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Investigation Of Aphrodisiac Potential Of *Blepharis edulis Linn.* (Utangan) Claimed By Tribals Of Malwa Region of Madhya Pradesh

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Abstract: The present study is aimed to investigate the effect of ethanolic extract of *Blepharis edulis* Linn. (family Acanthaceae) on general mating behaviour, libido, and adverse effects on sexually normal male albino mice. The suspension of the alcoholic extract was administered orally at the dose of 100, 250, and 500 mg / kg, to different groups of male mice (n = 6) once a day for seven days. The female Swiss Albino mice involved in mating were made receptive by hormonal treatment. The general mating behaviour, libido and potency were determined and compared with the standard reference drug sildenafil citrate. Hormonal parameter like testosterone was evaluated. The most appreciable effect of the extract was observed at the dose of 500 mg/kg. The results indicated that the ethanolic extract of *Blepharis edulis* Linn. (family Acanthaceae) produced a significant and sustained increase in hormonal levels of testosterone indication for the sexual activity of normal male mice without any adverse effects.

Keywords: *Blepharis edulis*. Aphrodisiacs. sexual behavior. testosterone.

Introduction

Blepharis edulis Linn. (family Acanthaceae) is a small gray pubescent or nearly glabrate perennial herb, found in Punjab, Western Rajasthan and malwa region of MP. In local Hindi language the plant is known as Utangan or Chaupatia where as in Ayurvedic system called as Shikhi. Stem is short and approximately 30 cm length. Nature of stem is rigid and leaves appear in-group of fours at the nodes. Shape of leaf is upper pair 5cm x 1 cm and lower pair smaller with oblong or narrow elliptic. The flower appears in blue colour in storability inflorescence. The fruit capsules are 5 cm long and two seeded. [1] The leaves and seeds are reported to be eaten. The herb forms a good fodder for sheep and camels. It has been identified as *Uchchata*- aphrodisiac drug in ayurvedic. The leaves commonly sold in Indian market, are reported to be useful in wounds, ulcers, nasal hemorrhages, asthma, throat inflammation, purgative, disorders of liver and spleen. The root is considered diuretic and beneficial in urinary discharges and dysmenorrhoea. [2] The seeds are considered to be diuretic, aphrodisiac, expectorant, deobstruent and useful in strangury and conjunctivitis. They yield bitter glycoside blepharin [3] and other research workers found dl alantoin, saponin and tannin catechol in samples from Allahabad variety. [4] It also showed presence of phytosterol in seeds as $C_{27}H_{42}O_3$. [5] In general, the entire plant had utility as animal fodder due to increase in milk production in cattle and as effective treatment in earache [6], contraceptive properties [7]. Its constituents include benoxazolone and blepharin [8], presence of galactose and fructose [9], and novel 4'-O-diglycoside of decarboxyrosmarinic acid [10]. The all above chemical constituents was reported in literature as shown and not any physical verification was carried out.

Materials and Methods

Plant material

The seeds of *Blepharis edulis* Linn. (family Acanthaceae) collected in and around Bhopal were identified in the Department of Pharmacy, Barkatullah University, Bhopal, India. A voucher specimen (No BUPH/4041D) was deposited in the department. Before start of animal studies permission of Internal animal ethics committee of Department of Pharmacy, Barkatullah University has been obtained by meeting on date 26/12/2006 Ref No BUPH/ IAEC / 7865. The Pharmacy Department of Barkatullah University is also approved for animal experimentation by (Letter No .444/01/C/CPSCEA date July 2001).

Chemicals used

Sildenafil citrate was purchased from Zydus Cadila, (Ahmadabad, India). Other drugs used were ethinyl oestradiol (Infar Limited, Calcutta, India), progesterone (Sun Pharmaceutical Industries Limited, Mumbai, India).

