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Antifertility Effects of Seeds of *Sapindus trifoliatus* on Histology of Testis and Epididymis of Male Albino Mice (*Mus Musculus Albinus*)

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Abstract: The present study was carried out as a preliminary study to determine the histological effects of seed powder of *Sapindus trifoliatus* on male albino mice. A total of 12 adult male albino mice were taken. Four groups each containing three mice were made. First group of mice served as control and other three groups were treated as experimental. The control mice were fed with normal diet, while experimental mice were administered with 10, 30 and 50 mg/kg body weight seed powder respectively for 30 days. After 30 days of experimentation all control and treated mice were dissected. The testis and epididymis were removed, their weights were recorded, fixed and processed for further histological technique. Administration of the *S. trifoliatus* to mice showed a significant increase in body weight, but testicular weight were unaffected. The present study also clearly demonstrates that the administration of seed powder of *S. trifoliatus* at all the tested doses caused insignificant reduction of the reproductive organs weight, testicular damage and complete arrest of spermatogenesis. At lower doses, a few layers of spermatogenic cells were noted but at later stage the spermatogenesis was stopped. At the highest dose i.e. 50 mg complete arrest of spermatogenesis and degeneration of remaining germinal cells could be seen. The atrophy of Leydig's cells was also noted at higher doses of treatment up to 60 days. The epididymis also showed degenerative changes in the epithelial cells at higher doses. Spermatozoa were not seen in the lumen of ductules of epididymis.

Keywords: *Sapindus trifoliatus*, epididymis, testis, spermatogenesis, Haematoxylin.

Introduction

The rapid growth of population, not only of human beings but among animals also, is a burning problem of the present century. Controlling the vast increasing population of the world is one of the biggest challenges of our times. Men has been in search of antifertility substances and the record of use of herbs as antifertility agent could be trace as far back as 1500 B.C. Numerous indigenous plants with antifertility properties have been reported by a number of workers and search for new plants having similar properties is still going on all over the world. The aim of the present study is to evaluate the effects of the extracts of some locally available plants in India on the fertility of male albino mice. A large number of medicinal plants growing in different parts of the world have been used by the native people for their antifertility efficacy. (Casey 1960, and Farnsworth et. al. 1975 a & b). In India a thorough search on contraceptive plants used by tribes is in progress (Chaudhary 1966, Saxena 1973, Kamboj and Dhawan 1982). Although there are a number of papers related to antifertility effects on male mice starting from the work conducted by East (1955a &b) but only few of them (Sinha 1991, Lohiya & Goyal 1992, and Garg et. al. 1992) are related to the laboratory-based results on the role of antifertility effects on rats. Khanna et. al. (1986) and Singh (1997) reported the antifertility effects of *Ocimum sanctum* L. leaves in male albino mice. Whereas, Garg et. al. (1992) and Bhatia et.al. (2010) reported the same results on male rats by oral administration of leaf extracts of *Azardirecta indica* and *Adiantum lunulatum*. On the other hand, Ozoko

et. al. (2018) and Mostafa et.al. (2021) revealed the antifertility effects on rats by using cotton seed oil, chloroform extract of *Gossypium barbadense*, the ethanolic extract of *Glycine max* and *Rosemarinus officinalis*. The present study is an attempt on laboratory experiments by oral administration of seed powder of *Sapindus trifoliatus* on histology of testis and epididymis of male albino mice.

Materials and Methods

Experimental animals

1. **Albino mice (*Mus musculus albinus*):** Healthy adult colony of bred swiss male albino mice weighing between 50 to 60 gm were selected and acclimatized to the laboratory conditions for 7 days prior to the commencement of experiment. The mice were kept in cages (60 cm x 45 cm x 45cm) under natural conditions of photoperiod at room temperature. Four groups each containing 3 mice were made, first group of mice served as control and other groups were treated as experimental. All mice were maintained on balanced homemade feed including bread, water soaked crushed Bengal gram, carrot, cabbage, etc. and water allowed at libitum.
2. **Plant material:** *Sapindus trifoliatus* L. (Sapindaceae) is a median sized deciduous tree growing wild in South India that belongs to the family Sapindaceae. It is known locally as soapnut tree. The pulp drying into a saponaceous wrinkled rind seeds are pea sized, enclosed in blackish, smooth, hard endocarp. The powdered seeds are employed for oral administration.
3. **Doses and administration:** Three doses 10, 30, and 50 mg/kg body weight were prepared. Each dose was macerated with 0.5% *S. trifoliatus* powder in distilled water. The volume was adjusted in such a way that 1 ml of solution correspond to 50 mg of crude powder. 1 ml of this solution containing 50 mg seed powder was fed to each mice per day. The rest of doses were prepared in similar manner and administered to the treated group of mice (group IInd, IIIrd, and IVth), the doses 10, 30 and 50 mg were given respectively for 30 days. The control mice (group Ist) were given 1 ml of distilled water only for 30 days. Administration of doses was made oral with specially designed knobbed feeding needle fitted into a syringe.
4. **Record of weight and histology:** The initial and final weight of the experimental as well as control mice were recorded. The mice from all groups of treated and control were sacrificed after 24 hours of the last dose under chloroform anesthesia and dissected quickly. The testis, epididymis and vas-a-deference were taken out freed from the adherent tissue and blood. Weight of testis and other organs was recorded. The testis, epididymis, and vas-a-deference etc. were fixed in Bouin's fluid, washed, dehydrated, embedded in paraffin wax, sectioned (6 micron) and stained with Haematoxylin-Eosin for histological study.
5. **Statistical analysis:** The significance of difference of weights between the treated and control mice was assessed by student 't' test, taking $P< 0.05$ as significance.

