Corrective Role of Argyreia Nervosa in Histological Architecture of Testis Against STZ Induced Experimental Diabetic Male Wister Rats.

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Research Article

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Abstract

Objective: The present study to findout the role of primary phytochemical constituents present in ethanol extracts of Argyreia nervosa resulting on a hyperglycemic males induced infertility. The objective of the study to evaluate the protective role ehanol extract against STZ induced histopathological changes testis.

Methods: Primilinary phytochemical analysis was done to findout the primary and secondary phytoconstituents persent in ethanol extracts of argyreia nervosa. In this study the diabetic model was created by given 40 mg/kg/bw of stz to animals. After the induction the animals blood glucose levels were monitored and noted the hyperglycemic level in blood. After confirmation of blood glucose level the ethanol extracts of argyeria nervosa and was given orally. The dosage of drug was (200 ,400 mg/kg/bw) Daily for 60 days. Total experimental period was 60 days. After the experimental period the tissues were collected and done histopathology study for testis.

Results: Ethanol extracts contains more phytochemical constituents such as tannins, phenolics, saponins, flavonoids, beta carotene, etc. The constituents contain more antioxidant property when compare with methanol and petroleum ether extract. End of the experiment, the blood glucose level was decreased in argyreia nervosa treated groups, when compare with untreated stz group. The serum testosterone levels were increased when compared with stz induced group. The histopathological study reveals that the argyreia nervosa (200&400 mg/kg/bw) treated groups shows significant changes in testis when compare with the stz induced diabetic groups.

Conclusion: From the present study, it was observed that the ethanol extract of argyreia nervosa lower the blood glucose level in hyperglycemic patients and the herbal have the beneficial effects against hyperglycemic induced infertility in male patients. Due to its aphrodisiac properties is well agreed with activites like adaptogenic, aphrodisiac and it might be contributed. The argyreia nervosa extracts contains potent antioxidants which is addition to enhancing the sperm density and sperm quality and minimize the damage to sperm cells reactive oxygen species by scavenging free radicals resulting from excessive oxidative damage.

Introduction

Now a days all age groups people are facing the male infertility issues due to problem in sexual performance, loss of libido and male sexual dysfunction. India is the 2nd largest population among the countries still facing the same problem. the environmental pollutant and changing of life style is one of the major part in infertility males[1]. The recent reports revealed that the diabetic also will cause the infertility issues among the young peoples. Peoples under the economic crisis they does not prefer the allopathic treatment like IVF (In-vitro fertilization) and AI (Artificial Insemination). They like traditional herbal plant treatments due to wide variety of treatment and less/ no side effects. The cost of treatment also very less. The present study explore the beneficial effects of ethanol extracts of Argyreia nervosa plant. This is used to cure many sorts of illness/ disease. The plant posses many properties like anti-

inflammation, anti-viral, anti- microbial, anti- diabetic and Aphrodiasic. The plant also contains many nutrients and minerals such as vitamin E, potassium, and zinc elements[2]. Due to its phytoconstituents such as flavonoids, tannins, saponins, polyphenols, lignins, quercetin it will protect the cell from oxidative stress and promote the testicular cells viable[3]. Ethanol extract have more action when compare with other extracts. 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 as contrast to modern medicine[4].

Materials And Methods

CHEMICALS:

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

PLANT COLLECTION:

Argyreia nervosa was collected from local areas in and around Tamilnadu. The collected sample was washed with saline and air dried under the shade. The dried material was grind into powder by using mechanical blender. The powder of sample was stored in the glass container for the next step of process. [5]

EXTRACTION:

The powdered sample was extracted by using ethanol solvent. The cold maceration techinique was used to increase the polarity. 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[6].

EXPERIMENTAL INDUCTION OF STZ

Experimental diabetic model was created by using a single (40mg/kg/body weight) interaperitoneal injection. The STZ was given by using 0.01 M (Citrate buffer). The P^H value is 4. After the induction the animals were observed and noted[7].

