylorus ligation induced ulcer model in rats

Most simply a peptic ulcer can be called as the irruption of the mucosal integrity of stomach/ duodenum dominant to excavation due to vigorous inflammation. It is mostly occurs in man when compared to women. It is considered as major suffering of human in the growing population. Ulceration mainly occurs in the antral *region* due to lesions in the gastric mucosa caused by pepsin and hydrochloric acid. It is characterized by innumerable episodes of spontaneous healing, frequent relapses. Gastro duodenal mucosal injury results

from an imbalance between the factors that damage the mucosa and those that protect it. Pathogenic processes of gastric ulceration are even more diverse than duodenal ulceration[12].

Ulcers were generated by pyloric ligation assigned to increased collection of gastric acid and also pepsin gives intimation to the auto digestion of gastric mucosa due to the surgery the stomach gets gigantic, the pressure on sensitive receptors at gastric mucosa increases and triggers the vagus-vagal reflex, thereby it causes increased gastric secretion[13]. Pre-treatment with EEMZL in pyloric induced ulcer model notably decreased ulcer index. There was notably increased in pH with depletion in volume of gastric secretion. Free acidity and total acidity in extract treated groups decreased in a dose dependent manner as compared to control group ($p<0.01$). The effect of EEMZL treatment was specified in the Figure No. 1.

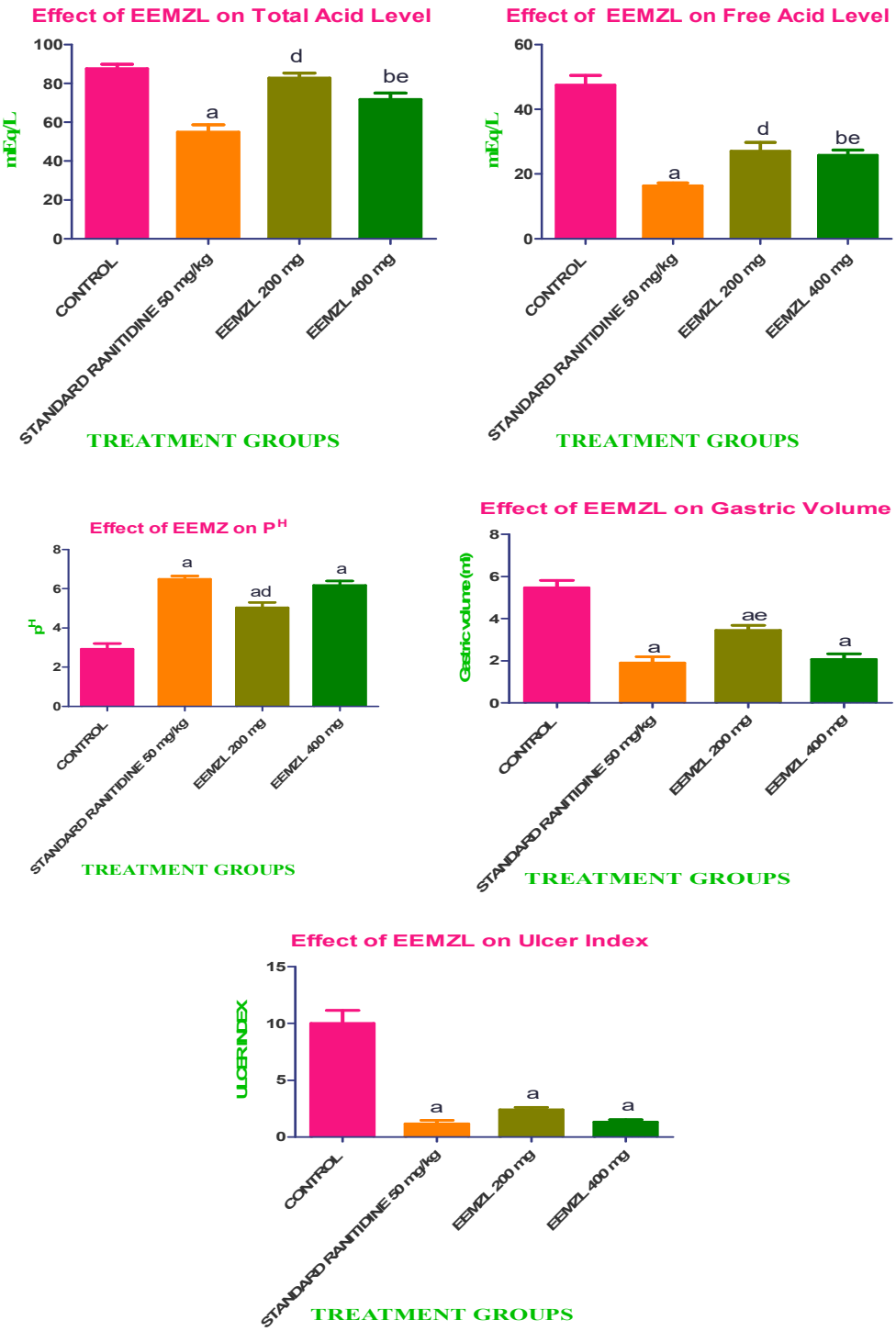
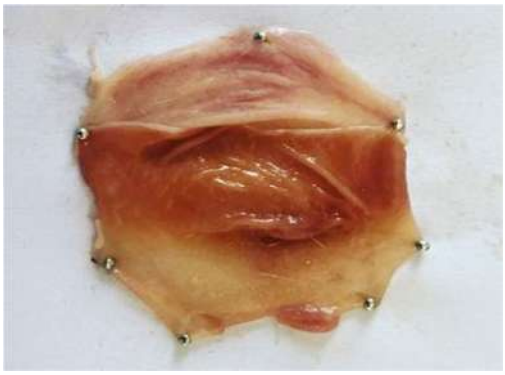


Figure No.1: **Effect of EEMZL treatment on pylorus ligation induced gastric ulcers.**

Values are represented as Mean ± SEM. Statistical analysis performed using one way ANOVA followed by post hoc Dunnett's test. [a]p<0.001 and [b]p<0.01 Vs Toxic control; [d]p<0.001, [e]p<0.01 and [f]p<0.05 Vs Standard Ranitidine.

