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Research Article

Evaluation of Antifertility Potential of *Ficus bengalensis* (Linn.) in Male Albino Mice

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ABSTRACT
The field of herbal contraceptives is getting popularized due to being cheap, easily available, natural with higher safety margins and lesser or no side-effects, and also for protection of privacy. Therefore, the search for an orally active, safe, and effective herbal contraceptive has become a matter of great interest in recent years. The present study was carried out to evaluate the antifertility potential of *Ficus bengalensis* (Linn.) in male albino mice after oral administration of 50% ethanolic leaf extract at 200 and 500 mg/kg body weight for 35 days. Testes in extract-treated mice showed degenerative histological alterations in seminiferous tubules, which more severe in those treated with *Ficus bengalensis* leaf extract at 500 mg/kg body weight than in those treated at 200 mg/kg body weight for 35 days. Further, sperm parameters were also adversely affected in extract-treated mice in a dose-dependent manner compared to controls. Treatment with 50% ethanolic leaf extract of *Ficus bengalensis* had no impact on libido, though, fertility of males treated at 500 mg/kg body weight for 35 days reduced significantly due to decreased number of live implants in impregnated females by pre-implantation loss. No significant alterations were observed in body weight, normal histoarchitecture of liver, kidney, adrenal gland and spleen, and in serum levels of alanine aminotransferase, aspartate aminotransferase and creatinine in extract-treated mice compared to controls. The results of the present investigation suggest that treatment with *Ficus bengalensis* causes suppression of spermatogenesis, sperm functions and fertility in male albino mice in a dose-dependent manner without any apparent toxic effects.
Keywords: *Ficus bengalensis*; Testis; **S**permatogenesis; Sperm motility; Libido

INTRODUCTION

The field of herbal contraceptives is getting popularized in both underdeveloped and developing countries due to being cheap, easily available, natural with higher safety margins and lesser or no side-effects, and also for protection of privacy [1]. Therefore, the search for an orally active, safe, and effective herbal contraceptive has become a matter of great interest in recent years [2,3,4]. A number of medicinal plants *viz. Azadiracta indica*, *Carica papaya*, *Hibiscus rosa-sinensis*, *Piper longum*, *Abrus precatorius*, *Aegele marmelos*, *Albizzia lebbeck*, *Barleria prionitis*, *Cassia fistula*, *Terminalia chebula*, *Ficus bengalensis etc*. have been investigated [5,6,7,8] and are known to be used for the purpose of fertility regulation both by male and female since long time as mentioned in ancient literature.

Ficus bengalensis (Linn.) frequently called as 'Banayan tree' is a well known and reputed laticiferous tree of the family *Moraceae*. It is found in the Sub-Himalayan tract and Peninsular India. In the traditional system of medicine, almost all parts of this plant are used in one or other way to cure various health problems and diseases. The leaves are used to cure ulcer, aerial roots are used to treat gonorrhea and seeds and fruits are used as tonic. The bark from stem and root, leaves and latex are commonly used in medicines to cure nervous disorders, increased blood sugar, hemorrhage, leucorrhoea, menorrhagia, diarrhea, and dysentery [10]. Various extracts prepared from the bark of *F. bengalensis* show anthelmintic [11], anti-inflammatory [12], anti-stress and anti-allergic [13] properties. The aqueous extract of bark of *F. bengalensis* has antidiabetic [14], hypocholesterolaemic and hypolipidaemic effects in rats [15]. However, antifertility potential of *F. bengalensis* is poorly studied except a few works where treatment with methanolic extract of bark at 250 mg/kg body weight for 21 days has been shown to cause histological alterations in the structures of ovary and uterus [16]. Further, there are no reports on antifertility potential of *F. bengalensis* in males. In view of the fact that the plants may play an important role in regulation of fertility, the present study was carried out to evaluate antifertility potential of *F. bengalensis* in male albino mice after chronic administration of 50% ethanolic leaf extract at 500 mg/kg body weight for 35 days.

MATERIAL AND METHODS

Plant material and preparation of extract

F. bengalensis was first identified by an expert in Taxonomy and a voucher herbarium specimen was deposited in the Herbarium at Department of Botany for future record. Fresh leaves of *F. bengalensis* were collected from local area. The leaf extract of *F. bengalensis* was prepared according to WHO protocols [17,18].The leaves were washed properly with distilled water, shade dried for one week, and then grounded into fine powder using an electric grinder. The powdered leaf material (100 g) was extracted with 50% ethanolic water (2000 ml, w/v 1:20) for 8 hours by a soxhlate apparatus. The extract was then filtered with a Watman filter paper and the filtrate, thus obtained, was concentrated in an oven at 40^0C to get a blackish extract (20 g) that was stored at 4^0C for future use [8].