Results

1. **Body and genital organ weights**: Table 1 shows the weights of body and genital organs of male mice treated with *S. trifoliatus* seed powder at various doses for 30 days along with control mice. Control mice administered with 0.5% gum-acacia powder dissolved in distilled water (1ml/day) as vehicle, did not reduce the body and genital organ weights throughout the experimental period. Experimental mice administered with *S. trifoliatus* seed all doses showed an increase in the body weight. The doses 10 mg and 30 mg/day for 30 days caused a little reduction of genital organ weight in comparison to control. An insignificant change

(reduction) in the genital organ weights were observed at the 50mg /day for 30 days.

Table 1: Effects of the administration of *Sapindus trifoliatus* on male albino mice body weight and testicular organs weight. Three animals were used in each group, values are mean ±S.E.

Treatment Groups	Dose mg/day	Body weight (g)		Body Weight Difference (g)	Weight change (%)	Organ's weight (mg)	
		Initial	Final			Testis	Epididymis
(Ist) (Control)	–	48.50 ± 16.26	56.20 ±10.02	7.7	13.70	242.00 ±3.40	88.20 ± 13.95
(IInd) *Sapindus trifoliatus*	10	45.60 ± 7.07	45.80 ± 12.04	0.2	0.43	235.20 ±7.20	84.60 ± 5.37
(IIIrd)	30	38.60 ± 5.36	45.80 ± 8.98	7.2	15.72	213.60 ±6.07	79.60 ± 2.10
(IVth)	50	37.20 ± 6.85	43.70 ± 6.63	6.5	14.80	211.80 ± 1.80	67.20 ± 1.70

P<0.05

2. Histopathological examination

(A) Effect on histology of testis

Control: The histology of testis of control mice showed a normal adult picture. The tunica propria was well developed. The seminiferous tubules have spermatogenesis by regular stages of spermatogonia cells which produce spermatozoa after spermatogenesis. The Leydig's cells were present in the interstitum. The vascularity of the organ was normal. (Fig.1)

Treated: The administration of 10 mg/day dose of *S. trifoliatus* tubers for 30 days caused an arrest of spermatogenesis at later stage. A few layers of spermatogenic elements could be seen in the seminiferous tubules. At places, leakage of germ cells was noted. The leaked germ cells were collected in the interstitial spaces. The Leydig's cells were atrophied in the interstitium (Fig. 2). The administration of 30 mg/day dose for 30 days also caused an arrest of spermatogenesis. The spermatogenesis could not proceed beyond the spermatogonia. The lumen of seminiferous tubules was filled with cellular debris. Degenerated cellular material was also seen in the interstitium. Leydig's cells were not seen in interstitium. The vascularity was affected (Fig. 3).

The administration of 50 mg /day dose caused severe degenerative changes in the seminiferous tubules leading to complete arrest of spermatogenesis. The degenerative cellular material included atrophy of spermatogenic elements, oedema and necrosis of the tubules. Only spermatogonia were visible with pyknotic nuclei. The lumen of the tubule was filled with oedematous fluid and cellular debris. Germinal epithelium at certain places was disintegrated. Interstitum was devoid of Leydig's cells and filled with leaked germ cells. The vascularity of the organ was also affected (Fig. 4).