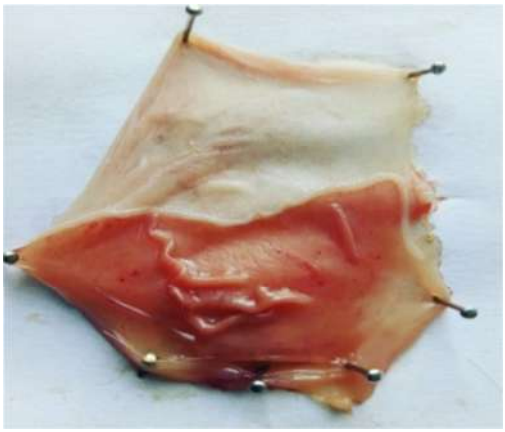
In the pyloric ligation model, accumulation of gastric secretion (5.47±0.347) with pH (3.21±0.21) as well as increased total acidity and free acidity (87.64±2.35 and 47.5±2.97) were observed in control group. Pre-treatment with EEMZL was significantly (p<0.05) reduced the volume of gastric secretion (1.9±0.293 and 1.17±0.307) at doses of 200mg and 400mg/kg b.wt respectively. The pH of gastric juice was significantly (p<0.05) elevated to 6.81±0.21 at a dose of 400 mg/kg b. wt of EEMZL. In addition, total (82.8±2.62 and 71.7±3.45) and free acidity (27.0±2.80 and 25.8±1.58) were also reduced significantly (p<0.05) in dose dependant manner. Further it was observed that pylorus ligation induced model has originate gastric ulceration and pre-treatment with EEMZL has reduced them significantly (p<0.05) in a dose dependent manner. The gastro protective recommended by the test extract was parallel to that of standard drug Ranitidine (50mg/kg b.wt, *p.o*).

Microscopical view of pylorus ligation induced ulcers



Group-I: Control group (Damgae of gastric mucosal layer)

Group-II:Standard Ranitidine (50mg/kg b.wt) (Protected of gastric Mucosal layer)



Group-III:Treatment with EEMZL (200 mg/kg b. wt) showed moderate protection of gastric mucosal layer.

Group-III:Treatment with EEMZL (400 mg/kg b. wt) showed significant protection of gastric mucosal layer.

CONCLUSION

The study concluded that ethanolic extract *Manilkara zapota* leaves significantly decreased pylorus ligation induced gastric ulcers in Wistar male albino rats. Therefore, Manilkara zapota leaves could be used for the treatment of gastric ulcers.

AUTHORS CONTRIBUTION STATEMENT

Dr. M. Raghavendra, Ms. I. SundarDeepthi and Miss. D. Pooja sri conceptualized and gathered all the results and data by performing research study. Dr. MV Kiran Kumar and Dr. K. Abbulu provided necessary suggestions for designing of work and manuscript.

ACKNOWLEDGMENT

We acknowledge special thanks to Mr. Ch. Gopal Reddy, Secretary and correspondent, CMR College of Pharmacy, Medchal-Malkajgiri district, Telangana State for providing necessary facilities for successful completion of the study.

CONFLICT OF INTEREST

Conflict of interest declared none.

References

1. Petrovska BB. Historical review of medicinal plants' usage. Pharmacognosy reviews. 2012 Jan;6(11):1.
2. Kuna L, Jakab J, Smolic R, Raguz-Lucic N, Vcev A, Smolic M. Peptic ulcer disease: a brief review of conventional therapy and herbal treatment options. Journal of clinical medicine. 2019 Feb;8(2):179.
3. Farrer L, Marinetti C, Cavaco YK, Costongs C. Advocacy for health equity: a synthesis review. The Milbank Quarterly. 2015 Jun; 93(2):392-437.
4. Kuehner C. Gender differences in unipolar depression: an update of epidemiological findings and possible explanations. ActaPsychiatricaScandinavica. 2003 Sep;108(3):163-74.
5. Chanda SV, Nagani KV. Antioxidant capacity of Manilkara zapota L. leaves extracts evaluated by four in vitro methods. Nature and science. 2010;8(10):260-6.
6. Barbalho SM, Bueno PC, Delazari DS, Guiguer EL, Coqueiro DP, Araújo AC, de Souza MD, Farinazzi-Machado FM, Mendes CG, Groppo M. Antidiabetic and antilipidemic effects of Manilkara zapota. Journal of medicinal food. 2015 Mar 1;18(3):385-91.
7. Osman MA, Aziz MA, Habib MR, Karim MR. Antimicrobial investigation on Manilkara zapota (L.) P. Royen. Int J Drug Dev Res. 2011 Jan;3(1):185-90.
8. Singh M, Soni P, Upmanyu N, Shivhare Y. In-vitro Anti-arthritic activity of Manilkara zapota Linn. Asian J Pharm Tech. 2011 Oct;1(4):123-4.
9. Konuku K, Karri KC, Gopalakrishnan VK, Hagos Z, Kebede H, Naidu TK, Noyola PP, Dogulas J, PALLETIe GR. Anti-inflammatory activity of Manilkara zapota leaf extract. Int J Curr Pharm Res. 2017;9(4):130-4.
10. Kumar RS, Venkateswar C, Samuel G, Rao SG. Phytochemical screening of some compounds from plant leaf extracts of Holopteleaintegrifolia (Planch.) and Celestrusemarginata (Grah.) used by gondu tribes at Adilabad district, Andhra Pradesh, India. International Journal of Engineering Science Invention 2013; 2: 65-70.
11. Dashputre NL, Naikwade NS. Evaluation of anti-ulcer activity of methanolic extract of Abutilon indicum Linn leaves in experimental rats. Int J Pharm Sci Drug Res. 2011;3(2):97-100.
12. Prabhu V, Shivani A. An overview of history, pathogenesis and treatment of perforated peptic ulcer disease with evaluation of prognostic scoring in adults. Annals of medical and health sciences research. 2014;4(1):22-9.
13. Brodie DA. The mechanism of gastric hyperacidity produced by pylorus ligation in the rat. The American journal of digestive diseases. 1966 Mar 1;11(3):231-41.

