

Full Length Research Paper

Histomorphological study to evaluate anti-fertility effect of *Abrus precatorius* L. in adult male mice

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This study was designed to find effects of ethanol extracts of *Abrus precatorius* seeds on male mice (Balb-C) fertility. The extracts were intraperitoneally administered with 20, 40 and 60 mg/kg doses for 20 days. Compared to the controls, there was no significant decrease in testicular and epididymal weight. Histological analysis showed disrupted arrangement of seminiferous tubules, loosening of germinal epithelium and low counts of leydig cells, germ cells and sperm cells. Thickness of tunica albugenia, seminiferous tubule diameter and germinal epithelial height reduced significantly in treated mice compared to controls. Histomorphology of the epididymus showed a decrease in tubule size, epithelial height and a reduction in sperm number in the tubular lumen. The epididymal corpus segment underwent dysplasia, intraepithelial vacuolation and decreased sperm counts. Plasma testosterone levels decreased significantly with a higher dose (60 mg/kg) compared to controls. This study suggests that *A. precatorius* seed extract with higher dose (60 mg/kg) tends to suppress spermatogenesis and is hence liable to cause infertility in male mice.

Key words: Histomorphological, anti-fertility, *Abrus precatorius*.

INTRODUCTION

In spite of great advances observed in modern medicine in recent decades, plants still make an important contribution to healthcare (Shu, 1998). Much interest, however, has been shown in recent years, to control male fertility by using plants (Chowdhury et al., 2001; Kamal, 2003). Numerous plants have been used historically to reduce fertility and modern scientific research has confirmed anti-fertility effects in at least some of the herbs tested (Oderinde et al., 2002; McNeil et al., 2003; Olabiyi et al., 2006). Although the toxicity profile of most medicinal plants have not been thoroughly evaluated, it is generally accepted that medicines derived from plant products are safer than their synthetic counterparts (Vongtau et al., 2005; Oluyemi et al., 2007).

Plant

Abrus precatorius L. (Fabaceae) is a high climbing, twining woody vine with herbaceous branches. The fruits

have more or less the shape of bean pods that contains seeds and are borne in clusters.

Traditional uses

The most widespread use of *A. precatorius* seeds is in the treatment of eye infections and as a potential contraceptive (Amudar et al., 1991). *A. precatorius* seeds are considered as poison which, when administered, inhaled or swallowed, is capable of acting deleteriously on the body (Parikh and Keith, 2007).

Chemical constituents

The active principle in the seeds of *A. precatorius* is abrin (Parikh and Keith, 2007). Cycloartane glycoside, designated abrusoside E, 3 β -O-[β -D-glucuronopyranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl] (20S, 22S)-3 β , 22-dihydroxy-9, 19-cyclolanost-24-en-26, 29-dioic acid δ -lactone (Kennelly et al., 1996).

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Previous investigations

The oral administration of ethanol extract of *A. precatorius* seeds in albino rats results in an absolute infertility in males which is reversible (Sinha, 1990). Methanol extract of *A. precatorius* seeds have inhibitory effects on the motility of washed human spermatozoa (Ratnasooriya et al., 1991). Aqueous extract of the leaves of *A. precatorius* show toxic effect in rats (Adedapo et al., 2007). It was found that the methanolic extract of *A. precatorius* through oral route induces functional sterility in adult male mice, without toxic symptoms (Bhatt et al., 2007). The seeds of *A. precatorius* can be used as a herbal source to isolate promising bioactive compounds with contraceptive capacity (Zia-Ui-Haque et al., 1983). The epididymis is a site which can be exploited for male contraception without undue side effects (Kirchoff, 1997).

Keeping the previous findings in view, the present study was designed to evaluate the histological alterations of testicular and epididymal tissues through various doses of parenteral (intraperitoneal injection) administration of *A. precatorius* in the male mouse reproductive system. Parenteral route of administration is selected because of its more rapid and predictable absorption than oral administration. This route is also advantageous because the drug is not inactivated or destroyed as may happen in the gastrointestinal tract and usually smaller doses are required because large concentrations of drug is achieved at the affected site.

MATERIALS AND METHODS

Plant material and extraction

Mature dried seeds of *A. precatorius* were procured from a local market in Rawalpindi, Pakistan. The seeds were washed to remove residual material and dried. An ethanolic extract was prepared using the cold extraction method (Adome et al., 2003). The seeds were coarsely powdered and 500 g of this powder was soaked in 1 L of 99.9% ethanol for seven days. On the eighth day the contents were filtered and ethanol was evaporated under reduced pressure in a rotary vacuum evaporator. This filtrate was dried at room temperature and dried mass was stored at 4°C. The yield of extract was 0.6% of the starting raw material. Three different doses of 20, 40 and 60 mg/ kg/ body weight/day were prepared from the stock solution by dissolving 3 g of the extract in 1000 ml of physiological saline.