EXPERIMENTAL DESIGN:

The present study the wister albino rats were used for this experiment. The rats were duly approved by Institutional animal Ethics Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) vide letter No. (IAECNO: KSRCT/BT/IAEC/2018/37). Totally 30 Animals were purchased weight between 170 ± 20 gms from the Mass biotec laboratory. After the induction of diabetes, all the rats were randomly divided into 5 groups. Each group consists of 6 rats.

Animal Grouping:

Group I - Control

Group II- STZ induced rats(40 mg/kg/bw)

Group III- STZ indued rats treated with Clomiphene citrate (5ml/kg/bw)

Group IV- STZ induced rats treated with Argyreia nervosa (200mg/kg/bw)

Group V – STZ indued rats treated with Argyreia nervosa treated rats(400 mg/kg/bw)

METHODS:

All animals were kept and maintain in animal house, erode college of pharmacy in erode. Animals was kept in separate cage according to the groups. After the confirmation of diabetic model all animals were divided into 5 groups. Each group contains 6 animals. From the day one of experiment the Ethanol extract of Argyreia nervosa was given orally by using the oral cavage tube. Group I: control without any treatment; Group II: STZ induced without any 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 0th, 7th, 21st, 45th, 60th day. The study was carried out for 60 days. End of the experiment the animals was sacrificed and the organs was collected and fixed with formalin for further process.

Results And Discussion

Phytochemical Study of Argyreia Nervosa:

The current study revealed the presence of phytoconstituents in ethanol, methanol, petroleum ether extracts of Argyreia nervosa such as flavonoids, quercetin, sterols, polyphenols, saponins, tanins, amino acids, steroids, fats, proteins, oils. etc[8]. Presence of this phytochemical will show in table No. 1.

Table No: 1. Phytochemical constituents of Argyreia Nervosa

Phytochemicals	Argyreia Nervosa		
	Methanol	Ethanol	Petroleum ether
Alkaliods	-	+	-
Saponins	+	++	+
Glycosides	+	++	+
Tanins	+	+++	-
Flavonoids	+	+++	+
Quercetin	-	++	-
Lignins	-	+++	+
Aminoacids	+	+	+
Proteins	+	+	++
Fats	-	-	+
Triterpenoids	+	+	+
Steroids	+	-	-

(+) = Present; (-) = Absent

Effects of Ethanol Extracts of Argyreia Nervosa on Blood Glucose Level:

After 60 days of experiment, diabetic rats shows significantly higher blood glucose level (451.32 ± 0.78) and compared to the control rats (101.32 ± 9.42) treatment with Argyeria nervosa 200 mg/kg/bw(187.43 ± 0.74) and Argyreia nervosa 400mg/kg/bw (125.87 ± 34.00) rats shows significant reduced glucose levels in blood stream. When compare with STZ – induced untreated diabetic rats. The values were shown in Table no.2.

TABLE No:2. 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

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:1 – 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 Argyreia nervosa (400mg/kg/bw).

SERUM TESTOSTERONE LEVEL:

End of the experiment, blood was collected from the rat by using retro orbital method to analysis the level of testosterone. The serum testosterone level markedly reduced in diabetic rat (17.00 ± 0.64) when compare with the control group. The argyeria nervosa treated groups showed significant recovery in the level of serum testosterone (271.46 ± 0.651) when compared with clomiphene citrate(126.2 ± 4.76) treated group.

TABLE No:3. 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

Table:3 Show the serum testosterone level in Group I- control, Group II- diabetic group (stz 40mg/kg), Group III-Diabetic rats treated with clomiphene citrate(5mg/kg/bw) Group IV- Diabetic rats treated with Argyeria nervosa (200mg/kg), Group V- Diabetic rats treated with Argyreia nervosa (400mg/kg).