SP-45

CLINICAL EVALUATION OF ADVERSE DRUG REACTIONS IN PROTRACTED ILLNESS PATIENTS IN A TERTIARY CARE TEACHING HOSPITAL

A. V. KISHORE BABU[1]* AND A. SRINIVASA RAO[1]

1.Department of Pharmacy Practice, Bhaskar Pharmacy College, Hyderabad- 500075, India.
***Email id: kishorebabu.pharma@gmail.com**

ABSTRACT

The study mainly evaluates the causality, severity, preventability of adverse drug reactions and associated factors with the development of ADRs in chronic disease patients from various departments of tertiary care teaching hospital.A Prospective observational study was conducted in a tertiary care teaching hospital at Hyderabad, India, for a period two years. All the patients were distributed according to their gender, age, number medications used, disease condition, and socioeconomic state. The reported ADRs were analyzed by WHO-UMC causality assessment, Hartwig's Siegel's scale and modified Shumock and Thornton criteria respectively. Descriptive statistics were used for data analysis. A total of 691 patients enrolled in the study, in that 391 patients reported with 510 ADRs. Of these 37.0% are in-patients and 62.9% are out-patients. Majority of the patients are from female category (58.0%) and 45.8% of ADRs reported from adult age group (41-60 years). 65.8% patients are non-adherent to their prescription medication. Life style habits, economic status and education are also found to be predictors in experiencing ADRs. WHO's ADR probability scale showed that 42.9% of ADRs were probable.Hartwig's and Siegel's the severity assessment scales shown that 13.1 % ADRs are severe followed by 33.7% moderate ADRs and 40% of ADRs were preventable.This study provides a database of ADRs due to commonly used drugs. Hence our study advises that there is a need of improvement in ADR reporting from health care professionals. This study also suggests further research in India for the improvement of possible intervention strategies to reduce burden and cost of ADRs.

Key words: Diabetes mellitus, Adverse drug reactions, Spontaneous reporting, Naranjo's and Hartwig's Siegel's Severity assessment.

INTRODUCTION

According to WHO Pharmacovigilance (PV) is defined as the science and activities relating to the detection assessment, understanding and prevention of adverse effects or any other drug-related problem. WHO established its Programme for International Drug Monitoring in response to the thalidomide disaster detected in 1962.[1] The objective of PvPI is, to monitor ADRs in Indian population, to create awareness amongst health care professionals about the importance of ADR reporting in India, to monitor benefit-risk profile of medicines, generate independent, evidence based recommendations on the safety of medicines, support the CDSCO for formulating safety related regulatory decisions for medicines, communicate findings with all key stake holders and create a national centre of excellence as par with global drug safety monitoring standards.[2]

It is generally recommended to treat each chronic condition in accordance with disease-specific guidelines. However, most clinical practice guidelines do not modify or discuss the applicability of their recommendations for older patients with multiple diseases and following all guidelines for each and every drug a patient is taking will inevitably lead to polypharmacy.[3]According to estimates, India has the highest number of adults with diabetes reported at 50.8 millions in 2010 which is expected to rise to 87 million by 2030.[4] The prevalence of diabetes has been reported to be rapidly increasing in both rural and urban India.[5] Prescriber's knowledge about pharmacokinetics and pharmacodynamic aspects of medicines and their interaction with normal aging physiology is critical in the management of diabetes mellitus. The knowledge is needed to minimize and even avoid the potentially adverse effects of hypoglycemia and side effects associated with the anti-diabetic drugs.[6]

In the year 2000, it was also found that the world was estimated to have 1 billion people with hypertension and predicted to increase to 1.56 billion by 2025.[7] Antihypertensive medications are frequently associated with Adverse Drug Reactions which may limit treatment options and reduce patient compliance, which may hinder Blood Pressure control. It was believed that different discontinuation rates for various classes of antihypertensive medications are probably related to their different rates of adverse symptoms.[8, 9] Treatment of TB requires the use of expensive and toxic anti-tubercular drugs which are given for a longer duration.[10, 11] The Revised National Tuberculosis Control Program (RNTCP) in India follows the internationally recommended directly observed treatments (DOTS) guidelines for treatment TB from August 2007 onward.[12]

The female gender, age (very young and very old), multiple medications and the co-morbid medical conditions, socioeconomic status, educational status and lifestyle habits are considered as the important risk factors for ADRs.[13]

People with diabetes have an increased risk of developing a number of serious health problems. Consistently high blood glucose levels can lead to serious diseases affecting the heart and blood vessels, eyes, kidneys, nerves and teeth. In addition, people with diabetes also have a higher risk of developing infections.[14] Globally 70% of diabetic patients are reported to be affected with hypertension and the risk of development of hypertension is twice for diabetic patients as compared to euglycemic subjects.[15] A high prevalence of DRPs has been observed in T2DM patients.[16]

MATERIALS AND METHODS

Present study was carried out in Bhaskar Medical College and General Hospital, a tertiary care teaching hospital located in Hyderabad. The study assessed the causality, severity and preventability of ADRs in chronic diseases. Also, the study determined the prevalence of ADRs associated with their educational status, medication adherence, occupation and socioeconomic factors of patients. Our institute is the recognized ADR monitoring center (AMC) under the "pharmacovigilance program of India." The AMC collects suspected ADR reports from physicians, clinical pharmacy interns, PG medical students as well as nearby teaching hospitals. We transmit reports to the "VigiFlow software "of the WHO for the global monitoring of ADRs provided by Indian Pharmacopoeia Commission, Ghaziabad, India.

