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Research Article

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Protective Effect ofEthanolic Extract of*Argyreia Nervosa* Leaves on Chronic-Diabetes Induced Testicular Alterations

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Abstract: Our objective is to evaluated the role of primary phytochemical constituents present in ethanol extracts of *Argyreia nervosa*, resulting in chronic diabetic males induced infertility. The current study aims to assess the protective role of ethanol extract of *Argyreia Nervosa* against Streptozotocin (STZ)caused alterations in blood glucose level and histopathology architectureof the testis. Phytochemical profiling of ethanolicextract*ArgyreiaNervosa* was carried out. The diabetic model was created by giving animals 40 mg/kg/BW of STZ(intraperitoneally). Further, *Argyreia nervosa* was administered orally after the induction of STZ and confirmed blood glucose levels in experimental subjects. The drug dosage was (200,400 mg/kg/BW) daily for 60 days. Histopathological analysis of the removed testis at specified intervals was carried out. Ethanol extracts contain more phytochemical constituents such as tannins, phenolics, quercetin, saponins, flavonoids, beta carotene, etc. The components have more antioxidant properties when compared with methanol and petroleum ether extract. The results showed that blood glucose levels significantly decreased and noted elevated serum testosterone levels in *Argyreia nervosa*(400mg/kg) treated groups compared with the untreated Streptozotocin (STZ) induced group. The histopathological study revealed that the *Argyreia nervosa* (200&400 mg/kg/BW) treated groups show significant changes in testis when compared with the STZ induced diabetic groups. This herbal has many antioxidants, anti-diabetic, anti-microbial, antifungal, and aphrodisiac. Ethanol extract of *Argyreia nervosa* lowers the blood glucose level in hyperglycemic patients due to its anti-diabetic property and aphrodisiac properties. Like an adaptogenic aphrodisiac, *Argyreia nervosa* extracts contain potent antioxidants, which enhance sperm density and sperm quality and minimize the damage to sperm cells by scavenging free radicals resulting from excessive oxidative damage.

Keywords: *Argyreia Nervosa*, Diabetes, Infertility, Quercetin, Blood Glucose, Testis.

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1. INTRODUCTION

Infertility is the major health issue in life, and approximately about 30% of this problem is due to malefactor.[1] Diabetes guides to chronic metabolic disorders involving failure in carbohydrate metabolism[2].Recent studies reported that diabetes is one of the primary etiologies of organic impotence. Diabetes may be a harmful male reproductive system in pre-testicular, testicular, and post-testicular components[3]. People belonging to the adult age group face male infertility issues due to problems in sexual performance, loss of libido, and male sexual dysfunction.[4] India, is 2nd largest populous nation but still faces the same problem. Environmental pollutants and lifestyle changes are also a part of infertility in males[5]. People under the economic crisis /do not prefer the allopathic treatment like IVF(In-vitro fertilization) and AI (Artificial Insemination). They have hope regarding traditional herbal plant treatments due to the wide variety of treatments and fewer/ no side effects.The cost of herbal medicine is also comparatively lower. *Argyreia nervosa* belongs to the Convolvulaceae family, called Hawaiian Baby Woodrose or Elephant creeper, used as traditional Ayurvedic medicine. Leaves of Argyreia nervosa consist of beta-sitosterol, Quercetin and 1- Triacontanol. The plant possesses more clinical effects such as antibacterial, antiviral, antifungal, anti-inflammatory, andAphrodisiacproperties[6]. Literature showsthat this plant's root is used for rheumatism, chronic ulcer,gonorrhea, and to cure the defect in the nervous system[7]. However many reports showed only the effects of root extracts, since leaf extract of *Argyreia nervosa* had been a very limited study. Moreover the ethanol extract contains more phytoconstituentssoluable in polar solvent like ethanol..Hencethe authors of the present study explored the beneficial effects of ethanol extracts from the *Argyreia Nervosa* plant; The plant also contains many nutrients and minerals, including vitamin E, potassium, and zinc elements. The presence of active aphrodisiac properties and phytochemical constituents such as flavonoids, tannins, saponins, polyphenols, lignins, and quercetin will protect the cells from oxidative stress after promote the testicular cell's viable. The previous study reported that Ethanol extract has more active properties when compared with other extracts[9]. The consumption of traditional medicine/ medicinal herbs/natural plants is mounting extensively due to their therapeutic efficiency, easy availability, social suitability, and low or no concomitant effects in contrast to modern medicine.[10]In this study , consumption of Ethanol extract of Aryyreia nervosa reduce the elevatedblood glucose level as well as it enhance the fertility rate in diabetic males.