Animals

Adult male mice of Balb-C strain (n = 24), were used. They were housed in microcolon boxes with standard laboratory diet and water was available. Animals in each group were housed in steel cages. They were kept for two weeks to acclimatize and then weighed. The study was conducted after obtaining Institutional Animal Ethical Committee clearance.

Dosage

The animals were randomly divided into four groups (n = 6) as

follows. Those in Group I served as controls, all the animals of this group were given saline. Animals in group II, III and IV received different doses of extract intraperitoneally (20, 40 and 60 mg/kg respectively) for 20 days. The body weight of adult mice was recorded throughout the treatment period and also at the time of autopsy.

Schedule of sacrifice

After 20 days of dosing, on the 21st day the animals were sacrificed under chloroform anesthesia. Blood was drawn through cardiac puncture and was centrifuged; plasma was separated and stored at -20°C until analyzed for testosterone. Serum testosterone concentration was measured by Enzyme immuno assay (EIA) kit (BioCheck. Inc.CA.94404). All samples were quantified in a single assay.

Organ weight and size

Testis and epididymis were dissected out, blotted free of blood and weighed with the help of a Sartorius digital balance. Length and width of testes and epididymis were measured by vernier callipers.

Histology

Testes and three parts of epididymis (caput, corpus and cauda) were fixed in alcoholic fixative and embedded in paraffin. Transverse sections of the organs were cut at 5 µm and stained with hematoxylin and eosin.

Light microscopic study

Five micrometer thick transverse sections of testis and epididymis were studied under light microscope (Nikon) at 20, 40 and 100 magnifications. Slides of all the groups were studied and photographed by Cannon digital camera. Mean ± SEM of morphological and histological data of control and *A. precatorius* treated groups were determined.

Statistical analysis

The data was analyzed and compared by one way ANOVA by using Graph-Pad Prism V. Level of significance has been considered at the level of P < 0.05.

RESULTS

The influence of the ethanolic extract of *A. precatorius* seeds on testes and epididymis of Balb-C mice was assessed in a concentration-dependent study over a 20 days period. Weight and size of the testis and epididymis in *A. precatorius* treated mice did not show marked differences compared to controls (Table 1). Within the treatment groups there was also a non significant (P > 0.05) difference and all the animals maintained a healthy appearance throughout the period of investigation. A significant decrease (P < 0.05) was noticed in serum testosterone level in high dose treated mice (60 mg/kg) compared to controls, while 20 mg/kg and 40 mg/kg

Table 1. Effect of various doses of *A. precatorius* extracts on the mean testicular and epididymal weight and size in control and treated mice.

Group	Testes	Epididymis		Total (Right and left Epididymal) weight	Total (Right and left Epididymal) length
	Total (Right and left Testicular) weight	Total (Right and left Testicular) length	Total (Right and left Testicular) width		
Control	0.16 ± 0.00	14.03 ± 0.49	8.42 ± 0.09	0.086 ± 0.00	37.54 ± 2.48
20 mg/kg	0.15 ± 0.00	13.33 ± 0.33	8.68 ± 0.23	0.077 ± 0.00	36.11 ± 1.23
40 mg/kg	0.15 ± 0.00	14.56 ± 0.34	9.44 ± 0.35	0.063 ± 0.00	32.78 ± 1.94
60 mg/kg	0.13 ± 0.00	11.98 ± 0.86	7.17 ± 0.81	0.068 ± 0.00	36.16 ± 2.07

Values (Mean ± SEM).

Table 2. Amount of testosterone estimated after injection of extract at various doses in controls and experimental mice.

Group	Testosterone Concentration (ng/ml)
Control	4.78 ± 1.57
20 mg/kg	3.31 ± 0.85
40 mg/kg	2.45 ± 0.23
60 mg/kg	0.51 ± 0.18

Values (Mean ± SEM)

Table 3. Histometrical parameters of testes in controls and male mice treated with *A. precatorius* extract for 20 days duration.