Study Design

Prospective observational longitudinal study with active pharmacovigilance reporting system.

Study Period

The study was conducted over a period of 2 years from May 2017 to April 2019.

Ethics committee approval

The study protocol was reviewed and approved by Institutional Human Ethical Committee of Bhaskar Medical College and General Hospital authority prior to the commencement of study.

Study criteria

Inclusion Criteria

- Patients of age from 18 years and both genders
- Both inpatients and outpatient
- Patients with any chronic disease and co-morbid medical condition

Exclusion Criteria

- Children's and pregnant women.
- Patients receiving medicines other than allopathic
- Patients who experienced adverse event to vaccines, blood and /or blood products.
- Adverse event to poisoning/ drug abuse and dependence

Statistical Analysis

Descriptive statistics were used for data analysis. All values were expressed in percentages and depicted using tables and charts. Data were subdivided based on age, gender, number of drugs used, drugs class, medication adherence, habits, economic status, education and occupation.

RESULTS & DISCUSSION

1. Study Population

691 patients met the study criteria were included in the study. Of which 37.0% (n=256) were inpatients and 62.9% (n=435) were outpatients.

2. Characteristics of the Study Population

Out of 691 study patients, 41.9% (n=290) and 58.0% (n=401) were male and female respectively. Majority of the patients were in the age group of 40 – 60 (45.8%). 46% of patients using drugs between 1-2 drugs. 65.8% of patients are non adherent to their medication. 34.5% of patients are both alcoholic and smokers. 50.6% patients are not educated. 25.4% of patients are unemployed followed by 20.1% patients are formers and 34% of patients are economically lower in class. The demographic details of the study population are given in Table.

a. Demographic details of the study population

b.

Characteristics		Inpatients (%) (n=256)	Outpatients (%) (n=435)	Total (%) (n=691)
Gender	Male	107 (41.7)	183 (42.0)	290 (41.9)
	Female	149 (58.2)	252 (57.9)	401 (58.0)
Age	Young Adult (19-39)	45 (17.5)	44 (10.1)	89 (12.8)
	Adult (40-60)	112 (43.7)	205 (47.1)	317 (45.8)
	Elderly (> 61)	99 (38.6)	186 (42.7)	285 (41.2)
No. of Drugs	1 – 2	116 (45.3)	202(46.4)	318 (46.0)
	3 – 4	89 (34.7)	169 (38.8)	258 (37.3)
	≥5	51 (19.9)	64 (14.7)	115 (16.6)
Medication adherence	Adherence	89 (34.7)	147 (33.7)	236 (34.1)
	Non	167 (65.2)	288 (66.2)	455 (65.8)
Social habits	Nil	39 (15.2)	80 (18.3)	119 (17.2)
	Alcoholic	57 (22.2)	110 (25.2)	167 (24.1)
	Smoker	59 (23.0)	93 (21.3)	152 (21.9)
	Alcoholic& Smoker	99 (38.6)	140 (32.1)	239 (34.5)
	Abuse	02 (0.7)	12 (2.7)	14 (2.0)
Education	Illiterate	141(55.0)	209 (48.0)	350 (50.6)
	Primary edu	66 (25.7)	102 (23.4)	168 (24.3)
	Secondary	32 (12.5)	75 (17.2)	107 (15.4)
	Pre university	13 (5.0)	34 (7.8)	47 (6.8)
	university	04 (1.5)	15 (3.4)	19 (2.7)
Occupation	Student	17 (6.6)	22 (5.0)	39 (5.6)
	Daily worker	55 (21.4)	87 (20.0)	142 (20.5)
	Homemaker	32 (12.5)	85 (19.5)	117 (16.9)
	Agriculture	62 (24.2)	77 (3.9)	139 (20.1)
	Salaried/ Busin	29 (11.3)	49 (11.2)	78 (11.2)
	Unemployed	61 (23.8)	115 (26.4)	176 (25.4)
Socioeconomic	Upper	03 (1.1)	18 (4.1)	21 (3.0)
	Upper middle	12 (4.6)	54 (12.4)	66 (9.5)
	Middle Class	51(19.9)	98 (22.5)	149 (21.5)
	Lower Middle	91 (35.5)	129 (29.6)	220 (31.8)
	Lower Class	99 (38.6)	1361.2)	235(34.0)

3. Adverse Drug Reaction

Out of 691 patients enrolled in the study 391 patients reported with 510 ADRs during the study period. The incidence of ADRs details are given in Table

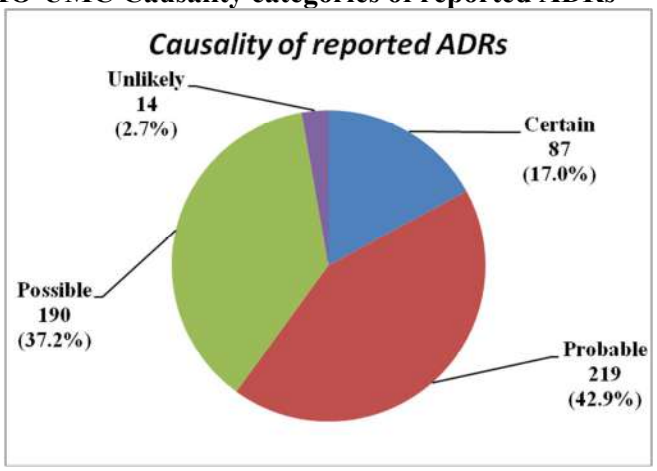
a. Incidence of ADRs based on patient characteristics

