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PRECLINICAL TOXICITY STUDIES OF AERIAL PARTS OF *WALSURA PISCIDIA* ROXB

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ABSTRACT

The present study was aimed to evaluate the safety of aqueous and ethanolic extracts of aerial part of *Walsura piscidia* Roxb. (AEWP & EEWP) by determining their potential toxicity after acute and 28-day repeated dose administration in Wistar Albino rats. Acute and 28-day repeated dose oral toxicity studies were performed by following OECD test guide lines 423 and 407 respectively. In acute toxicity study no treatment related death or toxic signs were observed with AEWP and EEWP administration. In repeated dose study no significant differences in bodyweight changes and hematology was observed between control and AEWP and EEWP groups. Conversely there was a decrease in serum glucose and cholesterol levels and increase in protein levels in treated rats compared to control. No gross pathological findings and difference in relative organ weights were observed between control and test drug treated rats. Histopathological examination revealed no abnormalities with the test drug treatment. Acute toxicity study reveals that LD_{50} of AEWP and EEWP is greater than 2000mg/kg bwt in fasted female rats and can be classified under category 5. The 28 days. repeated oral toxicity study justified that the No Observed Adverse Effect Level (NOAEL) of *Walsura piscidia* Roxb is greater than 800mg/kg bwt/day p.o in rats. There were no delayed effects in satellite group. In conclusion GS was found to be non toxic in tested doses and experimental conditions.

Key words: *Walsura piscidia* Roxb, acute toxicity study, repeated dose study, LD_{50}.

INTRODUCTION

Walsura piscidia Roxb is an evergreen tree widely distributed in the tropical areas of Asia, such as India, Southern China, Malaysia, and Indonesia (M. Suri Appa Rao et al 2012). The genus of Walsura Roxb belongs to family Meliaceae. It has comprised 10 species in India (Hooker J. D 1872; Ghosh R. B 1961). The plant is commonly known as Cheddavokko in Tamil, Male sagade in Kannada, Perillapacha in Malayalam.Traditionally the plant is reported for its Anti-microbial (K. Sri Rama Murthy, Nagamani Kandimalla 2008). Stimulant, Expectorant, emmenagogue and emetic properties. According to the literature tribal people uses this plant to treat various diseases like skin allergies, astringent and diarrhea (Pullaiah T, and Rani SS 1999). Despite the use of the plant in traditional, so far no scientific

evaluation was carried out on this plant for the toxicity profile preclinically. Therefore our study was therefore undertaken to screen phytochemical constituents and determine the toxicity profile of aqueous and ethanolic extracts of aerial parts of *Walsura piscidia* Roxb on Wistar Albino rats. The acute toxicity and 28-day repeated dose studies may be required to predict the safety and effects of long term exposure of a particular medicinal plant. This study therefore seeks to assess *Walsura piscidia* Roxb for its toxic effects by seeing body weight and organ weight changes and hematological and serum biochemical parameters and changes in histopathology.

MATERIALS AND METHODS

Plant material

Walsura piscidia Roxb was collected from the Western Ghats and identified by Dr. V. Chelladurai, Rtd Senior Research Officer, Tirunelveli, Voucher specimens have been deposited at Gokula Krishna College of Pharmacy, Department of Pharmacognosy, Sullurpet, Nellore Dist, Andhra Pradesh (Voucher No. GKCP-25).

Preparation of extract

About 200g of the powdered material was subjected to Soxhlet and exhaustively extracted with 80% ethanol for 20 hrs. The solvent was distilled off at low temperature under reduced pressure using a rotary flash evaporator. The semisolid mass obtained was dried in an oven at 40°C, and powdered (WHO, Geneva 2000, Ayurvedic Pharmacopoeia).

Selection of experimental animals

Male and female Wistar albino rats (130-160gm) were used in the study. Animals were housed individually in polypropylene cages in a ventilated room under ambient temperature of 22±2° C and 45-65 % relative humidity, with a 12 hour light followed by 12 hour dark. All the animals were acclimatized at least 7days to the laboratory conditions prior to experimentation .Tap water and food pellets were provided ad libitum. Food pellet was with held overnight prior to dosing. All rats were handled and maintained strictly as per guidelines of "Guide for the care and Use of Laboratory animals". (Institute of Laboratory Animals Resources, National Academic Press 1996: NIH Publication number # 85-23, revised 1996).