Animals

Twelve weeks old male and female albino mice of Wistar strain weighing 25–35 g were used for the study. They were housed singly in separate standard cages and maintained under standard laboratory conditions (temperature 24–28°C, relative humidity 60–70%, 12 h light-dark cycle) with free access to solid pellet diet (Gold Mohar, Lipton-India) and water *ad libitum* throughout the study except during the experiment. The ethical committee of the Department for animal cares and use approved the study design.

Preliminary Phytochemical studies

The powder of dried seeds of *Blepharis edulis* Linn. (family Acanthaceae) was subjected to continuous soxhlet extraction with various organic solvents such as petroleum ether (60-80^0 c), chloroform, benzene, methanol & ethanol respectively. The 1:3 ratio for drug to solvent was maintained for each solvent for successive solvent extraction method. After concentration and drying of each extract in vacuum desicator, identification of phytoconstituents was carried out using thin layer chromatography method by different detecting reagents. [11] The all extracts were then subjected to qualitative chemical tests for confirmation of phytoconstituents of interest. [12]

Since seeds of *Blepharis edulis* Linn. (family Acanthaceae) in ayurvedic medicine are orally administered, therefore, the ethanolic extract of *Blepharis edulis* Linn. (family Acantheceae) was suspended in distilled water using Tween 80 (1%) for oral administration. Sildenafil citrate and ethinyl oestradiol were also suspended in distilled water using Tween 80 (1%) separately, for oral use. Progesterone was dissolved in olive oil for subcutaneous injection. All the drug solutions were prepared just before administration.

Test for libido

The test was carried out by the method of Davidson [13], modified by Amin et al [14]. Albino mice were divided into six groups of 6 animals each and kept singly in separate cages during the experiment. Since the male animals should not be tested in unfamiliar circumstances, the animals were brought to the laboratory and exposed to dim light (in 1 w fluorescent tube in a laboratory of 14' × 14') at the stipulated time of testing daily for 6 days before the experiment. Group 1 represented the control group, which received 10 ml/kg of Tween 80 (1%) orally. Groups 2–4 received suspension of the extract orally at the doses of 100, 250 and 500 mg/kg, respectively, once a day in the evening (18:00 h) for 7 days. Group 5 served as standard and given suspension of sildenafil citrate orally at the dose of 5 mg/kg, 1 hr prior to the commencement of the experiment. Group 6 was given with 6% alcohol mixed with drinking water in drinking bottle. The female animals were artificially brought into oestrus (heat) by the Szechtman et al method [15] (as the female rats allow mating only during the estrus phase). They were administered suspension of ethinyl oestradiol orally at the dose of 100 µg/animal 48 h prior to the pairing plus progesterone injected subcutaneously, at the dose of 1 mg/animal 6 h before the experiment. The receptivity of the female animals was confirmed before the test by exposing them to male animals, other than the control, test and standard animals. The most receptive females were selected for the study. The animals were observed for the Mounting Frequency (MF), Intromission frequency (IF) on the evening of 7th day at 20:00 h. retracting the sheath exposed the penis and 5% xylocaine ointment was applied 30, 15 and 5 min before starting observations. After genital anaesthetization, this does away with the reinforcing effect of genital sensation thus, affording the study of pure libido or intrinsic sexual desire. Each animal was placed individually in a cage and the receptive female mouse was placed in the same cage. The number of mountings was noted. The animals were also observed for intromission and ejaculation. The MF in control, test and standard animals was statistically analyzed by employing one-way analysis of variance (ANOVA) method, followed by striking where the results are significant. Probability (Tukey Krammer) was considered at the level of 0.05.