MORPHOLOGY OF TESTIS

Effects of Argyeria nervosa treatment on testis weight:

The end of experiment all rats were sacrificed by using euthanasia method. After this procedure, the animals were dissected and organs were removed and washed with saline. Then the testis was kept in tissue paper and removed the remaining water from the organs. The testis was weighted 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, Argyeria nervosa (200 mg/kg/bw) and Argyreia nervosa (400mg/kg/bw).

Table:4. 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

HISTOPATHOLOGICAL ANALYSIS OF TESTIS:

The histopathological analysis of testis in control group showed normal architecture with standard size of the seminiferous tubules and normal spermatogenic series. While the STZ induced untreated diabetic group exhibited damages 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 mh/kg treated group showed mild damage in seminiferous tubules with reduced number of spermatozoa. Argyreia nervosa 400 mg/kg treated group showed the normal morphology with health seminiferous tubules and normal spermatogenic series.

Discussion

The present study demonstrated that diabetes makes testicular alteration and testis weight and Ethanol extract of argyreia nervosa oral administration improved these morphological alterations by providing protection against the impairment of seminiferous tubules and the reduced spermatogenic cell series[9]. In H&E sections of testis showed the administration of argyreia nervosa 200 mg/kg sections shows only reduction in the seminiferous tubules, when compared with the diabetic group. Diabetic group showed the heavy damage in seminiferous tubules and arrest of maturation. Clomiphene citrate treated group shows normal morphology nearly control group. argyreia nervosa 400mg/kg treated group shows regeneration of seminiferous tubules when compare with the clomiphene citrate group.

Administration of ethanol extract of argyreia nervosa will reduce the glucose level in blood against the STZ induction[10]. The blood glucose level was significantly reduced in argyreia nervosa 400mg/kg treated groups when compare with the stz induced untreated diabetic group[11–15].

In this study the preliminary phytochemical analysis showed ethanol extract of argyreia nervosa contains more phytochemival constituents when compare with the methanol and petroleum ether extract[16].

In this study weight of the testis markedly decreased in stz diabetic group when comare with all other group. The argyreia nervosa 400mg/kg treated group shows significant weight when compare with the argyreia nervosa 200mg/kg treated group.

Conclusion

In conclusion, STZ induced diabetes induced reproductive toxicity in male rats manifested by lowring the testosterone leve; decreased weight of gonads; changes in histological structure of testis. From this study the argyreia nervosa administration attenuated diabetic- related testicular alterations and histopathological changes. Argyreia nervosa renewed the activities of phytochemicals such as quercetin, vitamin E and saponins. These molecules reduce the diabetic induced oxidative DNA damages in spermatozoa cells and inhibit the apoptotic pathway. Aphordisiac property will improved the testosterone level. This study concluded that argyreia nervosa enhance the fertility against diabetes by preventing germinal cell damage.

Declarations

Conflict of interest

The authors declare that there are no conflict of interest.

Availability of data and material

Not applicable

Author's contribution

The corresponding author Saranya arunagiri was done the entire study. Dr. KRS author was suggested this journal to publish. Dr. MR author was helped to wrote the manuscript.

Funding:

None

Ethical approval

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

Consent to participate

All authours declare the consent.

Consent of publication

All authors declare the consent for the publication.