Group	Tunical thickness (µm)	Leydig cell nuclear diameter (µm)	Seminiferous Tubule Diameter (µm)	Epithelial height (µm)	Sertoli Cell Nuclear Diameter (µm)
Control	18.38 ± 0.66	7.02 ± 0.14	183.60 ± 4.63	45.79 ± 0.77	9.47 ± 0.17
20 mg/kg	16.48 ± 0.57 ^{a*}	5.60 ± 0.12 ^{a***}	174.71 ± 4.71	42.50 ± 1.1 ^{a***}	8.73 ± 0.19 ^{a*}
40 mg/kg	16.17 ± 0.51 ^{a**}	5.26 ± 0.11 ^{a***}	172.91 ± 2.83 ^{a**}	34.70 ± 0.83 ^{a***b***}	8.31 ± 0.17 ^{a**}
60 mg/kg	13.52 ± 0.48 ^{a**}	5.26 ± 0.08 ^{a***}	164.30 ± 4.44 ^{a***}	34.53 ± 1.04 ^{a***c***}	8.20 ± 0.17 ^{a***}

Values (Mean ± SEM) a = Controls vs 20 mg/kg, 40 mg/kg and 60 mg/kg. b = 20 vs 40 mg/kg, c = 20 vs 60 mg/kg, d = 40 vs 60 mg/kg. *P < 0.05, **P < 0.01, ***P < 0.001.

groups showed non significant (P > 0.05) decrease in testosterone concentration compared to controls (Table 2). The comparison of treatment groups exhibited a non-significant decrease in serum testosterone level.

For histomorphometry the following parameters were studied: Tunica albuginea thickness, seminiferous tubule diameter, epithelial height of seminiferous tubule, nuclear diameter of Leydig cells, Sertoli cells and germ cells (Table 3, 4 and 5). Cross sections of control testes revealed that it was surrounded by a thick tunica albuginea (Figure 1a). Within the testis seminiferous tubules were present which were compactly arranged towards periphery, adjacent to the tunica albuginea (Figure 2). Large number of round, oval or irregular shaped Leydig cells were present in groups around blood vessels possessing distinct oval or spherical shaped nucleus in their cytoplasm (Figure 3a). Active spermatogenesis was visible in nearly all the tubules (Figure 4a). Large sized Sertoli cells were observed in the seminiferous

tubule. Where as, dose dependent degenerative effects were seen in the testicles of treated mice.

Doses of 60 and 40 mg/kg body weight caused marked alterations in the histological appearance of the testis in treated mice, while 20 mg/kg of *A. precatorius* seed extract did not cause appreciable alterations in physical and histological appearances. Treated mice's testes showed alterations in the seminiferous tubules, and these group (60 mg/kg) compared to controls. Necrosis of primary spermatocytes was observed in the testes of male mice treated with 60 mg/kg/day and 40 mg/kg/day of *A. precatorius*.

Significant decrease in spermatogenesis activity was observed in seminiferous tubules. Treated rats testicular cell population showed a decrease in number of spermatocytes and spermatids and majority of the tubules were deformed or of irregular shape by extract administration. Interstitial space was comparatively loose. The tunica albuginea of *A. precatorius* treated testis was smaller in

Table 4. Nuclear diameter findings of testicular germ cells in controls and extract treated mice.

Group	Spermatogonium Diameter (μm)	Primary Spermatocyte Nuclear Diameter (μm)	Secondary Spermatocyte Nuclear Diameter (μm)
Control	6.40 \pm 0.17	8.87 \pm 0.15	6.65 \pm 0.09
20 mg/kg	5.76 \pm 0.15 ^{a*}	8.00 \pm 0.14 ^{a***}	5.25 \pm 0.09 ^{a***}
40 mg/kg	5.70 \pm 0.14 ^{a**}	7.92 \pm 0.15 ^{a***}	5.13 \pm 0.07 ^{a***}
60 mg/kg	5.50 \pm 0.11 ^{a***}	7.07 \pm 0.14 ^{a***c***}	5.11 \pm 0.07 ^{a***}