Characteristics	Number of patients (n=691)	Number of patients with ADR (n=391)	Incidence	Number of ADRs (n=510)	Percentage of ADRs(%)
Category					
Inpatients	256	106	41.4	143	28.0
Out patients	435	285	65.5	367	71.9
Gender					
Male	290	190	65.5	239	46.8
Female	401	201	50.1	271	53.1
Age (years)					
Young Adults	89	31	34.8	48	9.4
Adults	317	187	58.9	258	50.5
Elderly (> 61)	285	173	60.7	204	40.0
Number of Medications					
1-2	318	218	68.5	261	51.1
3-4	258	108	41.8	159	31.1
>5	115	65	56.5	90	17.6
Medication Adherence					
Adherence	236	113	47.8	169	33.1
Non Adherence	455	278	61.0	341	66.8
Disease condition ICD-10					
(A00-B99)	129	107	82.9	176	34.5
(C00-D48)	1	1	100	1	0.1
(D50-D89)	18	9	50	13	2.5
(E00-E90)	288	145	50.3	152	29.8
(F00-F99)	7	4	57.1	6	1.1
(G00-G99)	29	17	58.6	24	4.7
(H00-H59)	2	1	50	1	0.1
(H60-H95)	2	1	50	1	0.1
(I00-I99)	44	23	52.2	26	5.0
(J00-J99)	22	16	72.7	21	4.1
(K00-K93)	23	11	47.8	13	2.5
(M00-M99)	18	6	33.3	9	1.7
(N00-N99)	10	4	40	7	1.3
(O00-O99)	15	8	53.3	12	2.3
(R00-R99)	75	34	45.3	42	8.2
(S00-T98)	1	1	100	1	0.1
(Z00-Z99)	7	3	42.8	5	0.9

b. Causality assessment of reported ADRs

Majority of the ADRs belonged to 'probable' in their casual relationship, as assessed by WHO probability Scale [n=219 (42.9%)], similar with study done by Rajeshreddy SGSV et al.[17] The causality categories of reported ADRs are presented in Figure .

WHO-UMC Causality categories of reported ADRs

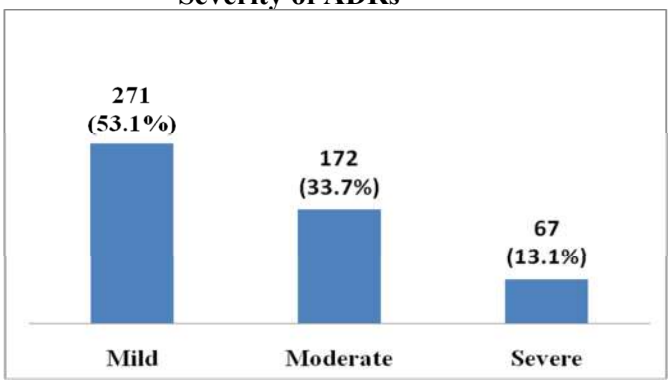


The WHO UMC proposed causality assessment is generally accepted method and most widely used method for causality assessment in clinical practice as they offered a simple methodology. Majority of the ADRs were assigned 'Probable' casual association between the adverse drug event and suspected drug.

c. Severity assessment of ADRs

Most of the reported ADRs were of 'Mild' in their severity and hence did not require withdrawal of the suspected drug especially when the benefits outweighed the risk. This finding coincide with ponnusankar et al., Dindayal Patidar et al. [18, 19] the details of severity of ADRs are given in Figure.

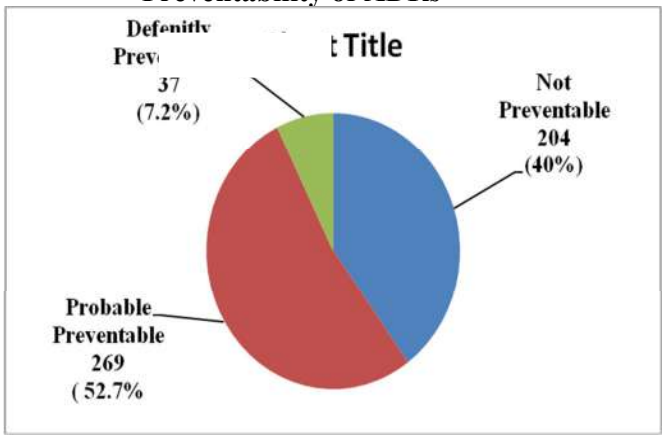
Severity of ADRs



d. Preventability of the ADRs

Of the 510 reported ADRs, 269 (52.7%) were classified as probable preventable, which is variance with the study done by ponnusankar et al.[18] The details of the preventability of ADRs are presented in Figure.

Preventability of ADRs



CONCLUSION

Our findings suggest students' reports were valuable and offered clinically relevant information. ADR monitoring through spontaneous reporting system helps to ensure patient safety through detection of new, serious, and rare drug reactions. Pharm.D interns and PG medical students as a future health-care professional should be exposed to ADR reporting during their clinical teaching posting. The present study relates to ADR profile of tubercular agents, antidiabetic drugs, cardiovascular and antibiotics, it is important to notice the physicians with latest adverse drug reactions of most commonly prescribed medicines in hospitals. Hence effective implementation of pharmacovigilance would result in better strict vigilance use of these drugs and their safety assessment which would ultimately result in better patient care.