(B) Effect on histology of epididymis

Control: The caput epididymis of the control group of mice presented normal histological features. It showed increased epithelial cell height, wide lumen of ductules packed with spermatozoa. The nuclei of the epididymal

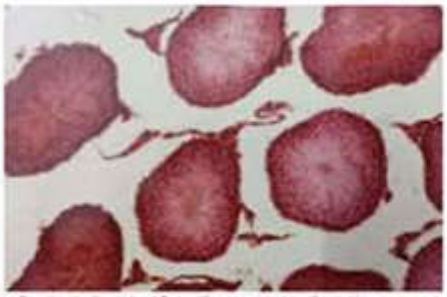

Fig. 1: T.S. testis of control mice (spermatogenic activity in seminiferous tubules andnormal Leydig's cells in interstitium)

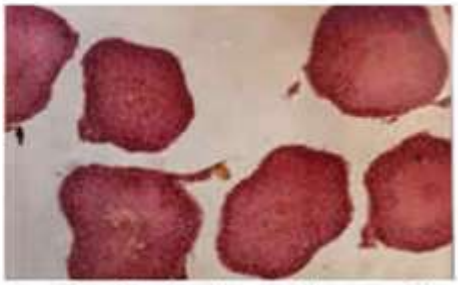

Fig. 2: T.S.testis of mice administered with 10 mg/day dose of S. trifoliatus for 30 days (the arrested spermatogenesis, leaked germ cells and atrophied Leydig"s cells in interstitium)

Fig. 3: T.S. of testis of mice administered with 30 mg/day dose of S. trifoliatus for 30 days (arrested spermatogenesis, lumen of seminiferous tubules filled with cellular debris. Absence of Leydig's cells in interstitium),

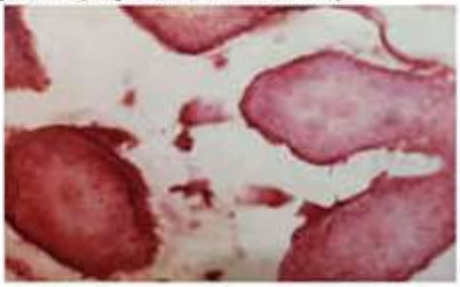

Fig. 4: T.S. of testis administered with 50 mg/day dose (complete arrest of spermatogenesis, spermatogonia have pyknotic nuclei, lumn filled with oedematous fluid and cellular debris, broken epithelium. Leydig's cells were absent , interstitium, filled liked germ cells)

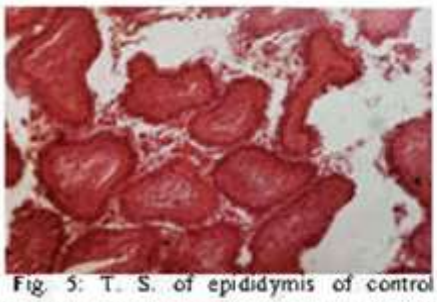

Fig. 5: T. S. of epididymis of control mice (normal epithelial cells, white lumen of ductule packed with spermatogoa. The sterecelia in large numbers bordering lumen. The vascularity of organ was normal),

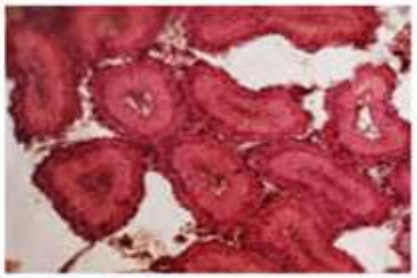

Fig. 6: T. S. of epididymis administered with 10 mg/day dose (normal histology, low sperm density, displaced nuclei in the epithelial cells.)

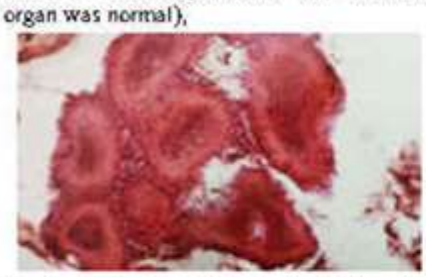

Fig. 7 : T. S. of epididymis administered with 30 mg/day dose for 30 days (broken epididymal epithelium in ductules, cell nuclei leaked into the interstitium, no spermatozoa in the lumen of ductules).

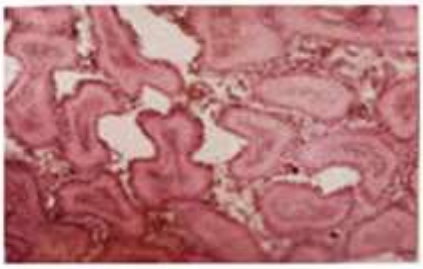

Fig. 8 : T. S. of epididymis administered with 50 mg/day dose for 30 days (produced epididymal height and lumen of ductules, nucleii of the epithelial cells became pyknotic, connective tissue of ductules change in the oedomatous fluid, with no spermatozoa).

epithelial cells were normal in position. The stereocilia were present in large numbers bordering lumen. Well-developed connective tissue material was seen between inter-ductular spaces or epididymal interstitium. The vascularity of the organ appeared normal. (Fig. 5)

Treated: The caput epididymis of mice treated with *S. trifoliatus* dose of 10 mg/day for 30 days showed no marked histological changes. There was no significant reduction in the size of the ductules but sperm concentration or density of spermatozoa in the lumen of ductules reduced greatly. A displacement of nuclei in the epithelial cells was also noted. Other details were comparable to the control mice (Fig. 6).