Animals and treatments

Fifteen adult (age 12-14 weeks) male and female mice of proven fertility weighing 30-34 g were used in the investigations. Animals were breed in an animal room under standard conditions (temperature 23±2^0C and 12 hours photoperiod with proper ventilation and 40-60% humidity) in polypropylene cages having dry rice husk as

the bedding material, following the guidelines of Laboratory Animal Care as provided by NIH [19]**.** Animals were given pelleted feed and fresh drinking tap water *ad libitum*. Mice were randomly allocated into three groups, each comprising of five animals, and were treated as follows:

Group I: Distilled water (0.5 ml/100g body weight) for 35 days;
Group II: *F. bengalensis* leaf extract (200 mg/kg body weight) for 35 days and
Group III: *F. bengalensis* leaf extract (200 mg/kg body weight) for 35 days.

F. bengalensis leaf extract was given orally, using an oral feeding needle, at 8:00 AM for 35 days. At the end of the treatment on day 36, animals in groups I, II and III were partially anaesthetized using diethyl ether and sacrificed by decapitation after recording their final body weights. Fresh blood was collected after decapitation and serum was obtained by allowing the blood to clot at room temperature for 30 minutes followed by centrifugation at 3,000 rpm for 20 minutes. The serum was stored at -20^0 C which was later used for estimations of alanine aminotransferase, aspartate aminotransferase and creatinine.

Histopathological study

For histopathological studies, testis, epididymis, a portion of liver, kidney, adrenal gland, and spleen were dissected out immediately after sacrificing the animal, blotted free of blood and fixed in freshly prepared aqueous Bouin's fluid for 3 hours. The tissues were then dehydrated in graded ethanol series, cleared in benzene, and embedded in paraffin wax (60-62^{0}C). The tissues were sectioned at 6µm, and sections were stained with periodic acid-Schiff (PAS) as a cytoplasmic stain and counter stained with Harris haematoxylin as a nuclear stain. The stained sections were examined under a Leitz (Germany) light microscope for qualitative and quantitative alterations. Under qualitative study, histological alterations occurred in seminiferous tubules of testis and in epididymis were studied. For qualitative analysis of testis, seminiferous tubules were identified as affected and normal ones. The affected seminiferous tubules were further categorized into degenerating and atrophic tubules depending upon the severity of the alterations. Quantitative alterations in testis were analyzed by counting the number of affected seminiferous tubules. Histological alterations in the seminiferous tubules were studied and described according to criteria given by Russell, Ettlin, Sinha Hikim, and Clegg [20].

For histopathological study, the epididymis was divided into five (I-V) segments. Segments I-III constituted the head (caput); segment IV, the body (corpus); and segment V, the tail (cauda) of the epididymis [21].

Sperm function tests

For sperm analyses, cauda epididymidis was taken out randomly from left or right sides of each of five animals in each group, and placed in a watch glass containing 0.5 ml of 0.9% normal saline maintained at 37^{0}C on a hot plate [8]. The tissue was then minced carefully to ensure complete extrusion of sperm from the cauda epididymidis. The tissue debris was removed and the sperm suspension was used for analyses of motility, viability, and number of sperm according to WHO protocol [22].

Biochemical tests

Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured [23] to determine the functional status of liver. Further, serum level of creatinine was measured using a commercial kit (Span Diagnostics Ltd., Surat, India) to determine the functional status of kidney.

Table 1 Effect of 50% ethanolic leaf extract of *Ficus bengalensis* on body weight and serum levels of ALT, AST and creatinine in male mice.

Groups /Treatments	Body weight (g)		ALT (U/L)	AST (U/L)	Creatinine (mg/100 ml)
	initial	final			
I Distilled water	31.2 ± 0.48	32.0 ± 0.31	23.45 ± 2.56	26.72 ± 0.17	1.55 ±0.18
II 200 mg/kg	31.2 ± 0.48	31.6 ± 0.50	20.45 ± 2.93	24.51 ± 0.86	1.59 ±0.06
III 500 mg/kg	30.8 ± 0.48	30.4 ± 0.24	31.07 ± 6.08	24.51 ± 3.11	1.99 ±0.10

Values are Mean ± SEM for five animals.