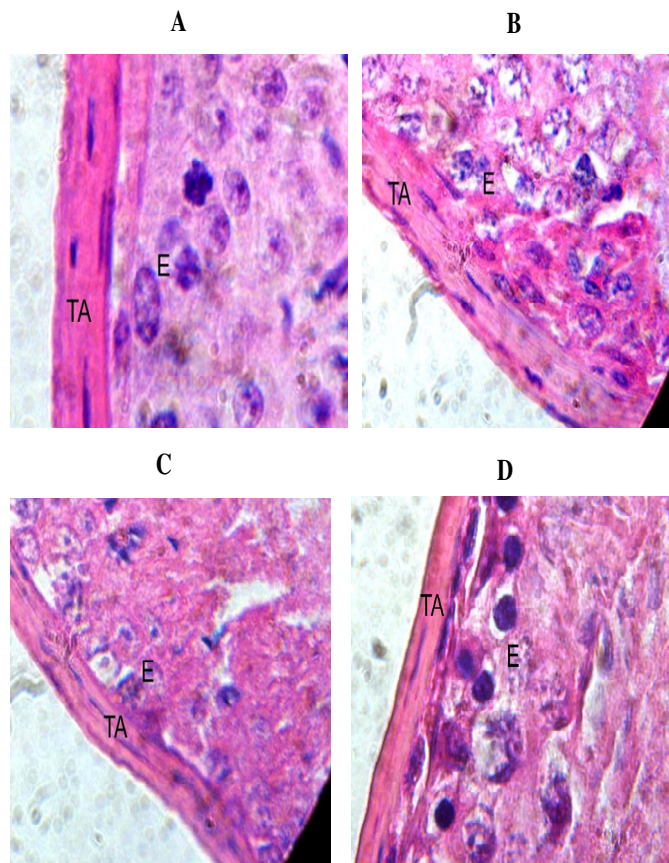
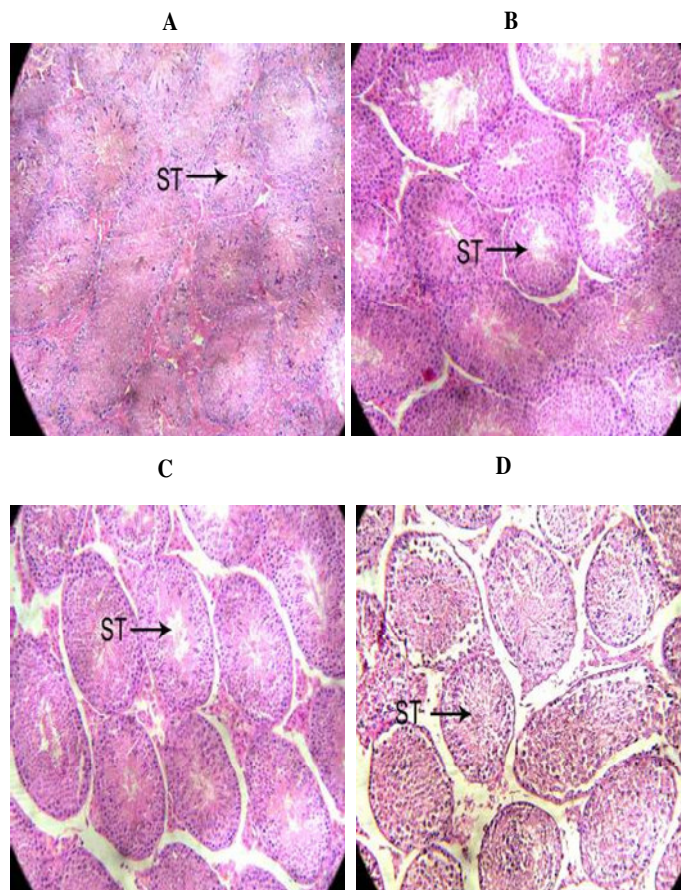
Values (Mean \pm SEM) a= Controls vs 20 mg/kg, 40 mg/kg and 60 mg/kg. b = 20 vs 40 mg/kg, c = 20 vs 60 mg/kg, d = 40vs 60 mg/kg.

*P < 0.05, **P < 0.01, ***P < 0.001.

Table 5. Histometrical parameters of epididymis in controls and male mice treated with *A. precatorius* extract for 20 days duration.

Group	Caput tubule diameter (μm)	Caput epithelial height (μm)	Corpus tubule diameter (μm)	Corpus epithelial height (μm)	Cauda tubule diameter (μm)	Cauda epithelial height (μm)
Control	99.68 \pm 1.49	23.56 \pm 0.50	141.50 \pm 1.74	20.20 \pm 0.40	155.11 \pm 3.40	21.00 \pm 0.46
20 mg/kg	99.41 \pm 1.12	23.20 \pm 0.63	139.51 \pm 2.96	18.40 \pm 0.40 ^{a*}	152.50 \pm 3.75	20.44 \pm 0.53
40 mg/kg	91.20 \pm 1.5 ^{a***b***}	23.20 \pm 0.49	135.80 \pm 4.40	18.16 \pm 0.43 ^{a**}	149.60 \pm 3.10	20.40 \pm 0.76
60 mg/kg	90.40 \pm 0.73 ^{a***c***}	22.44 \pm 0.56	129.50 \pm 4.61	17.80 \pm 0.37 ^{a***}	144.60 \pm 2.10	20.32 \pm 0.51