Acute oral toxicity study

The acute oral toxicity study was performed as per the Organisation for Economic and Cooperation and Development (OECD) 423 guidelines. Nine female rats (nulliparous and non pregnant; 130-160 gm bwt) were divided into three groups (3 per group) ie., control and two test groups. Control group received 0.5 % carboxy methyl cellulose as vehicle at a dose of 10ml/kg bwt while the test groups received an oral dose of 2000mg/kg bwt of AEWP and EEWP (10ml/kg bwt in 0.5% CMC). All the experimental animals were observed for mortality and clinical signs of toxicity (general behavior, respiratory patterns, cardiovascular signs, motor activities, reflexes and changes in skin and fur texture) at 30 min,1, 2, and 4 hours and thereafter once a day for 14 days following vehicle, AEWP and EEWP administration. Body weights were recorded once a week. On 15th day the overnight fasted rats (water allowed) were euthanized using CO_2 euthanasia chamber subjected to gross pathological examination of all the major internal organs such as heart, lung, liver, kidney and spleen. LD_{50} cut-off value of AEWP and EEWP was determined in accordance with Globally Harmonized System of Classification and labeling of chemicals (OECD 2001).

Repeated dose 28-day oral toxicity study

Methodology

A 28-day repeated oral toxicity was performed according to the OECD guideline, TG 407. In the present study, AEWP and EEWP were administered at three dose levels ie., at 200, 400and 800mg/kg/day. Both sexes of Wistar Albino rats (130-160gm) were divided into 7 groups with 10 animals (5 males+ 5 females) in each. Group I served as control and received 0.5 % CMC as vehicle orally at a dose of 10ml/kg b.wt. Remaining 6 groups received AEWP and EEWP at 200 (Group II & III), 400 (Group IV & V) and 800 (Group VI &VII) mg/kg/day, p.o, respectively (10ml/kg b. wt. in 0.5% CMC), for a period of 28 days. In order to determine the reversibility or recovery from toxic effects, additional satellite groups were preset. Group VIII served as satellite control (received 0.5 %CMC) Group IX and X served as treatment satellite groups which received AEWP and EEWP at 800mg/kg/day, p.o for a period of 28 days. Then the satellite groups were scheduled for follow-up observations for the next 14 days without vehicle or AEWP and EEWP administration (OECD 2008).

Observation

All the experimental animals were observed for mortality and morbidity twice a day, till the completion of treatment Clinical observations were made once daily to detect signs of toxicity. The focus of observation was same as described above for the acute toxicity study. Body weights of the animals were once a week.

Blood analysis

At the end of the stipulated treatment period, the overnight fasted animals were anesthetized, whole blood samples were collected by cardiac puncture for haematological and biochemical analysis. Haematological parameters such as red blood cell count (RBC), haemoglobin (Hb), hematocrit(HCT), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), platelets, white blood corpuscles (WBC) and lymphocytes were analysed by fully automated analyser. Biochemical parameters such as serum glucose, cholesterol, protein, bilirubin, Blood Urea Nitrogen (BUN), creatinine, alkaline phosphatease(ALP), aspartate transaminase (AST), alanine transaminase (ALT) and lactate dehydrogenase (LDH) were analysed.

Histopathology

Necroscopy was done in all the animals on 29th day except the satellite group for which it was done on 42nd day. After blood collection all the animals were euthanized for gross pathological examinations of all major internal organs. Organs such as heart, liver, lung, spleen and kidney were collected from all the animals for weighing and calculating relative organ weights and for histopathology. However as per OECD 407 guidelines histopathological examination should perform for the control and high dose initially, if any histopathological findings were observed with the high dose group, the low and mid dose group were to be studied. The organs were fixed 10% neutral buffered formalin, trimmed and a 5µ thickness of tissue sections were stained with hematoxylin and eosin for histopathological investigation.

Stastistical analysis

The statistical analysis were carried out by one way ANOVA followed by Dunnet's multiple comparision test for the control and treatment groups using Graph Pad prism 5.0. p value ≤ 0.05 was considered as significance.

RESULTS

Acute oral toxicity study

There was no treatment related death or signs of toxicity developed in the control, AEWP and EEWP treated rats through the study. Rubbing of nose and mouth on the floor of the cage and restlessness were the only behavioral signs of toxicity shown by the animals and these disappeared with in 24 hrs of extracts administration. During the study there were no significant changes in body weights of treated rats compared to control group. Further there were no gross pathological abnormalities in both control and treated rats. Thus the LD_{50} value was found to be greater than 2000mg/kg b.wt. with reference to the Globally Harmonized System of Classification and labeling the chemicals , *Walsura piscidia* Roxb can be classified as Category -5 and this provides the relevance for protecting human and animal health.

Repeated dose 28-day oral toxicity study

General behavior

There were no noticeable change in the general behavior; treatment related toxicity signs and mortality observed in both sexes of rats treated at 200, 400 and 800 mg/kg of both aqueous and ethanolic extracts orally for a period of 28 days and in the satellite groups of rats. No significant difference in the body weight gain was observed between control and treated groups during the study. The results are depicted in table 1.

Haemagram

Haematological parameters such a red blood corpuscles, haemoglobin, hematocrit, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin concentration, platelets, white blood corpuscles and lymphocytes were found to be well within the clinical range of rats (www.ahuedu 2011). in the experimental groups which are shown in table 2.