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Figures

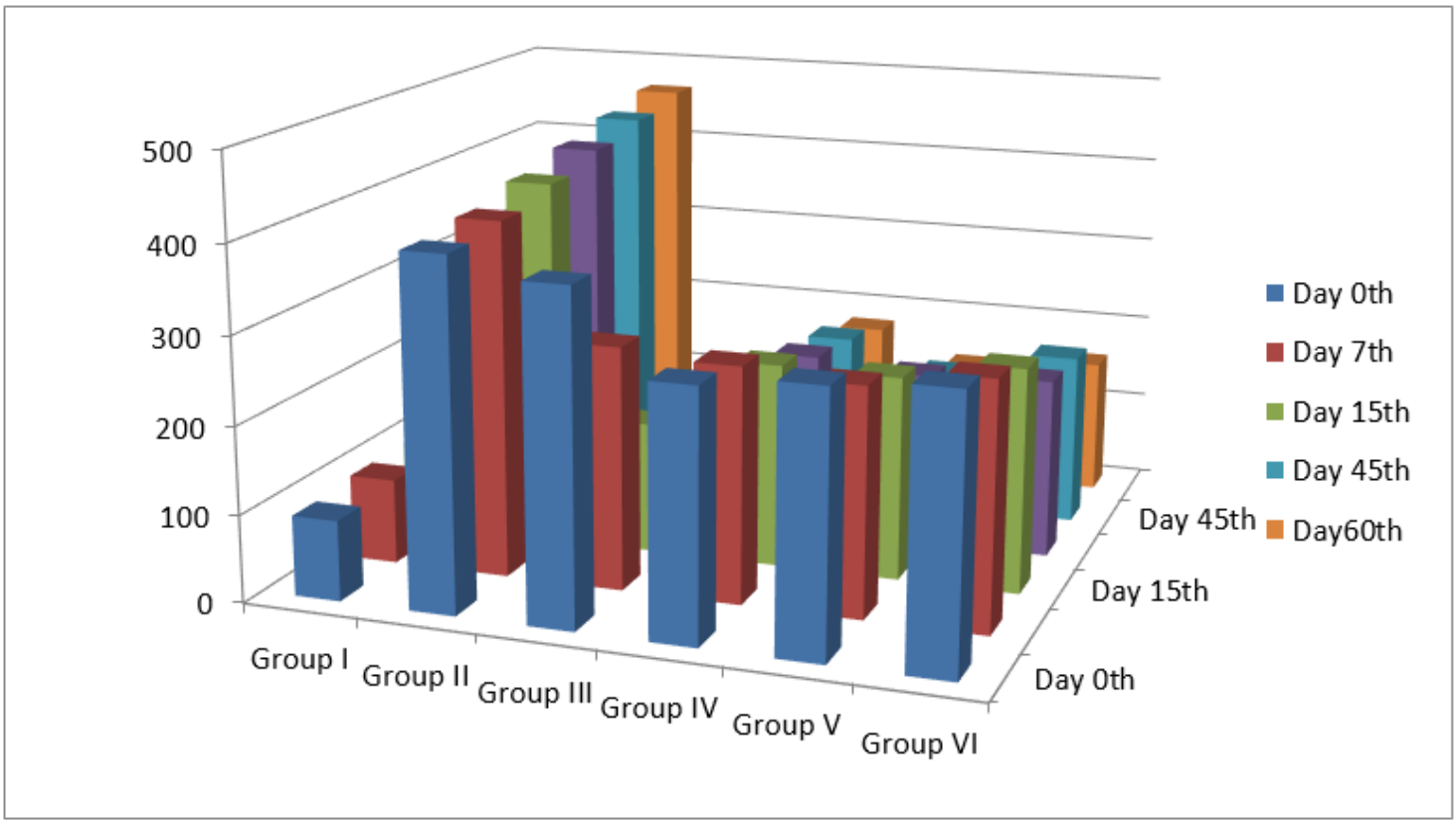

Figure 1

Effects of Argyreia nervosa on blood glucose level- STZ induced rats

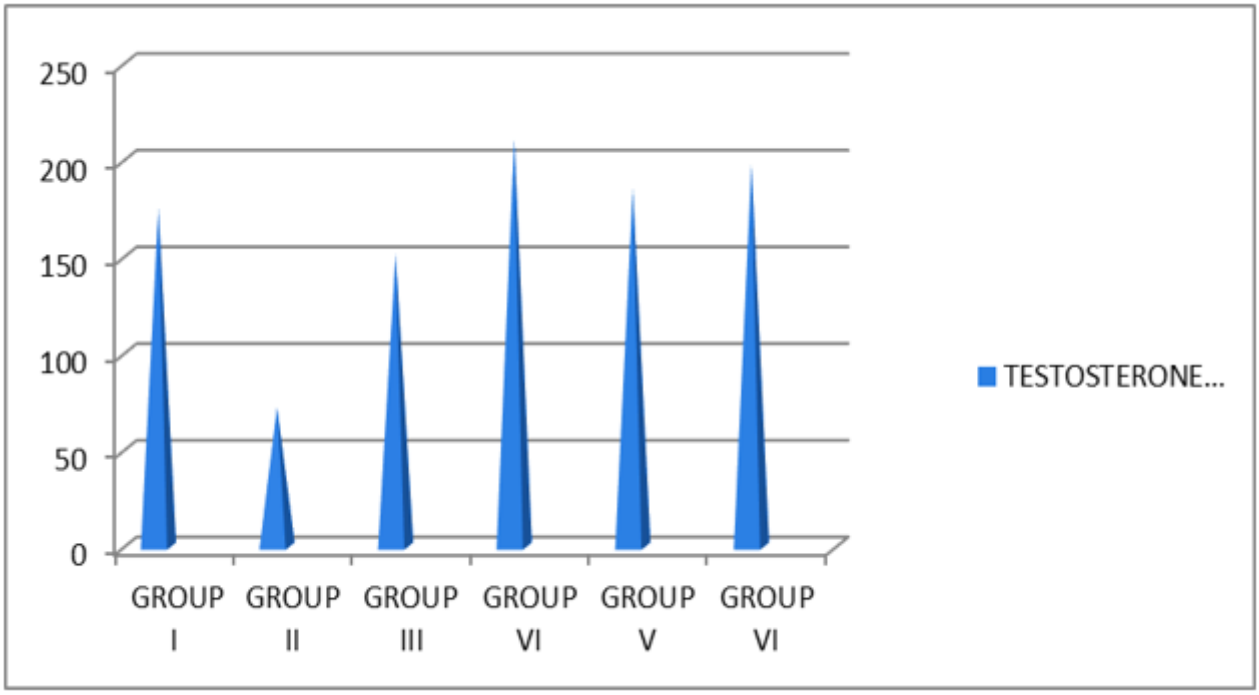

Figure 2

SERUM TESTOSTERONE LEVEL

Effects of ethanol extract of arygeria nervosa on testosterone levels of controls and diabetic rats, Mean ± standard deviation of all groups. **P lesser than 0.001, *Plesser than 0.01. P greater than 0.05 ns- non significance; Arygeria nervosa (200 &400 mg/kg/bw) treated groups in comparison to clomiphene citrate treated group show significant values.