Evaluation of Sex Hormone

As per the doses given in protocol (Table III) were administered to animals once daily for seven days. On 8th day under ether anesthesia, the neck areas were quickly cleared of fur and skin to expose the jugular vein. The jugular vein was slightly displaced from the neck region (to prevent contamination of the blood with interstitial fluid) and then cut with a sharp sterile blade. The mice were made to bleed into clean, dry corked centrifuge tubes, which were left at room temperature for 10 min. After that, the tubes were centrifuged at 33.5-× *g* for 15 min using Jyoti Laboratory Centrifuge (model SM800B, Gwalior, India). The sera were thereafter collected using Pasteur pipettes into clean, dry, sample

bottles and were then stored frozen overnight before being used for testosterone assay. [16]

The serum testosterone concentration was quantitatively determined using the direct human serum testosterone enzyme immunoassay kit as outlined in the manufacturer's protocol. The determination was based on the principle of direct assay of a limited (competitive) type following the general antibody-antigen reaction based on enzyme linked immuno absorbent assay as described by Tietz [17] using Serozyme IÔ Serono (Span Diagnostics, Surat, India). (Table No 4)

All treated mice were observed at least once daily for any overt sign of toxicity (salivation, rhinorrhoea, lachriymation, ptosis, writhing, convulsions and tremors), stress (erection of fur and exophalmia) and changes in behaviour (such as spontaneous movements in cage, climbing, cleaning of face). (Table no 5) After completion of the protocol of drug studied the testis of the animals removed and sent for the histopathology studies. One mouse was selected from each group for the histopathological studies. A small portion of testis blotted and kept in Bowins fluid (Picric acid: Formalin: Glacial acetic acid 75:25:5) for 12 hrs for fixation blocks were prepared in 100% paraffin wax followed by embedding of tissue in wax. 5-8 µm thick sections were cut on rocking microtone. The sections were then stained in Haemotoxylinoesin and were then hydrated.

Results

Seeds of *Blepharis edulis* Linn (family Acanthaceae) were extracted from different solvents and there successive solvent extraction values in various organic solvent tabulated in (Table No 1) The chemical test evaluation with various tests revealed that presence of bitter principles; saponin, coumarin, essential oil, phytosterol, amino acids and tannins were prominently observed. (Table No 2) Successive solvent extraction values in various organic solvent were observed as petroleum ether 3.53%, benzene 2.33%, chloroform 2.83%, acetone 2.66% ethanol 4.55%, and methanol 5.44% as shown in (Table No 3). The preliminary phytochemical studies with help of Thin Layer Chromatography method revealed that bitter principles in chloroform, acetone and methanol extract were seen prominently. Saponin was observed in methanol and ethanol extract respectively. Coumarin type compound was seen in acetone extract only. (Table No 4)

The results of studies undertaken suggest that ethanolic extract of seeds of *Blepharis edulis* Linn. (family Acanthaceae) causes significant effect, which is comparable to alcohol treated and standard treatment. The test extract of seeds of *Blepharis edulis* Linn. (family Acanthaceae) clearly indicated that no significant increase in testosterone as in the group treated with 250 mg dose 0.906$\pm$ 0.049 ng/dl and in the group treated with 500 mg 1.20$\pm$ 0.028 ng/dl as compared to control 0.85 $\pm$ 0.043 ng/dl. However, this activity was found high in the group treated with the standard drug 1.21$\pm$ 0.032 ng/dl and very lower or diminished in alcohol treated mice as 0.14 $\pm$ 0.056 ng/dl. (Table No 5)

Histopathological Profile

A) Control: The presence of thick collagenous connective tissue and vascular loose connective tissue has been present around the testicular cells. The seminiferous tubules embedded in interstitial connective tissue. The Leydig cells and blood vessels have been observed. Spermatogenetic cells forming a stratified epithelial and sperms are often found in clusters embedded in cytoplasm of sertoli cells. The acidophilic cells known, as Leydig cells are occasionally found in each seminiferous tubules spermatogenesis also observed. (Fig 1)

B) Standard: Cross section of testis reveled in the animal treated with standard drug presence of good seminiferous tubules with uniform arrangement of numerous sertoli cells. The connective tissue and the Leydig cells are highly appreciated. There is no evidence of testicular cell inflammation. (Fig 2)

C) BE/ UTG 100 mg: The collagenous connective tissue observed in some area but Sertoli and Leydig cells is absent totally. Very few sperm bunches observed. (Fig 3)