ACKNOWLEDGEMENTS

Authors would like to acknowledge ADR Monitoring Centre, Bhaskar Medical College and Bhaskar General Hospital, Hyderabad, Telangana for their kind support to conduct this study.

REFERENCES

1. WHO.https://www.who.int/medicines/areas/quality_safety/safety_efficacy/pharmvigi/en/
2. Pharmacovigilance Programme of India (PvPI) for Assuring Drug Safety. Available from http://cdsco.nic.in/pharmacovigilance_intro.htm
3. Boyd CM, Darer J, Boult C, Fried LP, Boult L, Wu AW. Clinical practice guidelines and quality of care for older patients with multiple comorbid diseases: implications for pay for performance. JAMA. 2005;294(6):716–24. doi: 10.1001/jama.294.6.716.
4. IDF. Diabetes Atlas. 4th ed. Brussels, Belgium: International Diabetes Federation; 2009.
5. Ramachandran A, Snehalatha C. Current scenario of diabetes in India. J Diabetes. 2009;1(1):18-28.
6. Coren J, Overbeck K, Filipetto F. Evaluating medications and adverse drug reactions in older patients, 2011. Available: http:// www.cecity.com/aoa/healthwatch/may_11/print2.pdf. cited on 2015, Nov 07].
7. Michel Joffres et al., Hypertension prevalence, awareness, treatment and control in national surveys from England, the USA and Canada, and correlation with stroke and ischaemic heart disease mortality: a cross-sectional study. British Medical Journal 2013; 3: e003423.
8. Degli Esposti E, Sturani A and Di Martino N: Long term persistence with anti-hypertensive drugs in new patients. Journal of Human Hypertension 2002; 16: 439-444.
9. Degli Esposti L, Degli Esposti E and Valpiani G: A retrospective, population- based analysis of persistence with anti-hypertensive drug therapy in primary care practice in Italy. Clinical Therapeutics 2002; 24: 1347-1357.
10. Iseman MD. Treatment of multidrug-resistant tuberculosis. N Engl J Med. 1993;329:784–91.
11. Drobniewski FA, Balabanova YM. The diagnosis and management of multiple-drug-resistant-tuberculosis at the beginning of the new millenium. Int J Infect Dis. 2002;6(Suppl 1):S21–31.
12. Shin SS, Pasechnikov AD, Gelmanova IY, Peremitin GG, Strelis AK, Mishustin S, et al. Adverse reactions among patients being treated for MDR-TB in Tomsk, Russia. Int J Tuberc Lung Dis. 2007;11:1314–20.
13. Bhabhor PH, Patel TK, Vahora R, Patel PB, Desai N. Adverse drug reactions in a tertiary care teaching hospital in India: Analysis of spontaneous reported cases. Int J Basic Clin Pharmacol 2014;3(6):1078-85.
14. https://www.idf.org/our-activities/care-prevention/cardiovascular-disease.html
15. Purohit P: A cross-sectional study of type -2 diabetic females at a higher CVD risk owing to a strong correlation of Systolic hypertension to CVD risk factors. Int J Biol Med Res. 2012; 3:1905-1909.
16. Haugbolle LS, Sorensen EW: Drug-related problems in patients with angina pectoris, type 2 diabetes and asthma–interviewing patients at home. Pharm World Sci 2006, 28(4):239–247.
17. *Rajeshreddy SGSV,* V., Lokesh V. Patil. Causality assessment and the severity of the adverse drug reactions in tertiary care hospital: a pharmacovigilance study, Int J Basic Clin Pharmacol. 2017 Dec;6(12):2800-2803.
18. S. ponnusankar, m. tejaswini and m. chaitanya. ADR Assessment Based on Spontaneous Signaling, Indian J Pharm Sci 2015;77(4):490-493.
19. Dindayal Patidar, Mithun S. Rajput, Nilesh P. Nirmal, Wenny Savitri. Implementation and evaluation of adverse drug reaction monitoring system in a tertiary care teaching hospital in Mumbai, India, Interdiscip Toxicol. 2013; Vol. 6(1): 41–46.

SP-46

A REVIEW ON CURRENT TREATMENT OPTIONS AND EVOLUTION OF TREATMENT STRATEGIES FOR PATIENTS WITH NON-SMALL CELL LUNG CANCER (NSCLC)

VESANGIKEERTHIVAISHNAVI*,TEKULASHIVASAI, DORANALAHARSHINI,SREENIVASPASULA
Department of Pharmacy Practice, Bhaskar Pharmacy College, Hyderabad- 500075, India.

ABSTRACT
Lung cancer is the most often diagnosed cancer and the largest etiological factor of cancer deaths globally are due to non-small cell lung cancer (NSCLC). Factors of risk for developing NSCLC have been established, with cigarette smoking of tobacco being a major commonest risk factor along with other climatic and hereditary risk factors. Depending on the stage of lung carcinoma, patients are categorized for certain treatments which include surgery, radiation therapy, chemotherapy, and targeted therapy. With the advancements of gene technology and biomarkers testing, the specific type of mutations can be identified in order to benefit each individual patient from target treatment along with the help of immunotherapy. Radiotherapy (RT) plays a vital role in the treating patients with lung cancer and is used at least once in all of patients as a part of both curative and palliative therapies. In thisarticle, wediscussed risk factors associated with NSCLC along with its pathological characterization and classification. Also we briefly mentioned about current treatments available along with surgical options, chemotherapy andimmunotherapy.

Keywords: Non-small cell lung cancer(NSCLC), Chemotherapy, Immunotherapy, Surgery.