Values (Mean \pm SEM) a = Controls vs 20 mg /kg, 40 mg/kg and 60 mg/kg. b = 20 vs 40 mg/kg, c = 20 vs 60 mg/kg, d = 40vs 60 mg/kg. *P < 0.05, **P < 0.01, ***P < 0.001

**Figure 1.** Tunical thickness (a) control, (b) extract treated group 20 mg/kg., (c) 40 mg/kg., (d) 60 mg/kg., Tunica albuginea (TA), seminiferous tubule epithelium (E).**Figure 2.** Seminiferous tubule (ST) arrangement in (a) control, (b) extract treated group 20 mg/kg., (c) 40 mg/kg., (d) 60 mg/kg.

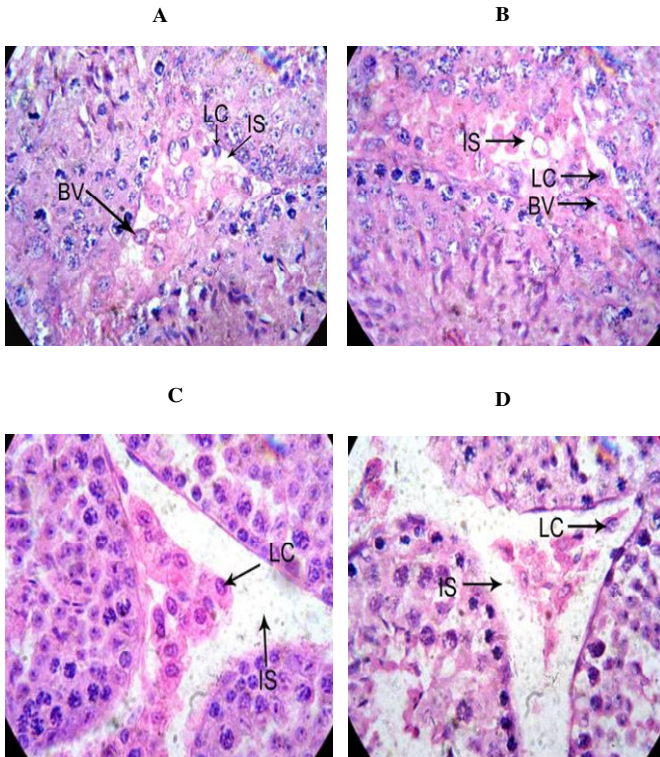


Figure 3. Interstitial space between seminiferous tubules in (a) control., (b) extract treated group 20 mg/kg., (c) 40 mg/kg., (d) 60 mg/kg. Leydig cells (LC). Interstitial spaces (IS).

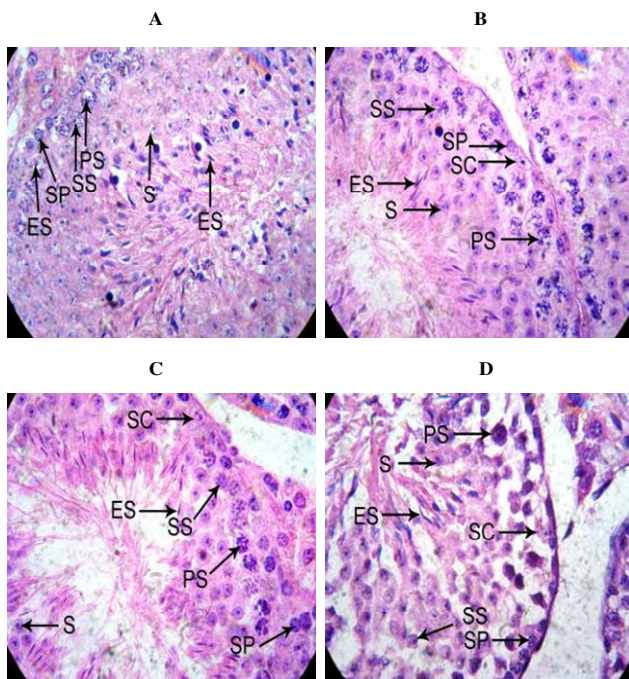


Figure 4. Different stages of spermatogenesis in seminiferous tubules (a) control., (b) extract treated group 20 mg/kg., (c) 40 mg/kg., (d) 60 mg/kg. Spermatogonia (SP), Primary spermatocytes (PS), secondary spermatocytes (SS), Elongated spermatids (ES), spermatozoa (S), Sertoli cells (SC).