D) BE/ UTG 250 mg: Transverse section of testis treated mice with test drug reveled those clear collagenous connective tissues. The well-differentiated cells in abundant quantity observed. The averagely populated sertoli cells producing sperms is observed. (Fig 4)

D) BE/ UTG 500 mg: The TS of testis treated with test drug reveled thick collagenous connective capsules at periphery. Highly populated seminiferous tubules closely connected and embedded in the interstitial connective tissues. A group of Leydig cells present in the interstitial sertoli cells is well differentiated and highly populated with producing a group of sperms. A group of sperms thread like on the center of seminiferous tubules is observed clearly. (Fig 5)

E) Alcohol: Cross section of testis reveled in the animal treated with alcohol presence of damaged seminiferous tubules with disturb arrangement of near about all sertoli cells. The connective tissue and the Leydig cells are highly constricted and fused. There is clear evidence of testicular cell inflammation. (Fig 6)

Discussion

Male sexual behaviour depends on the circulating levels of testosterone in the blood. The Sildenafil citrate was used as standard referent and alcohol, which reduce the testosterone levels, and ultimately sexual or aphrodisiac activity in mice was used as negative control for comparison purpose. Part of the physiological process of erection involves the parasympathetic nervous system causing the release of nitric oxide (NO) in the corpus cavernosum of the penis. NO binds to the receptors of the

enzyme guanylate cyclase which results in increased levels of cyclic guanosine monophosphate (cGMP), leading to smooth muscle relaxation (vasodilation) of the intimal cushions of the helicine arteries, resulting in increased inflow of blood and an erection. Sildenafil is a potent and selective inhibitor of cGMP specific phosphodiesterase type 5 (PDE5) which is responsible for degradation of cGMP in the corpus cavernosum. The molecular structure of sildenafil is similar to that of cGMP and acts as a competitive binding agent of PDE5 in the corpus cavernosum, resulting in more cGMP and better erections.

Improvement in mounting frequency, intromission frequency in the ethanolic extract of seeds of *Blepharis edulis* Linn. treated animals indicates that the drugs probably act by raising testosterone levels. The gonadotropin-releasing hormone (GnRH) from the hypothalamus acts on the anterior pituitary to release both the FSH, which stimulates gametogenesis and LH, which stimulates androgen secretion. The histological evaluation in this study also revealed an increase in FSH-LH-producing basophil cells in anterior pituitary thus indicating a possible role of the hypothalamo-pituitary-gonadal axis.

The present results indicated that the ethanolic extract of seeds of *Blepharis edulis* Linn. (family Acanthaceae) in dose of 500 mg per kg possesses potent aphrodisiac activity as compared to Sildenafil citrate in normal male albino mice without any gastric ulceration and adverse effects and provided scientific evidence in favour of the claims made in ayurvedic medicine that the *Blepharis edulis* Linn. (family Acantheceae) is clinically useful as sexual invigorator in males.

Tab 1: Successive solvent Extraction seeds of *Blepharis edulis Linn (Acanthaceae)*

S. No.	**Solvents used**	**Colour & Consistency**	**Average extractive values in % w/w on dry weight basis**
1	Petroleum Ether 40-60	Black green oily mass	3.53
2	Benzene	Black Green sticky mass	2.33
3	Chloroform	Light green residue	2.83
4	Acetone	Yellow	2.66
5	Methanol	Yellow blackish mass	5.44
6	Ethanol	Brown dry mass	4.55

Tab *2* ***Qualitative chemical examination of different extract of seeds of Blepharis edulis*** **(BE/UTG)**

S.No	Phytoconstituents	BE/ UTG
1	Alkaloid	-
2	Carbohydrate	+
3	Phytosterol	+
4	Protein & Amino acid	-
5	Saponin Glycoside/ glucoside	+
6	Fixed oil / fat	+
7	Gum/ mucilage	+
8	Flavonoid	+
9	Volatile oil	-
10	Amino acids	+
11	Tannin	+