Biochemical indices

There was a significance decrease in glucose and cholesterol levels in AEWP and EEWP treated rats and an increase in serum protein of rats treated with AEWP (800mg/kg/day) and EEWP (400 & 800mg/kg/day) compared to the control groups. No changes in other biochemical parameters like bilirubin, blood urea nitrogen, creatinine, alkaline

phosphatase, aspartate transaminase, alanine transaminase and lactate dehydrogenase observed between control and treated groups which are shown in table 3.

Organ weights

There were no significant differences in organ and relative organ weights of heart, lung, liver, kidney and spleen recorded between the control, AEWP and EEWP treated groups which are shown in table 4.

The macroscopic analysis of the target organs of the treated rats did not show changes in colour and texture when compared with the control group. In our study we performed histopathological examinations in control and high dose group. The organs revealed no abnormalities. Hence the authors didn't performed histopathological examination of low and mid dose groups. In addition, microscopic analysis did not show histological alterations in any of the organs examined.

Histopathology

Table-1

Effects of the extracts of Walsura piscidia* Roxb *on body weight gain of rats-repeated oral toxicity study

Treatment			Body weight in gms / week					
			1st	2nd	3rd	4th	5th	6th
Control Group	0.5% CMC		163.087±0.87	168.65±0.76	173.59±0.73	176.46±2.04		
W.P treated Group	200mg/kg P.O/day	Aq extract	162.600±0.92	167.74±1.07	172.38±1.31	175.82±1.29		
		Ethanolic extract	161.583±0.97	166.502±0.97	170.60±1.36	175.07±1.29		
	400mg/kg P.O/day	Aq extract	163.656±0.36	169..04±0.40	173.70±0.74	178.41±0.75		
		Ethanolic extract	162.111±0.93	167.96±0.77	172.46±1.51	177.34±1.08		
	800mg/kg P.O/day	Aq extract	163.739±0.53	169.01±0.59	174.03±0.54	175.74±1.16		
		Ethanolic extract	162.414±0.72	167.79±0.78	172.62±0.84	177.44±1.18		
Satellite Group	Control	0.5% CMC	164.341±0.58	169.62±0.57	174.70±0.72	178.58±1.06	182.09±0.95	185.91±0.80
	W.P (800mg/kg P.O/day)	Aq extract	163.301±0.45	169.17±0.39	173.16±0.85	177.72±0.83	180.04±.71	183.81±1.01
		Ethanolic extract	163.276±0.46	168.57±0.68	173.05±1.14	177.72±1.09	179.22±.93	182.79±1.19

Values are expressed as mean ± SEM; gm-gram; Aq-Aqueous; Eth-Ethanolic; Significance with Dunnet's test following one way ANOVA is evaluated as *p < 0.05, **p < 0.01and ***p <0.001 vs control group

Table -2

Effects of the extracts of Walsura piscidia* Roxb *on haematological parameters of rats – repeated oral toxicity study

Treatment			Haematological parameters			
			RBC(x10/cmm)	Hemoglobin (g/dl)	MCV(fl)	PCV (%)
Control Group	0.5% CMC		8.30±0.31	15.07±0.39	59.80±2.75	46.20±2.13
W.P treated Group	200mg/kg P.O/day	Aq extract	7.75±0.32	14.28±0.48	56.60±2.68	42.50±1.80
		Eth extract	7.78±0.26	13.80±0.35	53.90±3.03	44.40±1.97
	400mg/kg P.O/day	Aq extract	7.60±0.22	13.52±0.46	52.40±2.87	44.20±2.13
		Eth extract	7.87±0.29	14.31±0.43	54.30±2.46	43.66±2.17
	800mg/kg P.O/day	Aq extract	7.44±0.22	13.99±0.46	59.80±2.00	43.40±1.63
		Eth extract	8.16±0.29	13.96±0.46	56.40±1.88	42.90±1.74
Satellite Group	Control	0.5% CMC	8.14±0.33	14.58±0.54	60.30±2.45	46.60±1.64
	W.P(800mg/kg P.O/day)	Aq extract	7.65±0.24	13.83±0.28	57.50±2.20	45.70±1.73
		Eth extract	7.74±0.23	13.90±0.33	56.50±3.10	44.20±2.02