The administration of 30 mg /day for 30 days caused histopathological changes in the ductules of epididymis. At places the epididymal epithelium of ductules was broken and the cell nuclei were leaked into the interstitium. The connective tissues and vascularity of the organ were also affected. No spermatozoa could be seen in the lumen of ductules. (Fig.7)

The treatment with 50 mg /day dose for 30 days exerted severe degenerative changes in the epididymis. The epididymal height and lumen of ductules were reduced. Nuclei of the epithelial cells became pyknotic. Connective tissue between ductules was reduced and replaced by oedematous fluid. Spermatozoa in the ductule lumen were totally absent. The vascularity of the organ was greatly affected. (Fig. 8)

Discussion

The results of the present study clearly demonstrates that the administration of sapindus seed powder of *S. trifoliatus* at all the doses caused insignificant reduction of the reproductive organs weight, testicular damage and complete arrest of spermatogenesis. At lower doses a few layers of spermatogenic cells were noted but at later stage the spermatogenesis was stopped. At the highest dose i.e. 50mg/day complete arrest of spermatogenesis and degeneration of remaining germinal cells could be seen. The atrophy of leydig's cells was also noted at higher doses of treatment up to 60 days. The epididymis also showed degenerative changes in the epithelial cells at higher doses. Spermatozoa were not seen in the lumen of ductules of epididymis. All these pathological changes including reduction of genital organ weights are due to deficient nitrogen production as suggested by Paul et. al. (1953) and Nelson and Patanelli (1965). Absence of spermatozoa in the testis and epididymis suggests a sever effect of sapindus powder of *S. trifoliatus* on the process of spermatogenesis. Garg (1979) reported similar results after the administration of flower and root extracts of *Calotropis procera* on the male reproductive organs of Indian desert gerbil (*Meriones hurrianae* Jerdan).

The findings of the present studies concerning with *S. trifoliatus* tuber powder are in concurrence with the findings of Kholkute and Udupa (1974) who reported definite histopathological changes in the rat testis at higher doses (250 mg) of flower extract of *Hibiscus rosa sinensis* Linn. administration up to 60 days. The effects on spermatogenesis ranged from a mild damage in the testicular element to its total damage and arrest of spermatogenesis. In support of the results obtained by *S. trifoliatus* administration, similar observations were made by Pakrashi et.al. (1977a) after chronic administration of extract of *Aristolochia indica* Linn on male mice. Das (1980) also reported anti-spermatogenic changes in the testis of male albino rat without affecting the weight and fertility with the administration of crude powder of papaya seed (*Carica papaya*). Verma et. al. (1981) reported after 40 days of treatment with *Portulaca grandiflora* seed extract on the reproductive organs of male albino mice that it caused the arrest of spermatogenesis and mass atrophy of other cellular elements at high doses. There is a

close similarity between the findings of the present study and the studies of Roychoudhary et. al. (1983) and Singh (1985) who reported similar histopathological changes in the testis of mice after administration of *C. intybus* and *S. anacardium* seed powder for 10 days and 60 days.

Kaur et.al. (1988) studied the effect of gossypol on testis and epididymis of albino rats and found out many degenerative as well as necrotic changes in the seminiferous tubules in large percentage of treated rats are comparable to the present study with *S. trifoliatus.* The present findings are close to the Rao (1988) and Bhargava (1988) in the fact that both of them have reported the same results on testis and epididymis. However there is a difference with regard to plant extracts used *S. xanthocarpum* and *S. trifoliatus* respectively. Maina et.al. (2008) revealed the interstitial edema and congestion of blood vessels in the histological examination of the rat's testis treated with continuous and increase use of the herbal tea mixture including herbs i.e. *Gynostemma pentaphyllum, Radix polygoni multiflori*. *Semen cassiae,* Green tea and *Folium nelumbinus.*

The present findings are similar to the findings made by Patil et. al. (2022) who reported the decreased testis weight, reduced sperm count and motility as compared to the control rats administered with leaves extract of *Lagerstroemia speciose* and fruits of *Momordica dioica*. Taking the above observations into consideration it can be said that the seed powder of *S. trifoliatus* have the potentiality to regulate the male fertility by causing histopathological changes in the reproductive organs of mice, thereby reducing the fertility of animals

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