Table No 3 Thin layer chromatography scheme used to detect various extracts of seeds of *Blepharis edulis* Linn (Acanthaceae)

Solvent system used	Detection Reagent	Observation	Inference	P	B	C	A	M	E
Ethyl acetate: Methanol: Water (75.5:13.5:10)	KOH	Red. (Vis) Yellow	Anthraquinone Anthrone	-	-	-	-	-	-
	Vanillin sulphuric acid	Red/ yellow/brown/blue-green	Bitter principle	-	-	+	+	+	-
	Dragendorffs reagent	Orange Red (vis)	Alkaloid	-	-	-	-	-	-
	NP/PEG and UV	Yellow/green/orange	Flavonoid	-	-	-	-	-	-
	VS reagent	Blue (vis)	Saponin	-	-	-	-	+	+
Toluene: ethyl acetate (93: 7)	VS reagent	Red/ yellow/brown/blue-green	Essential oil	+	+	-	-	-	-
	Hcl/Acetic acid	Blue brown	Valepotriate	-	-	-	-	-	-
	NH3 / KOH	Light Blue brown	Coumarin	-	-	-	+	-	-

P= Petroleum ether, B= Benzene, C = Chloroform, A = Acetone, M= Methanol, E= Ethanol extract

Tab.4 Protocol for evaluation of Aphrodisiac activity of seeds of *Blepharis edulis*

S NO.	DRUG CODE	DOSE	ANIMAL AVERAGE WEIGHT	DOSE ADMINISTERED
1	BE/UTG	3 mg in 2 ml	25gm-35 gm	OD for 7 days
2	BE/UTG	3 mg in 2 ml	25gm-35 gm	OD for 7 days
3	BE/UTG	7.5 mg in 2 ml	25gm-35 gm	OD for 7 days
4	BE/UTG	7.5 mg in 2 ml	25gm-35 gm	OD for 7 days
5	BE/UTG	10 mg in 2 ml	25gm-35 gm	OD for 7 days
6	BE/UTG	10 mg in 2 ml	25gm-35 gm	OD for 7 days

Table 5 Effects of ethanolic extract of leaves of *Blepharis edulis* on testosterone levels in treated mice

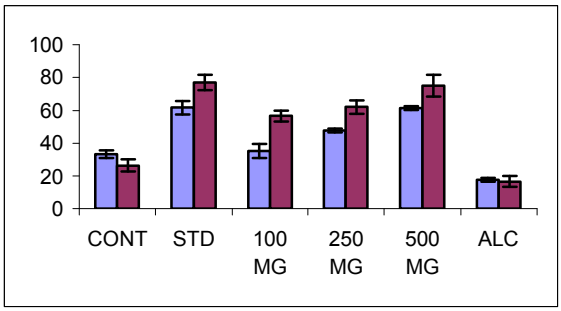
GROUPS	Mounting frequency (In No)	Intromission frequency (In No)	TESTOSTERONE ng/dl
I) CONTROL	33.60± 2.34	26.33 ± 3.84	0.85 ± 0.043
II) SILDANAFIL	61.80± 4.13	77.01 ± 4.75	1.21± 0.32
III) 100 mg DOSE	35.4± 4.34	56.67 ± 3.25	0.92 ± 0.023
IV) 250 mg DOSE	47.80± 1.24	62.12± 3.99	0.90± 0.049
V) 500 mg DOSE	61.4± 1.14*	75.15± 6.65*	1.20±0.028*
VI) Alcohol treated	17.44± 1.22	16.53 ± 3.24	0.14 ± 0.056

Values are mean ±SEM of six animals
Statistical significance: *= $p<0.01$ comparison was done with their respective control group

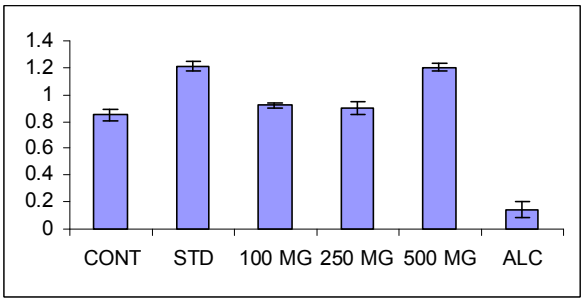
Table 6 Effect of on orientational activities towards female, towards environment & towards self on 7-day administration in normal mice and treated mice