Treatment			Haematological parameters			
			Lymphocytes (%)	MCHC(g/dl)	Platelets (x 10^3 /cmm)	WBC (x 10^3/cmm)
Control Group	0.5 % CMC		7.07±0.43	35.80±0.87	355.80±19.53	13.20±1.06
W.P treated group	200mg/kg P.O/day	Aq extract	6.64±0.47	35.10±0.78	317.40±26.10	13.20±1.15
		Eth extract	6.61±0.42	33.80±0.53	325.90±15.11	12.00±1.67
	400mg/kg P.O/day	Aq extract	6.75±0.29	34.40±0.81	326.50±16.26	11.80±0.99
		Eth extract	6.69±0.45	34.90±0.83	348.30±17.49	12.50±0.89
	800mg/kg P.O/day	Aq extract	6.86±0.43	34.80±0.55	333.00±16.09	12.40±1.28
		Eth extract	6.61±0.35	33.90±0.70	325.30±17.03	11.90±0.99
Satellie Group	Control	0.5 % CMC	6.99±0.38	35.40±0.70	368.10±17.72	13.70±1.01
	W.P(800mg/kg P.O/day)	Aq extract	6.27±0.29	34.90±0.73	321.10±15.32	12.00±1.03

		Eth extract	6.56±0.41	35.00±0.95	331.80±14.98	13.30±1.85

Values are expressed as mean ± SEM; Aq-Aqueous; Eth-Ethanolic; Significance with Dunnet's test following one way ANOVA is evaluated as *p < 0.05 **p < 0.01 and ***p <0.001 vs control group. RBC-Red blood cell; HCT-Hematocrit; PCV-Packed cell volume; MCV-Mean corpuscular concentration; MCH-Mean corpuscular haemoglobin; MCHC- Mean corpuscular haemoglobin concentration; WBC-White blood corpuscles.

Table 3

Effects of the extracts of Walsura piscidia Roxb *on biochemical parameters of rats-repeated oral toxicity study*

Treatment			**Biochemical parameters**				
			Glucose (mg/dl)	**Cholesterol (mg/dl)**	**Protein (g/dl)**	**Bilirubin (mg/dl)**	**BUN (mg/dl)**
Control group	0.5% CMC		97.20±4.63	97.20±4.63	6.73±0.24	0.45±0.03	17.31±0.58
W.P treated Group	200mg/kg P.O/day	Aq extract	93.60±3.24	91.80±3.88	5.85±0.23	0.37±0.02	16.89±0.33
		Eth extract	88.40±3.25	93.60±3.24	5.96±0.35	0.36±0.03	16.77±0.43
	400mg/kg P.O/day	Aq extract	88.40±2.88	88.10±3.25	6.65±0.25	0.39±0.03	16.68±0.39
		Eth extract	81.50±8.49	88.30±3.25	6.21±0.34	0.43±0.03	16.81±0.41
	800mg/kg P.O/day	Aq extract	94.00±3.79	88.40±2.88	6.47±0.31	0.422±0.01	16.88±0.51
		Eth extract	88.10±3.27	81.50±8.49	6.33±0.28	0.84±0.46	16.83±0.29
Satellite Group	Control	0.5% CMC	91.80±3.88	97.20±4.63	6.59±0.28	0.45±0.01	17.92±0.61
	W.P (800mg/kgP.O/day)	Aq extract	90.40±4.89	89.40±4.17	5.98±0.20	0.41±0.02	16.89±0.32
		Eth extract	88.60±3.97	88.80±4.00	6.24±0.26	0.39±0.03	16.54±0.29

Treatment			**Biochemical parameters**			
			Creatinine (mg/dl)	**ALP (IU/L)**	**AST(IU/L)**	**ALT(IU/L)**
Control Group	0.5 % CMC		0.56±0.04	44.20±1.34	64.03±3.96	25.04±0.78
W.P treated group	200mg/kgP.O/day	Aq extract	0.63±0.04	41.70±1.24	54.91±2.48	23.92±0.59
		Eth extract	0.62±0.05	43.80±1.72	54.54±2.54	24.76±0.31
	400mg/kgP.O/day	Aq extract	0.61±0.01	44.00±1.92	55.71±3.10	24.92±1.15
		Eth extract	0.64±0.03	42.90±1.62	58.45±3.69	24.20±0.32
	800mg/kgP.O/day	Aq extract	0.60±0.03	43.80±1.51	64.14±3.32	23.91±0.70
		Eth extract	0.64±0.03	42.90±1.74	56.38±3.07	24.34±0.67
Satellite Group	Control	0.5 % CMC	0.68±0.03	46.60±1.12	68.02±3.27	25.07±0.19
	W.P(800mg/kg P.O/day)	Aq extract	0.62±0.02	45.70±1.73	65.62±2.16	23.95±0.55
		Eth extract	0.62±0.04	44.70±1.33	67.92±2.00	23.79±0.57

Values are expressed as mean ± SEM; Aq-Aqueous; Eth-Ethanolic; Significance with Dunnet's test following one way ANOVA is evaluated as *p < 0.05 **p < 0.01 and ***p <0.001 vs control group. BUN-Blood urea nitrogen; ALP-Alkaline Phosphatase; AST-Aspartate transaminase; ALT-Alanine transaminase; LDH-Lactate dehydrogenase.