thickness compared to controls (Figure 1b, c, d). Leydig cell number and size was smaller compared to controls (Figure 3b, c.). Tubular epithelial height in treated mice was short in comparison to controls. Majority of the tubules possessed large lumen and was filled with detached spermatogenic cells (Figure 4c, d). Sertoli cells were observed in the seminiferous epithelium but their number and size was comparatively shorter than in controls (Figure 4 c, d).

Treatment caused a significant reduction in caput tubule diameter in 60 mg/kg ($P < 0.001$), 40 mg/kg ($P < 0.001$), groups compared to controls. The decrease in mean caput tubule diameter was more pronounced only at high dose levels. The comparison of treatment groups 60 mg/kg and 40 mg/kg with 20 mg/kg also exhibited a significant decrease ($P < 0.001$) in mean tubule diameter. There was a non-significant ($P > 0.05$) decrease in caput epithelial height in the three *A. precatorius* treated groups compared to controls as well as comparison within the groups. Non-significant ($P > 0.05$) decrease in corpus tubule diameter was observed in the three *A. precatorius* treated groups compared to controls and within the group. Whereas the comparison of treatment groups with controls exhibited a significant decrease ($P > 0.05$) in mean corpus epithelial height.

The study of cross sections of controls epididymis revealed that the majority of the tubules of ductus epididymis were compactly arranged, filled with sperm and were lined with pseudostratified epithelium consisting of principal cells and basal cells (Figure 5a). The principal cells appeared lined with stereocilia, the long branching microvilli. Tubules were small, rounded and filled with sperm in the caput portion of the epididymis whereas the large sized tubules were seen in corpus, also compactly arranged (Figure 6a) and nearly all were filled with sperm (Figure 6a). The Cauda portion had the largest tubule size, exhibiting an irregular contour. The extract treatment caused a dose dependent impairment of epididymis of the three extract treated groups compared to controls. In some tubules of caput and all the tubules of corpus segments of high dose treated mice disorganized epithelium (Figure 6b, c and d) vacuolation and reduction in the number of sperm in enlarged lumen was evident. In corpus epithelium of the high dose treated mice, the tubular epithelium had undergone dysplasia (Figure 6c, d). Where as epididymal tubules in low dose (20 mg/kg) mice were least affected (Figure 6b) the structural orientation of corpus portion of the low dose group mice was slightly affected (Figure 6b). The histology features of cauda epididymis were not affected in treated mice, except that there occurred a non-significant decrease in the tubular diameter and tubular epithelial height as compared to controls (Figure 7a, b, c, d).

DISCUSSION

A. precatorius ethanolic extract treatment in mice did not

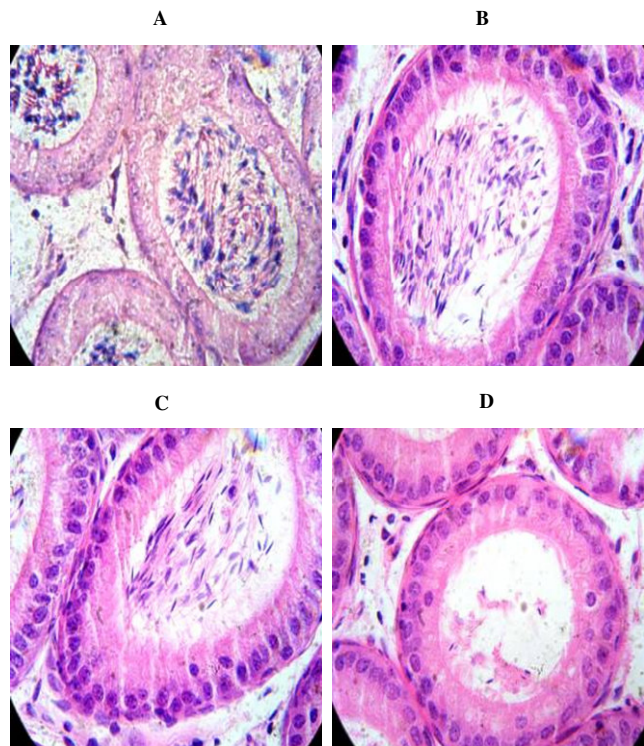


Figure 5. Caput region in the epididymis of adult mice showing in (a) control, (b) extract treated group 20 mg/kg., (c) 40 mg/kg., (d) 60 mg/kg; After treatment for 20 days.