Group (Dose\kg b. wt)	Towards female (60 minutes)		Towards environment (60 minutes)	Towards self (60 minutes)	
	No. of licking	No. of anogenital sniffing	***No. of climbing***	No. of genital grooming	No. of non-genital grooming
Control	9.33 ±0.33	9.83 ±0.54	32.33 ±0.85	16.50 ±0.95	29.50 ±0.67
Sildanafil	2.83 ±0.30	3.83 ±0.16	5.00 ±0.25	3.33 ±0.21	8.00 ±0.60
II) 100 mg DOSE (BE/UTG)	3.50 ±0.42	3.50 ±0.42	5.65 ±0.21	3.66 ±0.33	8.16 ±1.10
II) 250 mg DOSE (BE/UTG)	1.16 ±0.30	13.83 ±0.47	10.33 ±0.95	3.66 ±0.55	11.33 ±0.73
II) 500 mg DOSE (BE/UTG)	9.33 ±0.33	9.83 ±0.54	32.33 ±0.85	16.50 ±0.95	29.50 ±0.67
III) Alcohol	2.83 ±0.30	3.83 ±0.16	5.00 ±0.25	3.33 ±0.21	8.00 ±0.60

Values are mean ±SEM of six animals
Statistical significance: a= p<0.01 comparison was done with their respective control group



Graph No 1 Effects of Sexual behaviors in ethanolic extract of seeds of *Blepharis edulis*



Graph No 2 Effects of hormone testosterone in ethanolic extract of seeds of *Blepharis edulis*

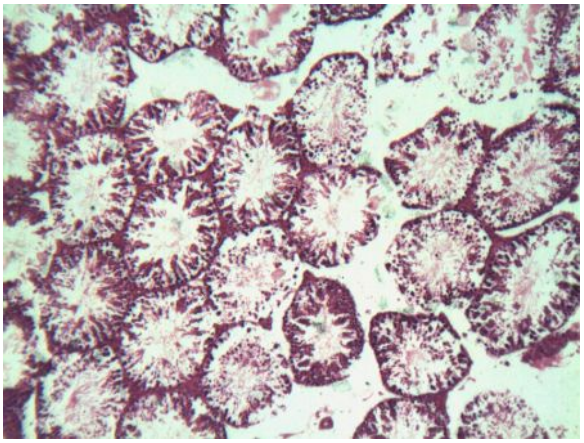


Figure 1.

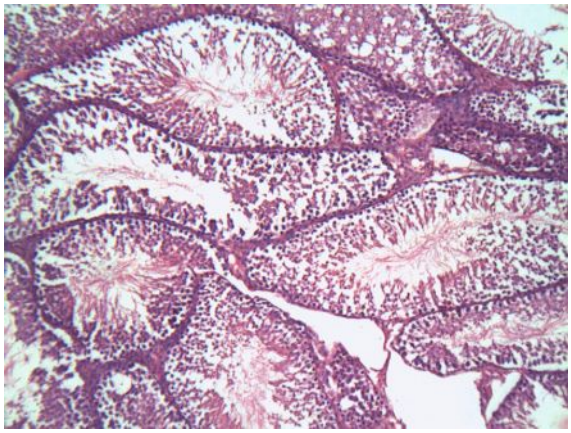


Figure 2.