2. MATERIALS AND METHODS

2.1 Chemicals

Biochemicals for this study were purchased from Sigma Aldrich and Mass biotech laboratory, Chennai.

2.2 Plant Collection

Argyreia nervosa was collected from local areas in and around Tamilnadu. Dr. Rakesh identified and authenticated the collected samples, Botanical Survey of India, Southern Regional Centre, and Coimbatore. The specimen voucher no. BSI/SRC/5/23/2020/Tech/799.The sample waswashed, air-dried, and ground into powder using a mechanical blender and stored in a glass container.

Fig:1 Argyreia Nervosa

2.3 Extraction

The powdered sample was extracted by using ethanol solvent. The cold maceration techniques were used .The extraction step was carried out 24 hrs for 7 Days.After extraction, the extracts were concentrated by a rotary evaporator and stored at 4°C for further study[11].

2.4 Proximate Physico chemical Analysis

2.4.1. Ash Values

Estimation of total ash value, acid insoluble ash, water – soluble ash might be useful indices for identification of the powdered drug was performed as per the reported methods. (Akhilesh k, 2013)

2.4.2. Extractive Values

Extracts were prepared with ethanol, methanol and petroleum ether solvents. Percentages of the extractive values were calculated.

2.4.3. pH Value

Standard glass electrodes were used to determine the pH value of solution. The powdereddrugs (5mg) were taken and dissolved in 100 ml distilled water and 10 g of powdered drugswere dissolved in 100 ml of distilled water. The electrodes were placed into the solution and the pH values were noted[12].

2.4.4. Quantification Of Water Content & Mineral Ashes

Healthy fresh leaves were grounded finely. In a clean beaker, 20g of aliquot was soaked in 80 ml of distilled water. The mixture was boiled for 15-20 minutes. After this the mixture was allowed to cool. After that the mixture was filtered through Whatman filter paper. The filtered material was further evaporated to dryness. The residue was reconstituted in solution with saline water to a known concentration[13].

2.5 Phytochemical Study

After the extraction, petroleum ether, ethanol and methanol extract were subjected to phytochemical analysis according to JaiswalBhagat Singh et al., 2018.

2.6 Experimental Induction OfStz

The diabetes was induced in animals by injecting STZ (40mg/kg/body weight) in 0.01 M Citrate buffer (pH 4) intraperitoneally. After the induction, the animals were observed and noted[14].

2.7 Experimental Design

The Wistar albino rats were used for this experiment in the present study.The rats were duly approved by the Institutional Animal Ethics Committee (IAEC) and the Committee for Control and Supervision of Experiments on Animals (CPCSEA) vide letter No. (IAECNO: KSRCT/BT/IAEC/2018/37). Thirty Animals have purchased weights between 150 ± 20 gmsfrom the Mass Biotech laboratory. After the induction of diabetes, all the rats were randomly divided into five groups. Each group consists of 6 rats.

2.8 Animal Grouping

Group I - Control
Group II- STZ induced rats(40 mg/kg/BW)
Group III- STZ induced rats treated with Clomiphene citrate (5ml/kg/BW)
Group IV- STZ induced rats treated with *argyreia nervosa* (200mg/kg/BW)
Group V – STZ induced rats treated with*argyreia nervosa* treated rats(400 mg/kg/BW)

2.9 METHODS

All animals were kept and maintained in the animal house, Erode college of pharmacy, Erode. Animals were kept in separate cages according to the groups. After confirming the diabetic model, all animals were divided into five groups. Each group contains six animals. From day one of the experiment, the Ethanol extract of *Argyreianervosa* was given orally using the oral gavage tube. Group I: control without treatment; Group II: STZ induced without treatment. Group III: STZ induced treated with clomiphene citrate (5 mg/kg/BW); Group IV: STZ induced rats treated with Ethanol extract of *Argyreia nervosa* (200 mg/kg/BW); Group V: STZ induced rats treated with Ethanol extract of *Argyreia nervosa* (400mg/kg/BW). The blood sample was taken from the experimental rats on the 0^{th}, 7^{th}, 21^{st}, 45^{th}, and 60^{th}day. The study was carried out for 60 days.