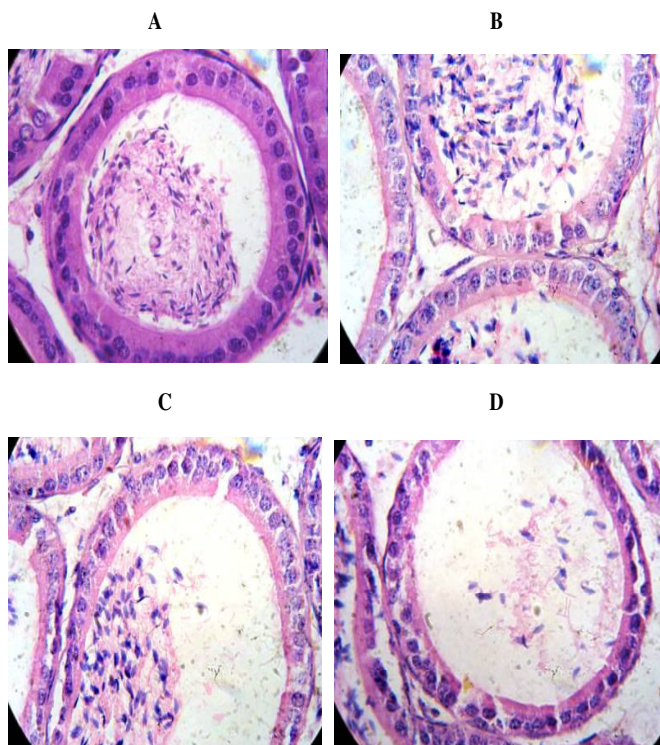


Figure 6. Corpus region in the epididymis of adult mice showing in (a) control., (b) extract treated group 20 mg/kg., (c) 40 mg/kg., (d) 60 mg/kg.

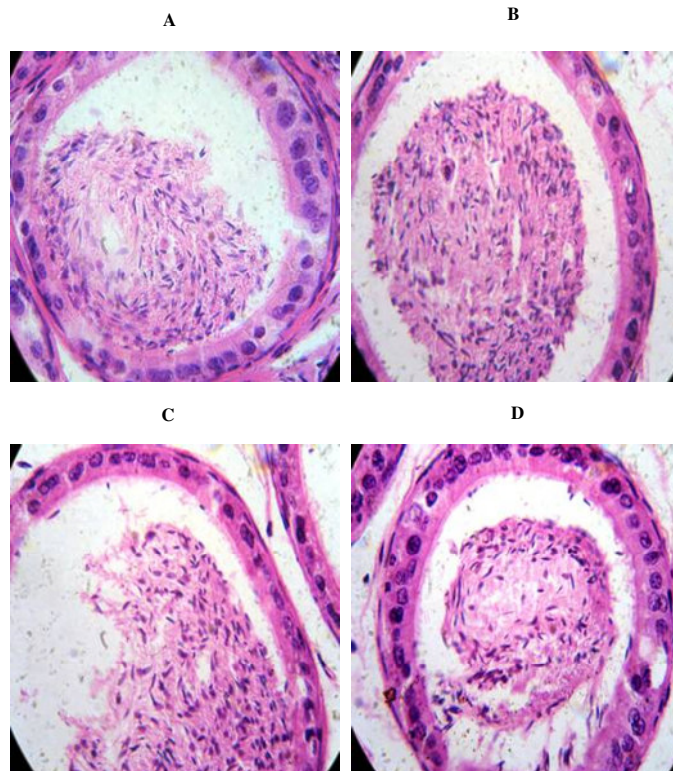


Figure 7. Cauda region in the epididymis of adult mice showing in (a) control., (b) extract treated group 20 mg/kg., (c) 40 mg/kg., (d) 60 mg/kg.

cause significant alterations in the testicular and epididymal weights but caused marked degenerative changes in their tubules. Previous findings also described the anti-fertility effect of aqueous seed extract of *A. precatorius* in rats, without a significant change in body and organ weight (Rao, 1987) which is in support of the present study. No effect on weight indicates that extract did not promote weight gain causing obesity and/ or water and electrolyte retention. In Parkes mice, marked degenerative changes in the seminiferous tubules without a significant change in testis weight were noticed, after treatment with gossypol tetra-acetic acid (Singh and Rath, 1990) aqueous leaf extract of *Azadirachta indica* (Mishra and Singh, 2005) and oral administration of *Bacopa monnieri* (Singh and Singh, 2008). A slight, non significant decrease in weights of testis and epididymis of mice at 60 mg/kg level could be attributed to a loss of spermatogenic elements in testis and absence of sperm in the epididymis. In Parkes mice, there were marked degenerative changes in the testis and epididymal tubules without a significant change in respective organ weight (Singh and Singh, 2008).