Table -4

Effect of the extracts of Walsura piscidia Roxb *on relative organ weights of rats –repeated oral toxicity study*

Treatment			**Organ weight (gms)**			
			Heart	**Liver**	**Kidney**	**Spleen**
Control Group	0.5% CMC		0.38±0.01	3.56±0.12	0.74±0.03	0.53±0.02
W.P treated Group	200mg/kg P.O/day	Aq extract	0.44±0.05	3.80±0.10	0.80±0.03	0.53±0.02
		Ethanolic extract	0.39±0.004	3.74±0.09	0.78±0.04	0.52±0.01
	400mg/kg P.O/day	Aq extract	0.39±0.008	3.58±0.13	0.75±0.02	0.53±0.01
		Ethanolic extract	0.37±0.01	3.71±0.09	0.75±0.02	0.53±0.01
	800mg/kg P.O/day	Aq extract	0.38±0.007	3.78±0.11	0.73±0.02	0.52±0.01
		Ethanolic extract	0.38±0.007	3.78±0.13	0.79±0.02	0.53±0.01
Satellite Group	Control	0.5% CMC	0.37±0.009	3.48±0.12	0.68±0.03	0.51±0.01
	W.P (800mg/kg P.O/day)	Aq extract	0.36±0.01	3.45±0.13	0.72±0.02	0.50±0.01
		Ethanolic extract	0.37±0.009	3.62±0.14	0.71±0.03	0.52±0.01

Values are expressed as mean ± SEM; Aq-Aqueous; Eth-Ethanolic; Significance with Dunnet's test following one way ANOVA is evaluated as *p < 0.05 **p < 0.01 and ***p <0.001 vs control group.

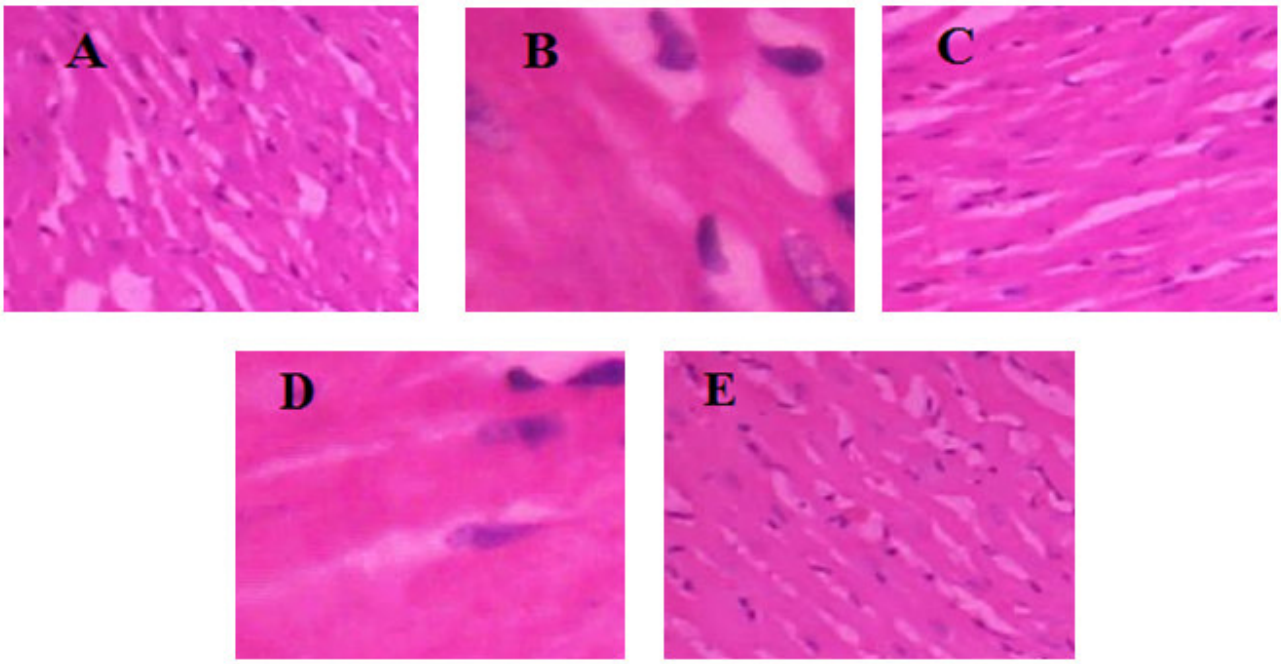

Figure 1
Photomicrographs of heart tissue: A-Control 0.5% CMC; B-AEWP 800mg/kg/day; C-EEWP 800mg/kg/day; D-Satellite AEWP 800mg/kg/day; E-Satellite EEWP 800mg/kg/day. (x 40 magnification)