2.10 *Serum testosterone analysis*

Before euthanasia the blood samples were collected, and the serum were separated and kept at 20^0C. concentration of testosterone was measured by means of radioimmunoassay technique. Serum concentrations of testosterone were measured by using a RIA kit.(Mahmetkanter- 2012).

2.11 *Euthanasia*

Euthanasia is as theproceduredone before sacrificing the experimental rats to stop heart rate and brain function within 5 minutes. Intravenous injection of either ketamine / Pentobarbital in one of the legs of the animal is targeted.

2.12 *Estimation of sperm count*

The sperm count was estimated by using a hemocytometer chamber under the light microscope. The haemocytometer pipette was used to withdraw the diluted (semen + normal saline) semen up to the mark 101 and then fluid was stained with eosin; after that the pipette content was shaken vigorously by holding the ends of pipette between the thumb and index finger. A fresh coverslip was placed over the haemocytometer counting chamber and a drop of diluted semen was spread between the haemocytometer chambers and its cover. Thereafter, the live sperms were counted in 5 large squares at 400X magnification. The sperm concentration was estimated by a multiple counted number of sperm by 100 (depth)and 1000 (dilution)[15].

2.13 *Collection of organs*

After euthanasia, the animals were sacrificed by dissecting the organs. The organs were removed from the animal, washed with saline, and poured into the container of 10% formalin. After the fixation, the tissue process was done for further study.

3. STATISTICAL ANALYSIS

The blood glucose level, serum testosterone, and weight of the testis were compared using one-way ANOVA followed by Dunnett's test. The statistical analysis was performed on commercial software INSTAT 3.0(Group Pad San Diego, CA).

4. RESULTS

Organoleptic evaluation of the plant material is shown in (Table 1). Physico-Chemical parameters of Argyreia nervosa shown in (Table.2). The pH values of powered drug solution at 5% and 10% concentration was found to be 6.01 & 7.98 respectively as shown (Table 3). The different solvent extractive values for *argyreia nervosa* as shown in (Table 4) respectively.The presence of phytoconstituents contents in , petroleum ether, ethanol, methanol, extracts of *Argyreia nervosa* shown in (Table 5.) such as flavonoids, quercetin, sterols, polyphenols, saponins, tannins, amino acids, steroids, fats,proteins,oils,etc[16].

Table1: Organoleptic Characters					
S No.	Name of Plant	Part used	Description		
			Color	Odor	Taste
1.	Argyreia nervosa	Leaf	Light green	No characteristic	Sweet

Table2:Physico- Chemical parameters of *Argyreia nervosa*		
S.No.	Parameters	Values obtained on dry weight basis(w/w)
1.	Loss of drying	5.76%
2.	Total Ash	9.8%
3.	Acid insoluble Ash	3.8%
4.	Water Soluble Ash	7.4%

Table3: pH Value of *Argyreia nervosa*		
S.No.	Solution of Different Concentration	pH values
1.	pH of 5% solution	6.01
2.	pH of 10% solution	7.98

Table 4:Yield of various fractions of *Argyreia nervosa*	
Solvents	% Yield of AN
Methanol	*35.98*
Ethanol	*47.43*
Petroleum ether	*9.75*
Aqueous	*8.43*

Table5: Phytochemical constituents of argyreia *nervosa*			
Phytochemicals	*Argyreia nervosa*		
	Petroleum ether	Ethanol	Methanol
Alkaloids	-	+	-
Saponins	-	++	+
Glycosides	–	++	+
Tannins	-	+++	+
Flavonoids	-	+++	+
Quercetin	-	++	-
Lignins	-	+++	-
Amino Acids	+	+	+
Proteins	++	+	+
Fats	-	-	+
Triterpenoids	+	+	+
Steroids	+	-	-

(+) = Present; (-) = Absent

Table 6: Mineral content analysis	
Mineral elements	Compositions
Calcium	46.6 ±1.16
Sodium	31.2±0.15
Iron	25.5±0.6
Copper	25.5±0.3
Zinc	21±0.11
Manganese	15±.5
Vitamin C	3.70±0.008
Vitamin D	0.070±0.02
Lead	0.050±0.02

P Values not aplicable for this values.

