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SCAN ME

Research Paper

EVALUATION OF CONTRACEPTIVE EFFICACY OF ETHANOLIC EXTRACT OF LYGODIUM FLEXUOSUM IN MALE ALBINO RAT: A HISTOLOGICAL APPROACH

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Abstract

E*thnobotanical uses of Lygodium flexuosum has been mentioned in the traditional and modern medicines. The present investigation deals with contraceptive efficacy of Lygodium flexuosum with its ethanolic extract on the male albino rat against control. The investigation involves the oral administration of sexually matured fertile male albino rat with an ethanolic extract of Lygodium flexuosum at the dose regimen of 50 mg/kg b. wt. /rat for 60 days as an experimental group whereas control group has received vehicle only wherein both the groups has 6 animals each. Rats were castrated for the histopathological investigations where tissues were fixed in the Bouin's fixative, dehydrated, sectioned and stained with hematoxylin and eosin. Treatment of Lygodium flexuosum in an experimental group have shown degenerated germinal epithelium lining of seminiferous tubule with spermatocytes, and spermatozoon debris in the lumen and tubules along with disoriented acini, shrunken cells with stromal and cellular degeneration in prostate, degenerative changes and reduced secretion in seminal vesicle, spermatozoan debris, degenerating epithelial layer, reduction in the lumen size of cauda epididymis, degenerating epithelial lining and distinctly visible spermatogonial debris in the lumen of caput epididymis. Also spermatozoon debris, reduction in lumen size of vas deferens was noted. The entire study was conducted by following the rules and regulation of CPCSEA at Agharkar Reseach Institute, Pune*.

Key words: *Lygodium flexuosum, ethnobotanical, ethanolic, contraceptive*.

INTRODUCTION

Growth of poor-developed countries and an undesirable consequences on environment and economic is badly affected by population explosion [1]. The detrimental effects of population explosion had badly affected life support system [2]. The rate of production of production of consumable contraceptives in males are limited and slow as compare to the females [3]. Various plants and their parts have been studied by various researchers for antifertility. Most of the synthetic chemicals have been studied as antifertility drugs which causes spermatogenic arrest and ultimately leads to the irreversible sterility [4]. According to the WHO about 80% of world's population is dependent on plant-derived medicines for their health care [5]. The reason behind selecting plant *Lygodium flexuosum* is its ethno botanical value. There are various compounds have been noted in the plant like drayocrassal, tectoquinone, kaempferol and stigmasterol [6]. Plant extract also shows presence of triterpene ester, anthraquinone, beta sitosterol [7]. The tribal communities are using this plant since long on gonorrhea, spermatorrhea, wound healing, headache, migraine, as pain killer and on eczema [8, 9, 10, 11]. *Lygodium flexuosum* is Malaysian native species of pteridophyte having presence of antifertility constituent [12].

Though the ethno botanical uses of *Lygodium flexuosum* have been well studied investigations on contraceptive efficacy have not been revealed in detail. So the present investigation contributes to the antifertility efficacy of the same.

MATERIALS AND METHODS

Sexually mature male albino rats of 8 to 10 weeks weighing 250-300 gms. with proven fertility has been used in the study. The animals were procured from CPCSEA approved laboratory at Agharakar Research Institute, Gopal Ganesh Agharkar Road, Pune, Maharashtra ARI/IAEC/2018/10. And were used for experiment and orally fed with dose regimen of 50 mg/kg animal body weight.

Animals were divided into two groups:

Group I …… Control.

Group II …… Plant extract
treated (Test) 50 mg/kg body wt.

On completion of experimental period the required animals were sacrificed in CO_2 chamber as per the guidelines strictly followed by the "Ethical regulations"

The required organs i.e. testes, seminal vesicle, prostate, epididymis and vas deferens of control as well as experimental animals were immediately excised, blotted, cleaned off the adhering fats and weighed and tissues were immersed in the Bouin's fixative for further processing of microscopic study. The stained slides were observed and photographed with the help of camera attached to the microscope.