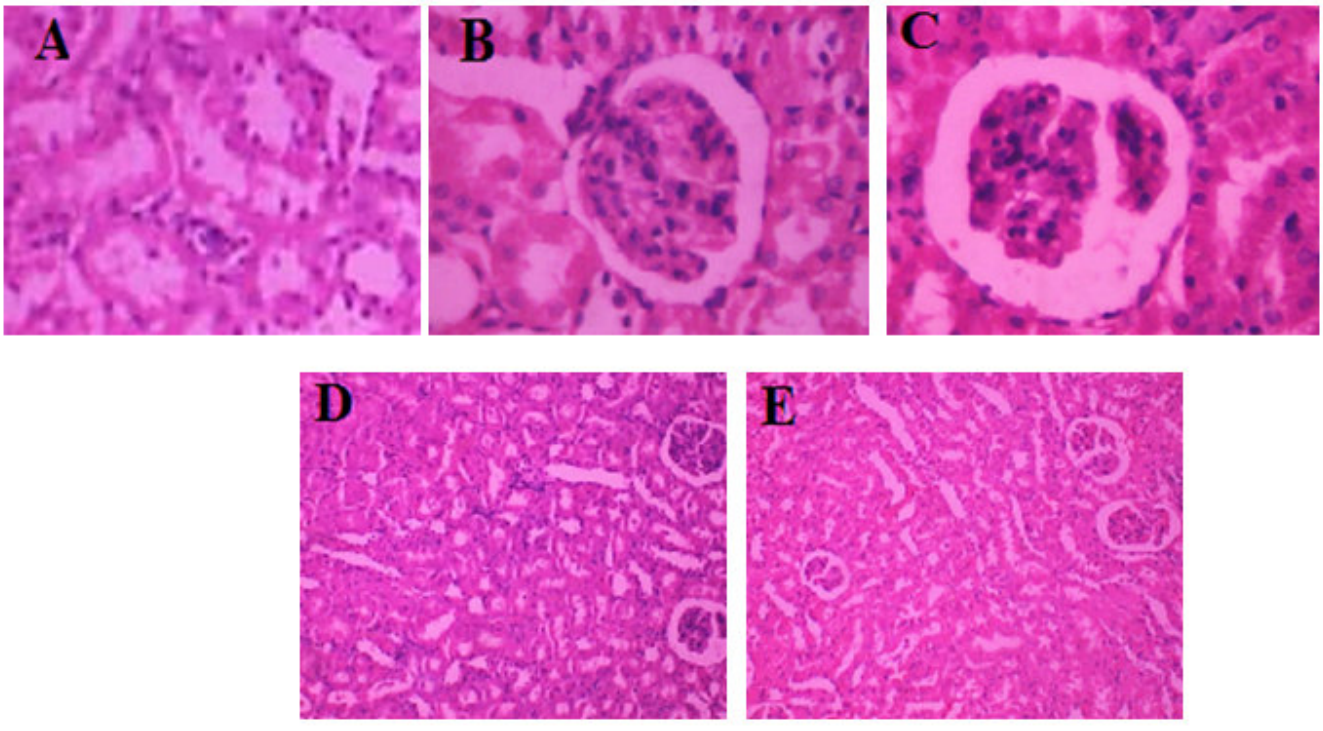

Figure 2
Photomicrographs of kidney tissue: A-Control 0.5% CMC; B-AEWP 800mg/kg/day; C-EEWP 800mg/kg/day; D-Satellite AEWP 800mg/kg/day; E-Satellite EEWP 800mg/kg/day. (x 40 magnification)