INTRODUCTION:
Lung cancer is the most prevailing and most vital cause of cancer mortality worldwide and it accounts for an estimated 1.6 million deaths each year. Among the various subtypes of lung cancers the non-small cell lung cancer(NSCLC) accounts for almost 85% of lung cancer cases[1]of which lung adenocarcinoma (LUAD) and lung squamous cell carcinoma(LUSC) are most common.[2]The passing rates because of lung malignant growths are higher than that of the other three most regular diseases (colon, bosom, and pancreatic) joined.[3]More than 33% of patients determined to have lung malignant growth experience death inside one year of finding and the survival rate of these people was found to be around 17.8%.[4]Advances in treatment and development of new drug therapies for NSCLC have been eased due to improved understanding and estimation of pathogenic genomic alterations that occur in the NSCLC[5]and also the use of biomarkers to know patients who probably can respond to immune checkpoint blockade therapy.[6]Treatment for NSCLC depends mostly on the grade of cancer and the patient's physical fitness. Radiotherapy (RT) is used in all types of lung cancer treatment and is required at least once in all patients for cure of the disease.[7]This review provides an overview of the different therapies and the evolving role of radiation therapies for patients with locally advanced or metastatic NSCLC**.**

RISK FACTORS:
Smoking is the principal hazard factor for lung diseases.[2]At the point when cigarettes turned into the significant tobacco item produced during the1900s, lung malignancy turned out to be increasingly normal. Increase in the quantity of years or the quantity of packs smoked every day caused an increase in the level of hazard for lung disease.[8]Smoking for the most cases causes 80 percent of lung malignant growth deaths around the world.[9]One of the commonest causes of lung cancer is due to occupational exposure to different chemicals. The use of asbestos in industry and its manufacturing caused an increased incidence of lung cancer.[10,11]In urban areas and other big cities with traffic congestion, long term exposure to air pollution(including emissions which are composed of polycyclic aromatic hydrocarbons) has proven to cause lung cancer.[12]Air pollution has also been associated with an eight percent increased risk of causing all types of lung cancer mortality.[13]Inheritance of lung cancer also serves as a risk factor to develop lung carcinoma.[14]

PATHOLOGICAL CHARACTERISATION AND CLASSIFICATION OF NSCLC:

HISTOLOCIAL CLASSIFICATION:

Histological analysis is mandatory to diagnose NSCLC. Generally, it helps in classifying into subtypes and molecular analysis of the lung tumors. The two most prevalent histological subtypes include adenocarcinoma(60%) and squamouscell carcinoma(15%). Mixed histology tumors and non-small cell carcinoma are uncommon variants.[15]The safety and therapeutic benefits of few chemotherapeutic agents vary by tumor histology.[16]

MOLECULAR CHARACTERIZATION:

Moleculartesting identifies patients with metastatic non-small cell lung cancer (NSCLC) as to indicate therapy who may benefit from targeted therapy or immunotherapy [i.e., immune checkpoint inhibitor treatment for patients with high tumor mutational burden (TMB)]. Molecular testing detects the presence of gene mutations or rearrangements in the body [for which the US Food and Drug Administration (FDA) has approved therapies]. These cellular alterations/mutations include epidermal growth factor receptor (EGFR) gene mutations, anaplastic lymphoma kinase (ALK) gene rearrangements, ROS proto-oncogene receptor tyrosine kinase 1 (ROS1) rearrangements, and neurotrophic receptor tyrosine kinase(NTRK) gene fusions.[17,18]

CURRENT TREATMENT OPTIONS :

1)SURGERY :

Patients who have grade I, II, and IIIA NSCLC ought to be exposed to medical procedureso as to isolate the tumor cells in order to know if the tumor is operable or not and if the patient is capable withstand the medical procedure. During medical procedure, the influenced flap might be expelled from the lung orthe segment of the lung containing the malignant growth cells can be evacuated. To know whether the tumor is resectable or not, imaging studies and biopsies are prescribed . It likewise helps in the assessment of the patient components to decide operability.[19]

2)ADJUVANT THERAPY:

A few patients who have experienced a medical procedure may profit by adjuvant treatment in lessening the danger of lung disease repeats. Adjuvant therapy may include radiation type, chemotherapy type, and targeted type therapy. Betterment in the overall survival (OS) and pCRis not observed with adjuvant therapy alone. This adjuvant therapy help prevent recurrences in majority of patients. Patients with IIA, IIB, and IIIA stage of non-small cell lung cancer are usually administered with chemotherapy after surgery in order to kill any other remaining cancer cells so that the survival rate of the patient can be prolonged.[20]

3) CHEMOTHERAPY:

Nearly 40% of lung cancers diagnosed are stage IV. The long term goal is to enhance survival, quality of life and decrease the disease-related adverse events. For stage IV NSCLC, combinational chemotherapy is used as first line option but therapeutic considerations can be varied based upon patient age, any other associated co morbidities and performance status(PS).[21] Cisplatin is irrelevant sensibly stunning platinum compound stood apart from different mixes. In any case, it has shown to have high threatening impacts. For patients with a PS of 2, they may require just one drug, which is ordinarily not a platinum compound.[22]On a very basic level after four treatment cycles, on the off chance that the treatment isn't improving the state of the patients, by then the treatment must be changed in like manner.[23,24] Patients with a PS of 3 genuinely don't react to chemotherapy considering consistently compromising occasions related with chemotherapy. For these patients enduring consideration is regularly given.[25]

CONCLUSION:

In this review, we have provided information on the biology and different molecular subtypes of non-small cell lung cancer (NSCLC) and different treatment options available for patients with NSCLC. Recent advances in these therapies have led to more biomarker mediated treatments for patients with metastatic disease. Also, the advances in imaging, tumor localization, and RT technology have facilitated the

development of stereostatic ablative radiotherapy(SART) for early-stage NSCLC and allowed the treatment of large volume NSCLC. The recent advances have also led to development of immunotherapy drugs that stimulate your immune system to identify and destroy cancer cells and acts by blocking the PD1 and PD-L1 pathways. All these types of therapies have led to improved overall survival and quality of life for patients.