RESULTS

OBSERVATIONS OF HISTOLOGY:

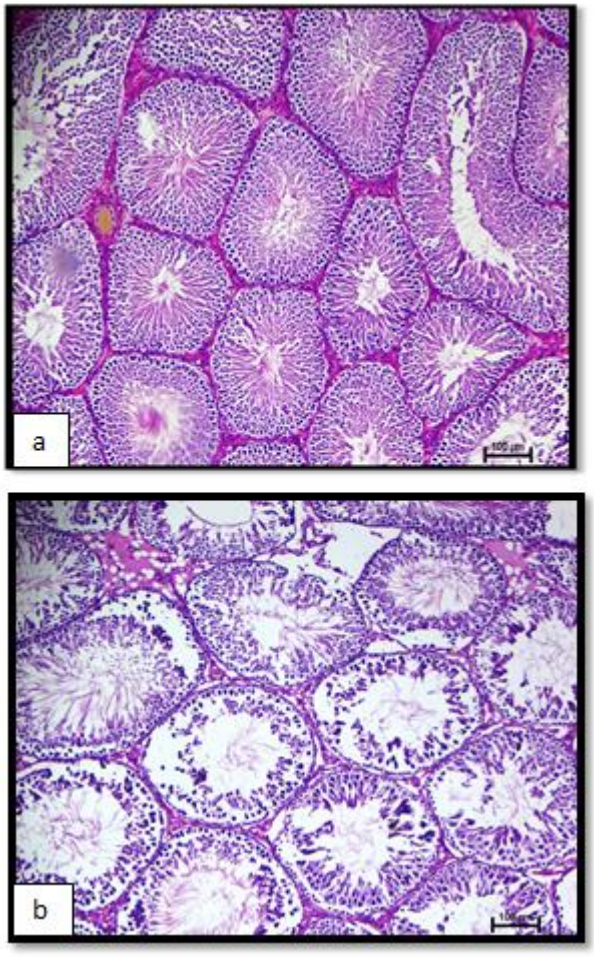

FIG. 1. PHOTOMICROGRAPH OF TESTES (X 100 H. E.) a) Control b) Test (Gr. II)

a) Control slide showing the normal histoarchitecture with well organised seminiferous Tubules showing germinal epithelium, spermatocytes, spermatids and spermatozoa.

b) Test slide showing disorgnised seminiferous tubule with eroded germinal epithelium and spermatozoan debris in treated groups (50 mg/kg of body wt.).

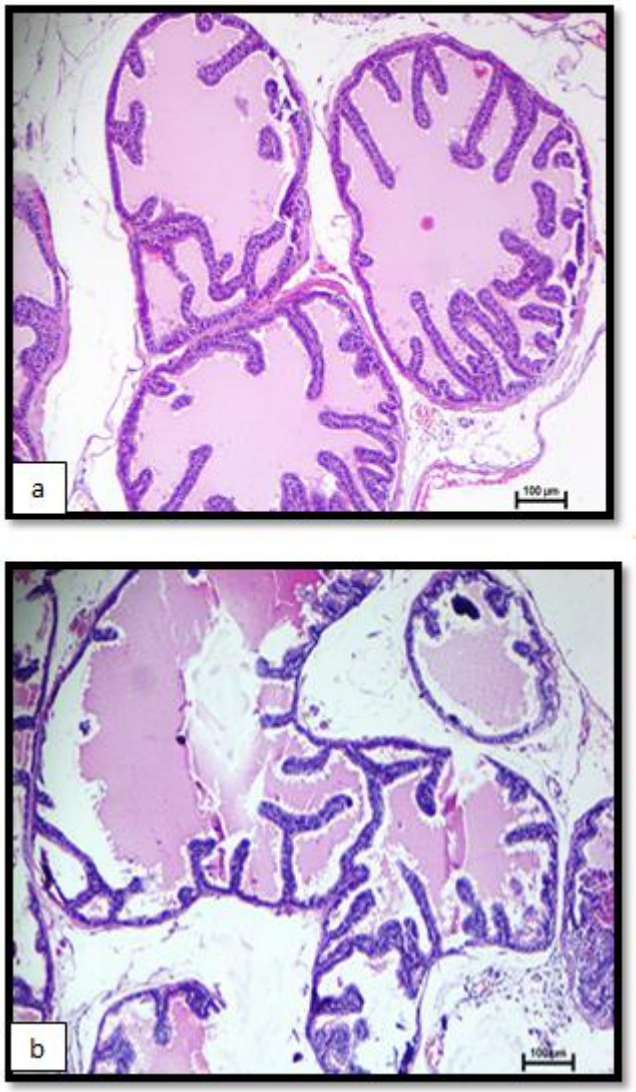

FIG 2. PHOTOMICROGRAPH OF PROSTATE GLAND (X 100 H E.): a) Control b) Test (Gr. II)

a) Distinct secretary zones of prostate acini are seen with fibromuscular stroma and compact smooth muscle fibers and basement membrane in the prostate gland of control rat .

b) Prostate gland of treated (50 mg/kg of body wt.) group shows disoriented acini, shrunken cells with stromal and cellular degeneration.