4.1. Effects of Ethanol Extracts of Argyreia Nervosa on Blood Glucose Level

After 60 days of the experiment, diabetic rats showed significantly higher blood glucose levels (451.32±0.78) and compared to the control rats(101.32±9.42) treatment with *Argyreia nervosa* 200 mg/kg/BW (187.43±0.74) &400mg/kg/BW (125.87±34.00) rats shows significantly reduced glucose levels in the bloodstream, when compared with STZ – induced untreated diabetic rats. The values are shown in (Table .7)

Table:7. Effects of *Argyreia nervosa* on blood glucose level- STZ induced rats

Groups	Day 0th	Day 7th	Day 21st	Day 45th	Day 60th
I	95.13±24.95	91.9±0.709	93.234±10.11	96.22±02.09	101.32±9.42
II	307.33±87.567	423.33±69.43	431.00±76.21	442.81±41.76	451.32±078
III	335.24±24.424	206.5±87.098	156.83±24.08	145.09±33.44	102.59±535
IV	290.657±23	267.98±54.567	234±23.56	225.86±30	187.43±74
V	365.67±55.24	297.87±98.00	241.67±56.9	284.87±12.13	125.87±34.00

Table7 Data were described as Mean ± S.D values of all groups, *P lesser than 0.01**P lesser than 0.001, P greater than 0.05 ns- non significance; Herbal treated groups were compared with STZ- induced diabetic group and control. Group:I – control; Group:2- Diabetic induced STZ (40mg/kg/bw), Group3: Diabetic group treated with clomiphene citrate, Group 4: Diabetic treated with *Argyeria nervosa*(200mg/kg/bw), Group 5: Diabetic treated with *Argyreianervosa*(400mg/kg/bw).

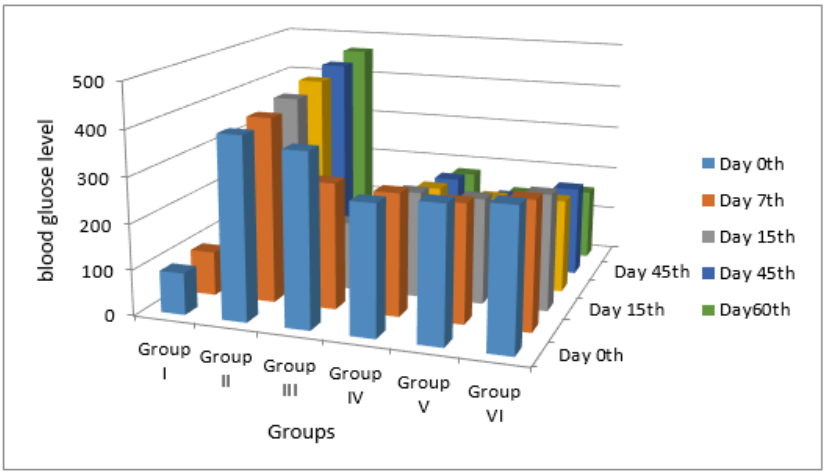


Fig:2 Effects of *Argyreia nervosa* on blood glucose level- STZ induced rats

4.2. Serum Testosterone Level

The serum testosterone level was markedly reduced in the diabetic rat (17.00±0.64) compared with the control group. The *Argyria nervosa s* treated groups showed significant recovery in the level of serum testosterone (271.46±0.651) when compared with the clomiphene citrate(126.2±4.76) treated group led in Table No:3.

Table: 8 Serum Testosterone Level

Groups	Serum Testosterone Levels (ng/dl)
Group I	43.25±0.26
Group II	17.00±0.64
Group III	126.2±4.76
Group IV	271.46±0.65
Group V	185.29±0.54

Table8Show the serum testosterone level in Group I- control, Group II- diabetic group(stz 40mg/kg),Group III- Diabetic rats treated with clomiphene citrate(5 mg/kg/bw) Group IV- Diabetic rats treated with *Argyreia nervosa* (200 mg/kg), Group V- Diabetic rats treated with *Argyreia nervosa* (400 mg/kg).