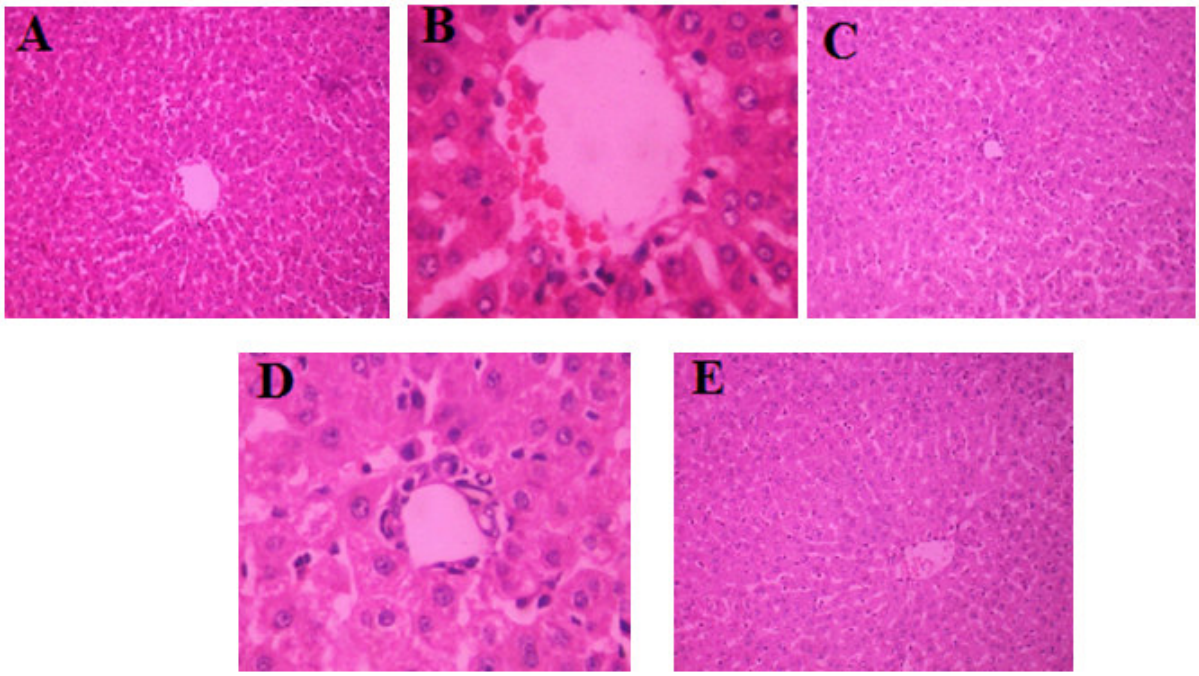

Figure 3
Photomicrographs of liver tissue: A-Control 0.5% CMC; B-AEWP 800mg/kg/day; C-EEWP 800mg/kg/day; D-Satellite AEWP 800mg/kg/day; E-Satellite EEWP 800mg/kg/day. (x 40 magnification)

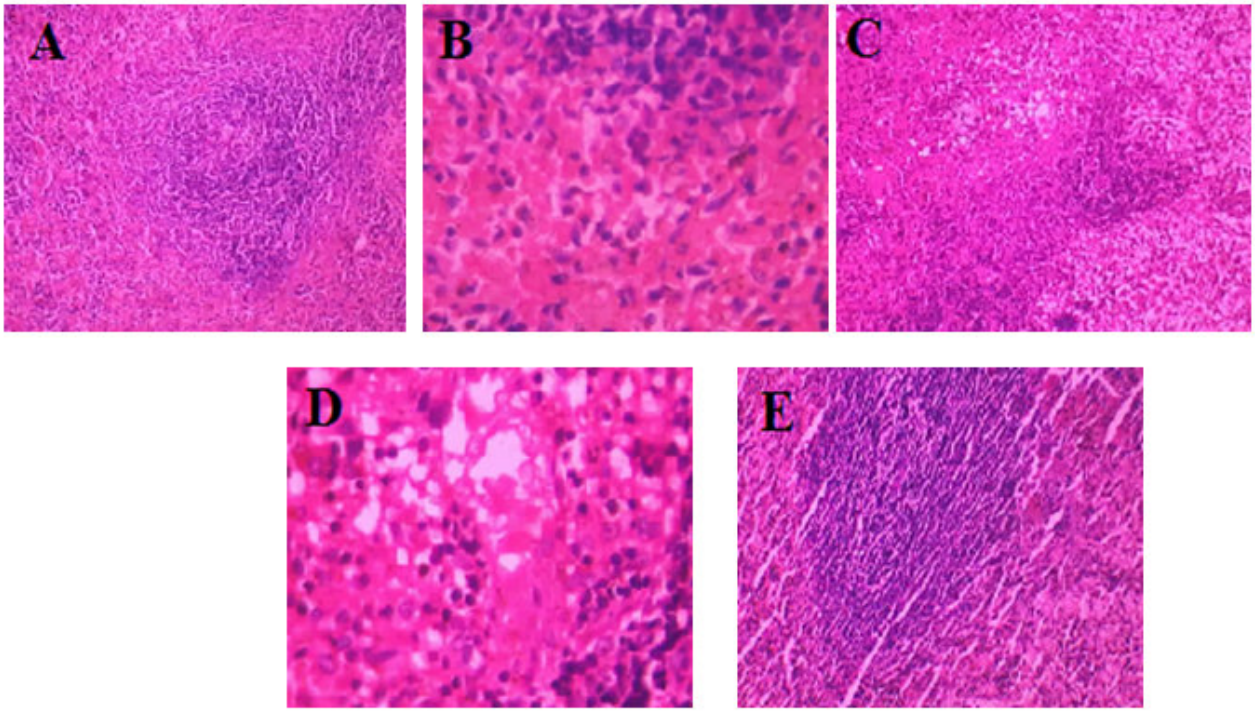

Figure 4
Photomicrographs of Spleen: A-Control 0.5% CMC; B-AEWP 800mg/kg/day; C-EEWP 800mg/kg/day; D-Satellite AEWP 800mg/kg/day; E-Satellite EEWP 800mg/kg/day. (x 40 magnification)