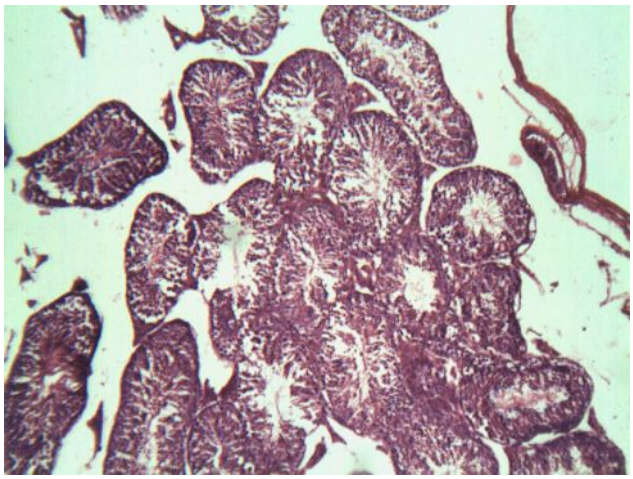


Figure 3.

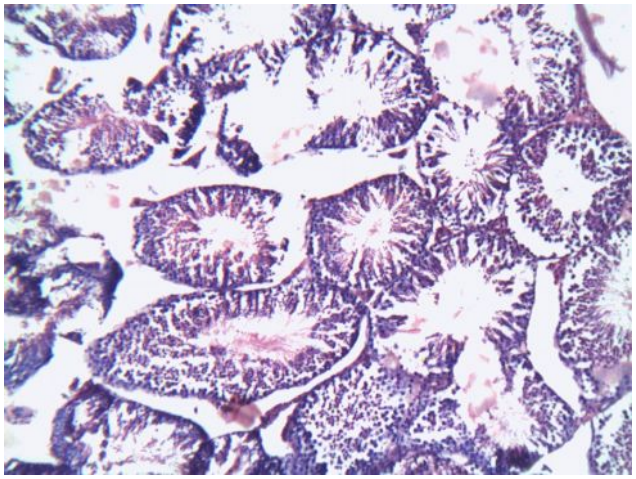


Figure 4.

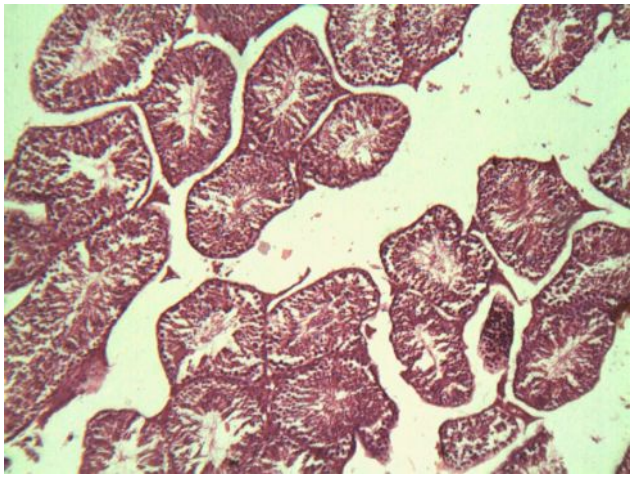


Figure 5.

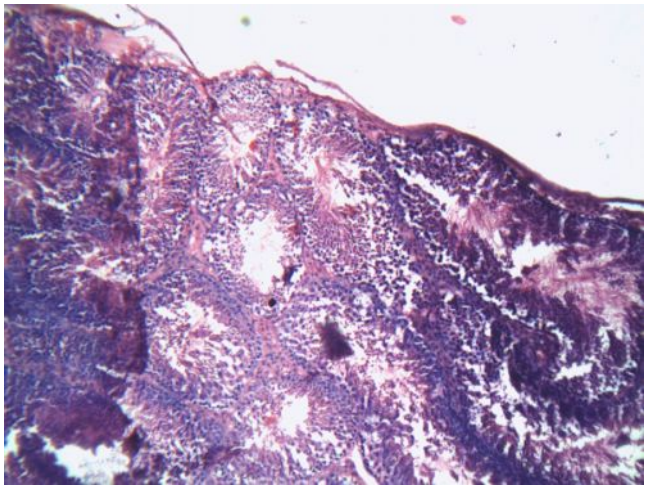


Figure 6.

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