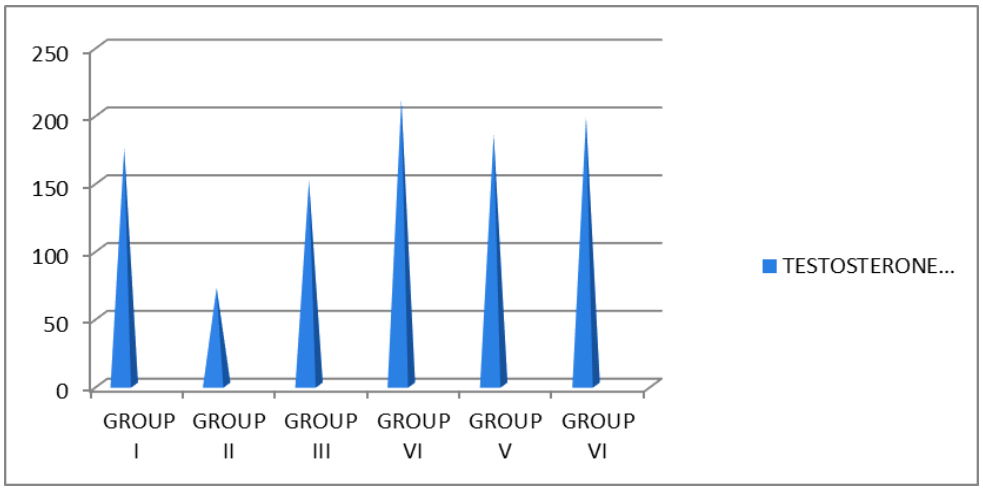


Fig :3 Serum Testosterone Level

4.3. *Sperm count Analysis*

Sperm count was markedly reduced in STZ (40 mg/kg) treated rats when compared with *argreia nervosa* (200& 400 mg/kg) treated rats and control rats. The clomiphene citrate group shows slight variation when compared with control and treated groups. The values were shown in Table No.4. respectively.

Table:9. Sperm Count	
Groups	**Sperm concentration (x10^{6}m/L)**
Group I	57.41±0.87
Group II	31.28±7.5
Group III	48.85±0.62
Group IV	68.10±0.97
Group V	52.00±9.39
Group VI	82.17±00

4.4. *Morphology Of Testis*

4.4.1. *Effects of Argyreia nervosa treatment on testis weight*

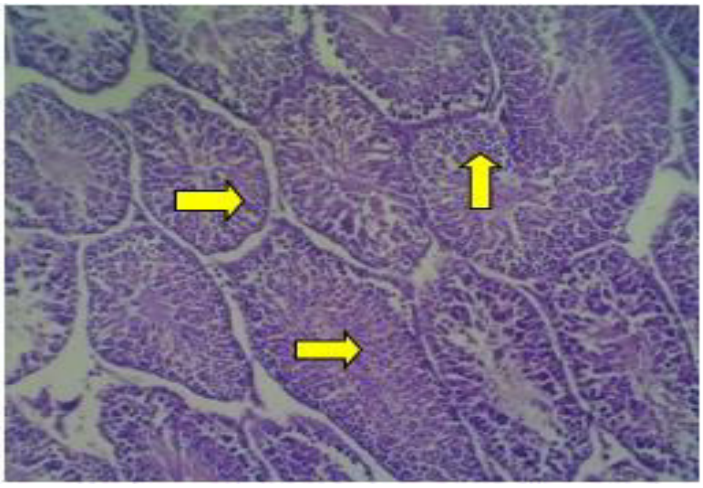
All rats were sacrificed using the euthanasia method at the end of the experiment. After this procedure, the animals were dissected, and organs were removed and washed with saline.Then the testis was kept in tissue paper, and the remaining water from the organs was removed. The testis was weighed and noted from all groups. The diabetes-induced(STZ 40mg/kg/BW) rats, clomiphene citrate (5mg/kg) treated rats shows decreased testis weight when compare with the control, *Argyreia Nervosa*(200 mg/kg/BW) and *Argyreia nervosa* (400mg/kg/BW).The values are shown in table no. 4