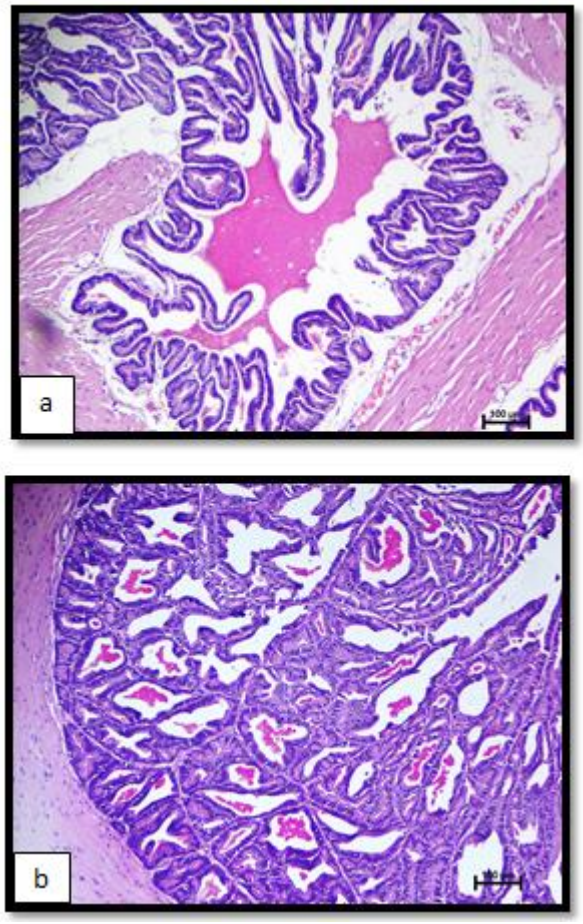

FIG. 3. PHOTOMICROGRAPH OF SEMINAL VESICLE (X 100 H. E.): a) Control b) Test (Gr. II)

a) Histology of Seminal vesicle in control group shows irregular branched lumen. The wall shows fibrous connective tissue with elastic fibers. It also shows columnar, non-ciliated cells, possessing basal nuclei, secretary granules with colloidal secretions.

b) The histological sections of Seminal vesicle after the 60 days treatment of extract (50 mg/kg of body wt.) shows degenerative changes involving reduction in epithelial lining and cell height with disruption of connective tissue and reduced secretion.

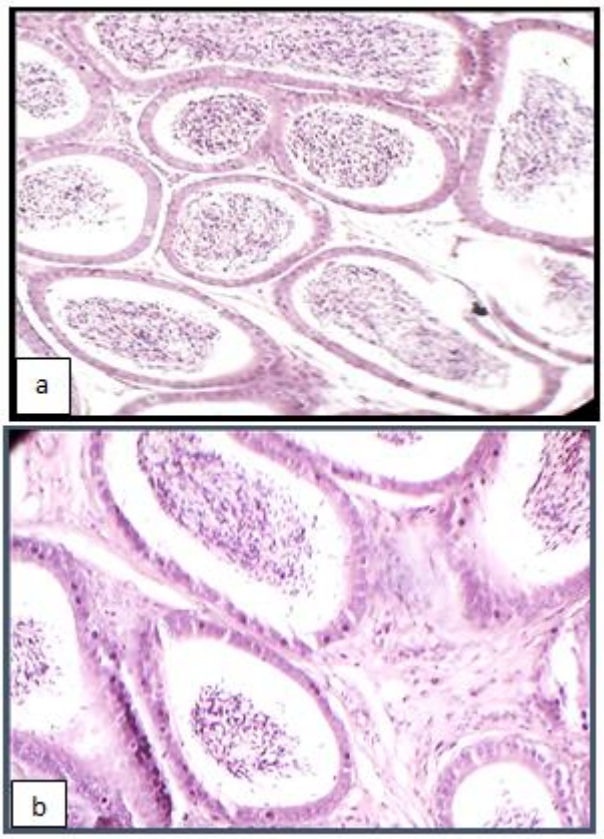

FIG. 4. PHOTOMICROGRAPH OF CAUDA EPIDIDYMIS (X 100 H. E.): a) Control b) Test (Gr. II)