The histological changes were more pronounced at high doses. Examination of testes of male mice treated with high doses (40 and 60 mg/kg/day) of extract mainly revealed disruption in the arrangement of seminiferous

tubules and very low Leydig cell count. Low Leydig cell number was also noticed (Nabil and Ziyad, 2005) in mice on treatment with aqueous extract of *Ruta graveolens*. Similar histologic changes have also been reported in testes of Parkes mice after treatment with aqueous leaf extract of *A. indica* and other anti-spermatogenic agents such as gossypol tetra-acetic acid (Singh and Rath, 1990). In high dose groups, the seminiferous tubules were lined by only few necrotic germ cells with scattered Sertoli cells which suggest that the treatment caused alterations in kinetics of spermatogenesis.

The reduction in the number of spermatozoa in *A. precatorius*-treated mice appeared to be due to the suppressive effect of the treatment on spermatogenesis, as it appears to possess a strong compound or principle mainly toxalbumin, that decreases fertility mainly by decreasing the circulating androgen levels. A significant decrease was noticed in serum testosterone level particularly at high dose (60 mg/kg) compared to controls and low doses. It appears to be due to very low Leydig cell count in (60 mg/kg) which seems to be a probable cause for testicular and epididymal function as a result of androgen deprived effect. Hence sperm production and maturation process in both respective organs can be severely affected leading to the loss of fertility in treated mice. There are also reports that describe the anti-fertility effects of alcoholic seed extract of *A. precatorius* in rats as it registers a significant reduction in serum testosterone levels that leads to functional sterility (Bhatt et al., 2007). In present study at 40 mg/kg dose marked degenerative changes in testicular and epididymal structures but no significant decrease in serum level of testosterone compared to controls suggests that it might be possible that the abrin may have a direct damaging action on the tubules. Intra vascular administration of Neem oil resulted in a block of spermatogenesis without affecting testosterone production (Upadhyay et al., 1993). Depletion of Sertoli cells at high doses indicate that it might be possible that the anti-spermatogenic action of *A. precatorius* extract in mouse testis is mediated via Sertoli cells but is not clear from the present study. It is well known that Sertoli cells play a major role in sustenance of spermatogenesis and that any damage to these cells would result in arrest of the spermatogenic process.

Histomorphology of the three segments of epididymis in high dose treated mice was affected after treatment involving a decrease in tubular size, epithelial heights and reduction in the number of sperm in the lumen compared to controls. Epididymal corpus segment of the high dose treated groups was most adversely affected as its tubular epithelium had undergone disorganization and intraepithelial vacuolation along with decreased sperm count. The mechanism of seed extract was probably via altering the hormone levels and causing androgen deprived effect to the target organ and *A. precatorius* seed extracts are known to contain abrin, an amino acid derivative which may generate a reactive oxygen species

thus inducing lipid peroxidation of the membranes and causes disruption in the form of intraepithelial vacuolation.

Similar observation was obtained in *Abrus* seed extract fed to rats (Sinha and Mathur, 1990). In the present study the changes in the epididymal corpus segment in a high dose groups was more pronounced but cauda remained unaffected. Similar observations are also reported that cauda portion of epididymis presents nearly normal histological features in mice treated with neem oil and Brahmi but cause marked alterations in caput and corpus portion of epididymis (Dehghan et al., 2006; Singh and Singh, 2008).

The present results suggest that parental *A. precatorius* extract treatment of 40 and 60 mg/kg/ by weight employed in the study causes impairment of testicular and epididymal structures. Significant decrease in spermatogenesis activity in seminiferous tubules and disorganized corpus epithelium accompanied by the reduction in the number of sperm in tubular lumen indicates that the action of seed extract is via action of its strong toxalbumen; abrin which causes depletion of Leydig cells in tubular interstitial thus reducing serum testosterone level and testicular and epididymal dysfunction might be due to this androgen deprived effect. Hence sperm production and maturation process in both respective organs is affected by the extract administration leading to the loss of fertility in treated mice without toxic symptoms.

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