Table:10 Testis Weight	
Groups	**Weight Of The Testis(gms)**
I	1.860±1.005
II	0.210±0.765
III	1.530±0.919
IV	1.560±0.571
V	1.470±0.031
VI	1.614±0.4

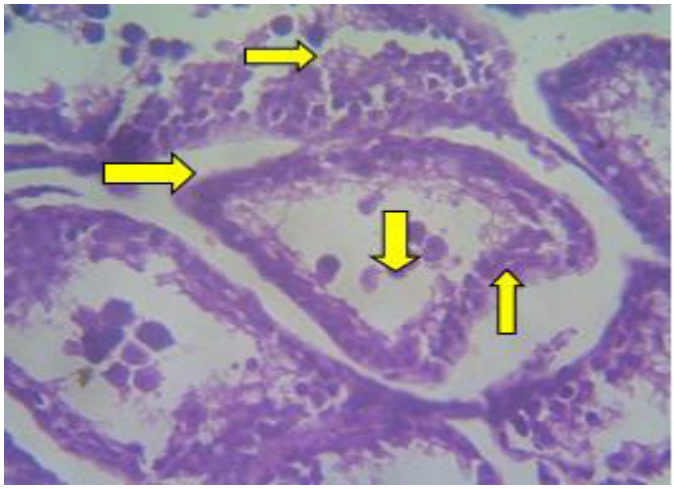
Table 10Shows the weight of the testis. The STZ-induced diabetic rats show a lightweight of the testis. The clomiphene citrate group rats showed significant weight compared to the control group. *Argyreia nervosa* (200mg/kg/BW & 400mg/kg/BW) shows markedly increased testis weight when compared with diabetic group.

4.4.2. *Histopathological Analysis of Testis*

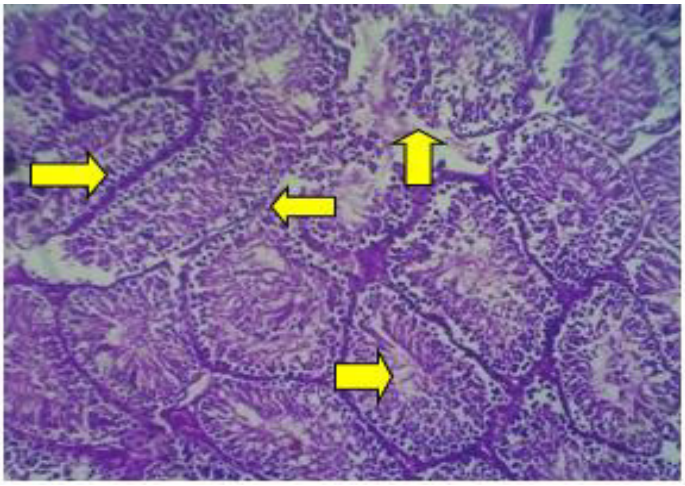
The histopathological analysis of testis in the control group showed typical architecture with standard size of the seminiferous tubules and regular spermatogenic series. In contrast, the STZ-induced untreated diabetic group exhibited damage in seminiferous tubules with maturation arrest. The STZ-induced Clomiphene citrate group shows normal morphology of seminiferous tubules with healthy spermatogenic series. The *Argyreia nervosa* 200 mg/kg treated group showed mild damage in seminiferous tubules with fewer spermatozoa. *Argyreia nervosa* 400 mg/kg treated group showed the normal morphology with healthy seminiferous tubules and regular spermatogenic series.