a) The cauda epididymis of control group shows normal histology with tubular lining containing pseudo-stratified epithelial cells, lumen with large spermatozoa. They shows well organized and richly inverted smooth muscle coat.

b) The cauda epididymis of treated (50 mg/kg of body wt.) groups shows abnormal histology with darkly stained spermatozoan debris, degenerating epithelial layer, reduction in the lumen size with absence of stereo cilia. It also shows reduction in tubular diameter and isolated inter tubular tissue.

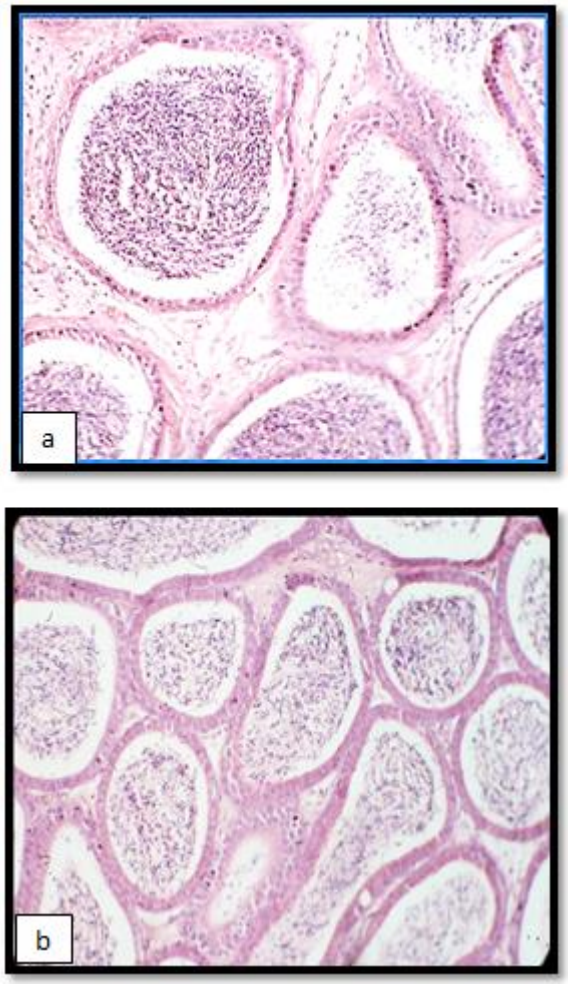

FIG. 5. PHOTOMICROGRAPH OF CAPUT EPIDIDYMIS (X 100 H. E.): a) Control b) Test (Gr. II)

a) The Caput epididymis of Control group shows normal histological architecture. The tubular lining shows tall columnar epithelial cells possessing long, tuft stereo cilia. The lumen shows presence of spermatozoa.

b) The Caput epididymis of 60 days treated (50 mg/kg of body wt.) rat shows reduction in tubular size, decrease in stereo cilia, sperm count, and reduction in epithelial cell height, degenerating epithelial lining and distinctly visible spermatogonial debris in the lumen.