DISCUSSION

The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and protective /disease preventive properties (Builders. M. I et al 2012). Hence herbal drugs have received greater attention as an alternative to clinical therapy and the demand for these herbal remedies has greatly increased recently. Their utilization is often based in long term clinical experience. Despite the usage of the plants in folklore medicine over the ages, only lately has pharmacology and toxicology of these plants begun to receive attention from scientists. Hence to validate their claimed pharmacological properties and investigate their possible toxicity, preclinical toxicity studies were carried out initially on the aqueous and ethanolic extracts of aerial parts extract of *Walsura piscidia* Roxb in Wistar Albino rats. In the present study, during acute toxicity evaluation, there were no mortality and toxicity signs observed at 2000mg/kg. *Walsura piscidia* Roxb can be classified under category -5 and LD_{50} value was greater than 2000mg/kg in accordance with Globally Harmonized System of Classification and Labeling of chemicals and this provides us a direct relevant for protecting human and animal health. A 28 day repeated oral toxicity study was performed following OECD test guideline 407 in both male and female Wistar Albino rats. Since examination of clinical signs plays major role in toxicological testing, mortality and morbidity were recorded twice a day throughout the study. AEWP and EEWP did not produce ant alterations in the feed and water consumption and insignificant in their body weights compared to that of control. This reveals that it does not adversely affect the basic metabolic processes of the experimental rats.

The haemopoietic system serves as an important target for toxic chemicals and is a sensitive index for pathological conditions both in humans and animals .In the study, treatment with AEWP and EEWP did not produce any alteration in haematological parameters (ie. RBC, haemoglobin, HCT, PCV, MCV, MCH, MCHC, Platelets, WBC and lymphocytes) which indicate that WP did not affect blood cells and their production. In biochemical evaluation the extracts treated groups showed a reduction in serum glucose levels. This suggests that WP could produce some hypoglycemic effects. A number of investigators have shown that coumarin, flavonoids, triterpenoids and a host of secondary plant metabolites including arginine and glutamic acids posses hypoglycemic effects in various experimental animal model.(Akah P.A et al 1992). Earlier researcher says that Aqueous and ethanolic extract of *Grewia serrulata* DC exhibited reduction in cholesterol levels, prossesses lipid lowering activity and also some beneficial effect on the cardiovascular risk factor. the lipid lowering activity may dye to the presence of flavonoids.(Karimulla Shaik 2013) The plant of *Walsura piscidia* Roxb contain a active chemical constituents like triterpinoids, flavonoids polyphenols and coumarine etc may also show reduction in cholesterol, possesses lipid lowering activity and also some beneficial effects on the cardiovascular risk factors. Several researches conducted had indicated that many plant sterols reduce serum cholesterol absorption.(Susrutha K 2006). There was a significant increase in protein levels in AEWP (800mg/kg/day) EEWP (400 & 800mg/kg/day) treated rats compared to control groups which may be due to its property of increased protein synthesis.

The insignificant difference in urea and creatinine levels between the treated groups and the control group probably suggests that the extract did not interfere with the renal capacity to excrete the metabolite. Indeed creatinine is known as a good indicator of renal function. Any rise in creatinine levels is only observed if there is a marked damage to functional nephrons. (Mukinda JT 2010). Histopathological slides of kidney structure showed normal structural features suggesting the preserved renal integrity of AEWP and EEWP treated rats. Ordinarily, liver cell damage is characterized by a rise in the enzyme levels like AST, ALP, ALT, LDH etc. *Walsura piscidia* Roxb did not induce hepatic cellular changes. Arise in serum alkaline phospatase (ALP) level is usually a characteristic finding in cholestatic liver disease.(Builders.M.I 2012) Insignificant difference between the control and treated rats justifies that no possible cholestasis occurred at dose levels tested. Certain drugs and other substances are known to affect and influence circulating bilirubin. Elevation of bilirubin suggests

an increase in haemolysis.[15] The aqueous and ethanolic extracts of *Walsura piscidia* Roxb did not alter the bilirubin levels in treated rats compared to the control. Histopahtological studies of heart, lung, liver, spleen and kidney of the treated rats did not demonstrate significant changes in morphology indicating the protective effect of AEWP and EEWP on these tissues (Orisakwe O.R et al 2003).

CONCLUSION

This Present study has shown the assortment in toxicity as well as the chemical constituents of the aerial part of plant extract of *Walsura piscidia* Roxb in relation to the extraction solvent. The LD_{50} value was found to be greater than 2000mg/kg b.wt. With reference to the Globally Harmonised System of Classification and labeling the chemicals, *Walsura piscidia* Roxb can be classified as Category-5 and this provides the relevance for protecting human and animal health. The No Observed Adverse Effect Level (NOAEL) of WP was estimated to be greater than 800mg/kg/day. This study provides the basis for further study on the detailed toxic and pharmacological effects of the aerial part of plant extract of *Walsura piscidia* Roxb and their active component(s).

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