ACKNOWLEDGMENT:
We thank A.Srinivas Rao, [Principal, Bhaskar Pharmacy College] for providing support, computer laboratory facility, online accessibility of articles and other resources. With pleasure we wish our sincere thanks to Dr.A.V.KishoreBabu [B.pharm ,PharmD (P.B), Head of the department , Department of pharmacy practice] for his advice and constant encouragement. We would like to thank Dr.SreenivasPasula [Pharm D (PhD), Department of pharmacy, Bhaskar Pharmacy College] who guided us through this article.

REFERENCES:

1. GovindanR, PageN, MorgenszternD,etal. Changing epidemiology of small-cell lung cancer in the United States over the last 30years: analysis of the Surveillance, Epidemiologic, and End Results database.JClinOncol.2006;24(28):4539- 4544. doi:10.1200/JCO.2005.04.485
2.Roy S. Herbst1, Daniel Morgensztern2 & Chris Boshoff1,3The biology and management of non-small cell lung cancer.
3. American Cancer Society. Cancer Facts and Figures 2015 Available online: http://www.cancer.org/acs/groups/ content/@editorial/documents/document/a cspc-044552.pdf.
4. National Cancer Institute. SEER Cancer Statistics Review, 1975-2011. Available online: http://seer.cancer.gov/ csr/1975_2011.
5. JordanEJ, KimHR, ArcilaME,etal. Prospective comprehensive molecular characterization of lung adeno carcinomas for efficient patient matching to approved and emerging therapies.Cancer Discov. 2017;7(6):596-609.doi:10.1158/2159- 8290.CD-161337.
6. KathrynC.Arbour,MD;GregoryJ.Riely,MD ,PhD Systemic Therapy for Locally Advanced and Metastatic Non–Small Cell Lung Cancer A Review.
7. Kong F-MS, Zhao J, Wang J, Faivre-Finn C. Radiation dose effect in locally advanced non-small cell lung cancer. J Thorac Dis 2014; 6: 336–47. doi: https:// doi. org/ 10. 3978/ j. issn. 2072- 1439. 2014. 01. 23.
8. Hecht SS. Tobacco smoke carcinogens and lung cancer. J Natl Cancer Inst 1999;91:1194-210
9. Cecilia Zappa, Shaker A. Mousa Non-small cell lung cancer: current treatment and future advances The Pharmaceutical Research Institute, Albany College of Pharmacy and Health Sciences, Rensselaer, New York, USA.
10. Hodgson JT, Darnton A. The quantitative risks of mesothelioma and lung cancer in relation to asbestos exposure. Ann OccupHyg 2000;44:565-601. 20.
11. Berman DW, Crump KS. A meta-analysis of asbestosrelated cancer risk that addresses fiber size and mineral type. Crit Rev Toxicol 2008;38 Suppl 1:49-73.
12. Vineis P, Husgafvel-Pursiainen K. Air pollution and cancer: biomarker studies in human populations. Carcinogenesis 2005;26:1846-55. 25.
13. Pope CA 3rd, Burnett RT, Thun MJ, et al. Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. JAMA 2002;287:1132-41.
14. Sellers T, Yang P. Familial and genetic influences on risk of lung cancer. In: King R, Rotter J, Motulsky A. editors. The Genetic Basis of Common Diseases. New York: NY, Oxford University, 2002:700- 12.
15. National Cancer Institute. Surveillance, Epidemiology, and End Results Program: cancer of the lung and bronchus: cancers at facts.https://seer.cancer.gov/statfacts/html/l ungb.html. AccessedJuly27,2019.
16. JohnsonDH, FehrenbacherL, NovotnyWF,etal. Randomized phaseII trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small cell lung cancer.J ClinOncol. 2004;22(11):2184-2191. doi:10.1200/ JCO.2004.11.022.
17. TravisWD, BrambillaE, RielyGJ. New pathologic classification of lung cancer: relevance for clinical practice and clinical trials,JClinOncol2013;31(8): 992-1001.doi:10.1200/JCO.2012.46.9270.
18. Van Loon AJ, Kant IJ, Swaen GM, et al. Occupational exposure to carcinogens and risk of lung cancer: results from The Netherlands cohort study. Occup Environ Med 1997;54:817-24.

19. Howington JA, Blum MG, Chang AC, et al. Treatment ofstage I and II non-small cell lung cancer: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 2013;143:e278S-313S.
20. Chemotherapy in non-small cell lung cancer: a metaanalysis using updated data on individual patients from 52 randomised clinical trials. Non-small Cell Lung Cancer Collaborative Group. BMJ 1995;311:899-909.
21. Ramalingam S, Belani C. Systemic chemotherapy for advanced non-small cell lung cancer: recent advances and future directions. Oncologist 2008;13 Suppl 1:5- 13.
22.Gridelli C, Ardizzoni A, Le Chevalier T, et al. Treatment of advanced non-small-cell lung cancer patients with ECOG performance status 2: results of an European Experts Panel. Ann Oncol 2004;15:419-26
23.Pisters KM, Evans WK, Azzoli CG, et al. Cancer Care Ontario and American Society of Clinical Oncology adjuvant chemotherapy and adjuvant radiation therapy for stages I-IIIA resectable non small-cell lung cancer guideline. J ClinOncol 2007;25:5506-18.
24.Scott WJ, Howington J, Feigenberg S, et al. Treatment of non-small cell lung cancer stage I and stage II: ACCP evidence-based clinical practice guidelines (2nd edition). Chest 2007;132:234S-242S.
25.Cecilia Zappa, Shaker A. Mousa Non-small cell lung cancer: current treatment and future advances. http// dx.doi.org/10.21037//tlcr.2016.06.07.