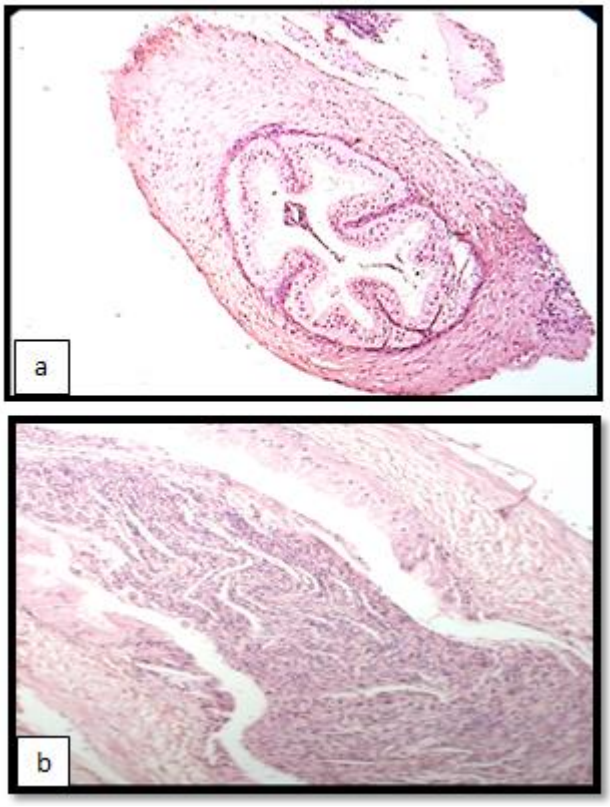

FIG. 6. PHOTOMICROGRAPH OF VASA DEFERENTIA (X 100 H. E.): a) Control b) Test (Gr. II)

a) The thick walled well organized muscular tubule of Vas deferens shows normal inner and outer longitudinal layer of muscle and intermediate circular layer of muscle. The inner lining also shows presence of delicate cilia towards the lumen which is filled with mature spermatozoa.

b) After the treatment of extract (50 mg/kg of body wt.) for 60 days, histoarchitecture shows darkly stained spermatozoon debris, reduction in lumen size and scanty stereo cilia.

DISCUSSION

Androgen is the key component responsible for proper maintenance of structural and functional integrity of reproductive organs in males hence findings of the present work are in agreement with [13, 14, 15, 16, 17] hence circulating androgen levels in the body of the animal should been sufficient to maintain the reproductive organs histoarchitecture and its normal functionality. Process of spermatogenesis is prominently dependent on protein content and hence observed degenerative changes in the histology of gonad showed degenerated germinal epithelium lining of seminiferous tubule with spermatocytes, and spermatozoon debris in the lumen and tubules which might be because of affected spermatogenesis, [18, 19, 20]. Secretary activity of seminal

vesicle and prostate is dependent upon the androgen secretion and this serves as a biological marker of androgen activity, [22, 22, 23] hence disoriented acini, shrunken cells with stromal and cellular degeneration in prostate, degenerative changes and reduced secretion in seminal vesicle might be due to the androgenic insufficiency. Epididymal secretions are dependent upon the protein content and this also corresponds to androgen level,[24,25] observed spermatozoan debris, degenerating epithelial layer, reduction in the lumen size of cauda epididymis, degenerating epithelial lining and distinctly visible spermatogonial debris in the lumen of caput epididymis along with spermatozoon debris, reduction in lumen size of vas deferens was also noted this might have affected the normal functioning of epididymis due to deprived androgen production. Accumulation of cholesterol in the testes gives a direct evidence for antagonistic action of the test substance which might lead to infertility, it inhibits androgenesis by hyperlipidimia causing alteration in biochemistry and histology of reproductive organs and causes inhibition of spermatogenesis via Leydig cells [26]. Administered extract in the test group of animals might have shown the same kind of effects. Decreased ascorbic acid content leads to the hypo functioning of testes causing degeneration of germinal epithelium [27] which was noticed in the histology of testes.

CONCLUSION

It can be concluded after the observations and results that treatment of 50mg/kg of body weight ethanolic extract of *Lygodium flexuosum* in male albino rat have shown degenerative changes in the histoarchitecture of the gonad and accessory reproductive organs and have shown its prominent contraceptive efficacy. Hence, *Lygodium flexuosum* carries an ideally emerging plant based contraceptive agent for males which is orally active, non-steroidal, toxically safe and cheap that meet all essential criteria framed by WHO task force.

The present investigation is just a small step towards the clinical development of natural, safe, reliable, orally active male contraceptive which can be used in the reduction of over burden of females who are contributing major role in family planning though they had to face severe consequences associated with it not only at physical level but mentally too…

Long duration and antifertility efficacy of *Lygodium flexuosum* ethanolic components has to be tested on other animal models, in the view of possible development of potential contraceptive of natural origin for clinical application can be warranted for better acceptability.

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CONFLICT OF INTRESTS.

Conflict of interest declared none.

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