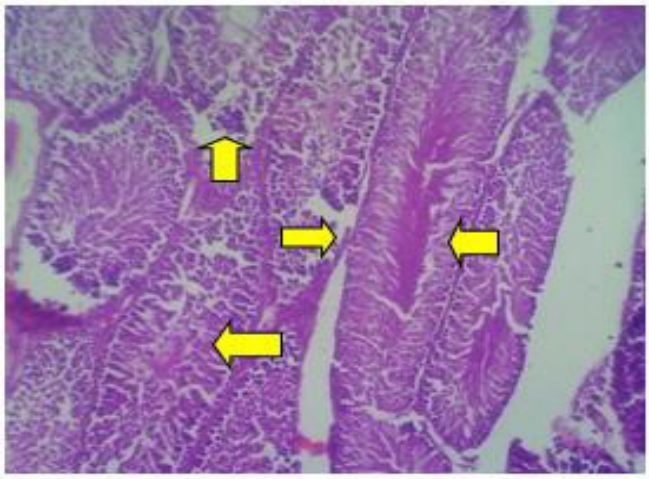
I) Control



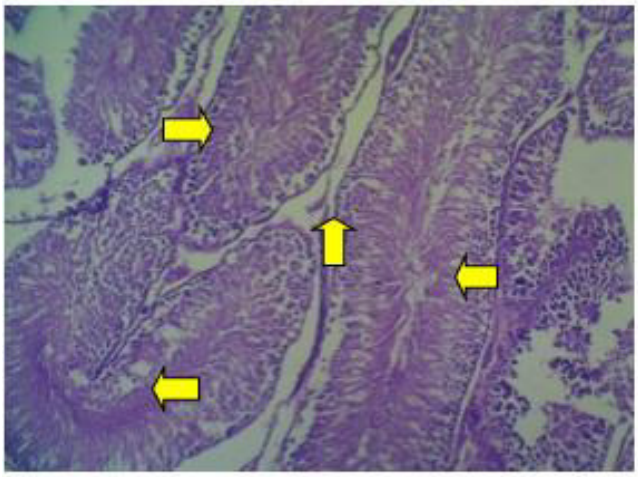
(II) STZ -Diabetic



(III) Clomiphene citrate(5mg/kg)



(IV)*Argyreia nervosa*(200mg/kg)



(V)*Argyreia nervosa*(400mg/kg)

Histology of testis in experimental rats after 60 days of treatment: (I) Control- shows normal structure of testis with seminiferous tubules showing normal spermatogenesis with varying stages of maturation; (II) STZ induced diabetic group- seminiferous tubules showing maturation arrest with epithelioid distortion. Reduced Leydigcell number. (III) Clomiphene citrate group- shows nearly normal architecture of testis with mild epithelial distortion;(IV) *Argyreianervosa*(200 mg/kg) treated group – shows normal round seminiferous tubules with healthy spermatogenesis series; (V) *Argyreia nervosa* (400 mg/kg) treated group- noted leydig cells shows no significant pathology. There is no evidence of fibrosis/atrophy/malignancy seen in this section.

5. DISCUSSION

The present study demonstrated that diabetes causes testicular anatomy alterations, and depleted testis weight, sperm count. Oral administration of ethanol extract of*Argyreia nervosa*(400mg/kg/BW) improved testis morphological alterations by protecting against the impairment of seminiferous tubules and the reduced spermatogenic cell series. A previous study reported that alcohol root/flower extract (200 mg/kg) of *Argyreia nervosa* resulted in a remarkable increase in mating behavior in male mice due to its Aphrodisiac property(A.Subramoniumet. al., 2007)[17].JaiswalBhagat Singh, et.al,.2018 Alcohol extract of root, flower & leaf of the plant at 200mg/kg showed an increase in mounting behavior in mice[18]. Mohd, AzeemuddinMukram, et. al.,.2013 reported that the combined form of *Argyreia nervosa* at the doses of 300,600 & 900 mg/kg body weight reversed the Cyclophosphamide-(CP) induced damage in reproductive organs[19].P.V.Hubbu et., al,.2008 reveals the ethanolic& ethyl acetate extracts possessed strong antioxidant activity against CCl4 induced hepatotoxicity[20]. A previous study reported that 32 mg/kg root extract of AN improved the sperm count in alcohol-exposed rats. (SK. Mitra, et.,al,.1996).[21]According to Ganesh DattatraySaratale et., al,. two thousand seventeen silver nanoparticles of AN possess strong anti-diabetic potential[22]. Ethanol & ethyl acetate extract (200 & 400 mg/kg) showed hepatoprotective activity against carbon tetrachloride-induced hepatotoxicity. (V.J. Galani et,.al,.2010).[23]The present study revealed ethanol extracts of *Argyreia nervosa*(400mg/kg) showed significant alterations in testis testosterone levels. Several reports demonstrated *Argyreia nervosa* possesses a lot of activities, including the presence of primary phytochemical constituents and anti-oxidative activity. (Krishna Veni et al.,.2009)[24]. Leaves of *Argyreia nervosa* possess more flavonoids and phenolics and antioxidant capacity(Abhay Prakash Mishra et al.,2015)[25].According to Niraj, Vyas et al. reveal. The alkaloidal fraction of Argyreia nervosa reported mounting frequency, increment in serum testosterone & serum cholesterol level in a dose-dependent manner[26].Vivek P, Jayakumar D, et al.,2016 evaluated the anti-diabetic activity of *Argyreia nervosa* against Alloxan-induced diabetic rabbits; the results revealed that the plant is less effective in decreasing the blood glucose level when compared with the standard