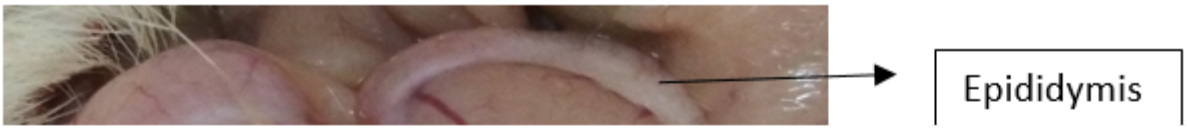

Figure 3

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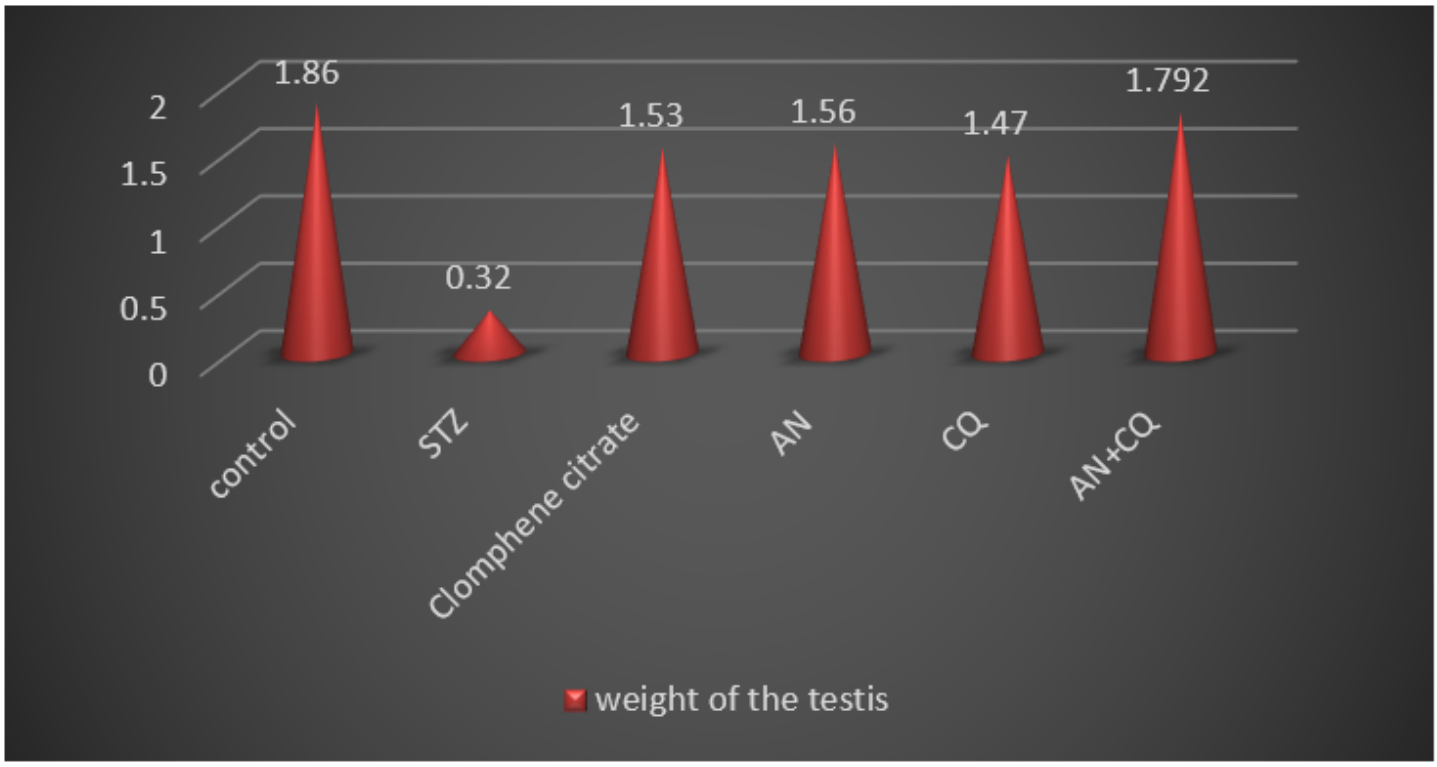

Figure 4

Fig:3 TESTISWEIGHT

Figure:3 shows the weight of the testis. The stz induced diabetic rats shows very low weight of the testis. The clomiphene citrate group rats showed significant weight when compare with the control group. Argyreia nervosa (200mg/kg/bw & 400mg/kg/bw) shows markedly increased testis weight when compare with diabetic group.

Figure 5

Fig:2 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 epitheloid distortion. Reduced Leydig cells number. (III) Clomiphene citrate group- shows nearly normal architecture of testis with mild epithelial distortion;(IV) Arygeria nervosa (200 mg/kg) treated group – shows normal round seminiferous tubules with healthy spermatogenesis series; (V) Argyreia nervosa (400mg/kg) treated group- noted leydig cells shows no significant pathology. There is no evidence of fibrosis/atrophy/malignancy seen in this section.

Supplementary Files

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