drug metformin[27].Administration of ethanol extract of *Argyreia nervosa* will reduce the glucose level in blood against the alloxan-induced diabetes mellitus (AliS. A,Hamed, et al.,2011)[28].The study reveals that the blood glucose level markedly decreased in *Argyreia nervosa* 400 mg/kg treated rats against STZ-induced diabetic models.Sreedharan C.S.et. al., in his study, reported that aqueous extract possessed more antioxidant activity in a concentration-dependent manner[29]. L. Ronnerbg (1980) reported the clomiphene citrate treatment for 60 days has increased the sperm count. The present study reveals the same[30]. Mohamed Kanter et,.al,.2012. The reported administration of quercetin is a potentially beneficial agent for reducing the testicular damage in STZ- induced diabetic rats.[31] The present study reveals that ethanol extract possesses more quercetin due to this agent, which might reduce the testicular damage[27].Histopathological study of testis (treated with *Argyreia nervosa* 200 mg/kg) sections showed the only reduction in the seminiferous tubules compared with the diabetic group. The diabetic group showed severe damage in seminiferous tubules and maturation arrest. Clomiphene citrate treated group shows normal morphology nearly control group.However, *Argyreia nervosa*400 mg/kg treated group showed regeneration of seminiferous tubules compared with the clomiphene citrate group.The blood glucose level was significantly reduced in *Argyreia nervosa* 400mg/kg treated groups compared with the STZ induced untreated diabetic group. In this study, the preliminary phytochemical analysis showed ethanol extract of *Argyreianervosa* contains more phytochemical constituents when compared with the methanol and petroleum ether extract[19]. The weight of the testisremarkably decreased in STZ diabetic group when compared with all other groups. The sperm count also significantly increased in argyreia nervosa group when comparedwith the STZ induced group. The *Argyreia nervosa* 400mg/kg treated group shows significant weight compared with the *Argyreia nervosa* 200mg/kg treated group.

6. CONCLUSION

Management of diabetes without/fewer side effects is still a challenge that led to increased demand for natural traditional herbal products with anti-diabetic activity. In conclusion, STZ-induced diabetes rats manifested "reproductive toxicity" in male rats by lowering the testosterone level, decreased gonads, and changes in the histological structure of the testis. Surprisingly, the *Argyreia nervosa* administration attenuated diabetic-related testicular alterations and histopathological changes. ThePpossiblemechanism is *Argyreia nervosa* renewed the activities of phytochemicals like quercetin, vitamin E and saponins either by scavenging diabetic-induced oxidative DNA damages or inhibiting apoptosis in spermatozoa cells. The aphrodisiac property will improve the testosterone level. This study concluded that the administration of *Argyreianervosa* supplement might enhance the fertility against diabetes by preventing germinal cell damage. The study concluded that the Argyreianervosa might act as drug for anti-diabetic and Anti- infertility, due to the presence of Aphrodiasic property. Its easy availability the common man can utilize the beneficial effects of Argyreia nervosa.

7. AUTHOR'S CONTRIBUTION STATEMENT

The corresponding author Saranyaarunagirihas done the entire study. Dr. KRS author suggested this journal to publish. Dr. MR author was helped to write the manuscript.

8. ETHICAL APPROVAL

The protocol of this study was approved by Institutional Animal Ethical Committee No.[KSRCT/BT/IAEC/2018/37]

9. CONFLICT OF INTEREST

Conflict of interest declared none.

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