Fertility tests

Fifteen adult (age 12-14 weeks) male and female mice (Groups I, II & III) were employed in the fertility tests. The fertility of control and extract-treated (*F. bengalensis* leaf extract at 200 and 500 mg/kg body weight for 35 days) males was tested on day 36, at 24 hour after cessation of the treatment. This was done by allowing each male to cohabit with one coeval, virgin female of proven fertility in proestrus phase for overnight. Successful mating was confirmed by presence of vaginal plug in the mated female. Further, it also indicates that libido is not affected. After 12/13 days of mating, the females were autopsied to record the total number of implantation (live, dead, and resorption) and corpora lutea in both the uteri and ovaries respectively. The males were considered fertile if the females impregnated by them showed live implants. The resorption sites were counted be putting the uterus in 10% ammonium sulphide solution [24]. Fertility parameters such as libido in treated males, pre- and post-implantation losses and number of live implants in impregnated females were determined.

$$\text{Index of libido} = \frac{\text{Number mated}}{\text{Number paired}} \times 100$$

$$\text{Pre} - \text{implantation loss} = \frac{\text{Total no. of corpora lutea} - \text{total no. of implantations}}{\text{Total no. of corpora lutea}} \times 100$$

$$\text{Pre} - \text{implantation loss} = \frac{\text{Total no. of implantations} - \text{total no. of viable implantations}}{\text{Total no. of implantations}} \times 100$$

Statistical Analyses

All data, except those of body weight, were analyzed by one-way analysis of variance (ANOVA), followed by

Neuman-Keuls' multiple range test. Data on body weight were analyzed by Student's t-test. Values were expressed as Mean ± SEM. Results were considered significant at $p< 0.05$ level.

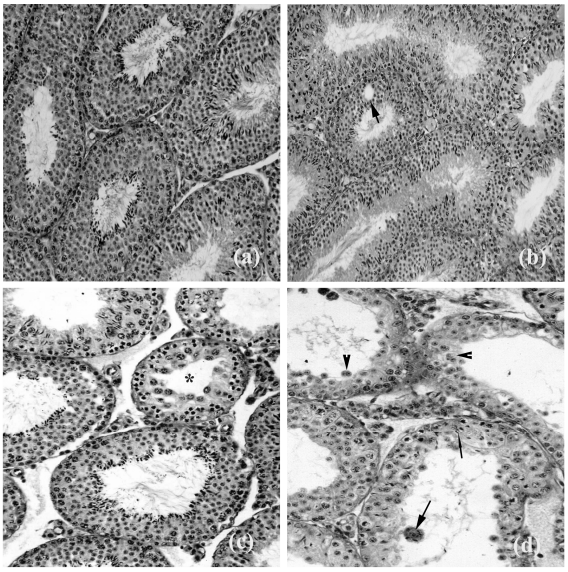

Figure 1 PAS-H stained sections of mouse testis (Magnifications: a-c: X 200; e: X 252). (a) Control to show normal spermatogenesis. (b) After treatment with *F. bengalensis* leaf extract (200 mg/kg body weight for 35 days) to show intraepithelial vacuolation (arrow). (c) After the same treatment as in figure b to show presence of an affected tubule (asterisk) (d) After treatment with *F. bengalensis* leaf extract (500 mg/kg body weight for 35 days) to show multinucleated giant cells containing round (arrow heads) or containing elongated spermatids (arrow). Note the active phagocytosis (small arrow) by Sertoli cells.

RESULTS

Histopathological observations

Histological observations of testes in distilled water-treated controls showed normal spermatogenesis (Figure 1 a) in nearly all the seminiferous tubules except in a few (see Table 2). On the other hand, obvious alterations were noticed in normal histoarchitecture of testes in mice treated with 50% ethanolic leaf extract of *F. bengalensis* at 200 and 500 mg/kg body weight for 35 days (Figures 1 b-d). In general, affected tubules in testes of extract-treated mice showed intraepithelial vacuolation, exfoliation of germ cells, phagocytosis of elongated spermatids and presence of spermatids of different stages of spermatogenic cycle in the same tubule (Figures 1 b-c). Alterations induced in testis were non-uniform as both affected and normal seminiferous tubules were observed in the same testis sections (Figure 1 c). Further, individual differences were also evident as some animals showed more severe alterations in seminiferous tubules than others in same treated groups (Groups II and III). Furthermore, treatment with 50% ethanolic leaf extract of *F. bengalensis* resulted in a dose-dependent degeneration in the seminiferous tubules, since mice treated at 500 mg/kg body weight (Group III) showed more severe histological alterations in their testes than those treated at 200 mg/kg body weight (Group II) for 35 days. The testes in mice treated at 500 mg/kg body weight for 35 days (Group III) often showed formation of multinucleated giant cells containing round or elongated spermatids in seminiferous tubules (Figure 1 d). When quantitatively analyzed, testes in *F. bengalensis* extract-treated mice showed a significant ($p<0.05$) increase in frequency of affected seminiferous tubules compared to controls (Table 2). Further, frequency of affected seminiferous tubules was significantly higher in testes of mice treated with *F. bengalensis* leaf extract at 500 mg/kg body weight (Group III) than in those treated at 200 mg/kg body weight (Group II) for 35 days (Table 2).

Table 2 Effect of 50% ethanolic leaf extract of *Ficus bengalensis* on motility, viability and number of sperm in caudae epididymidis, and on frequency of affected seminiferous tubules in testes of male mice.

Groups / Treatments	Sperm parameters			Frequency of affected seminiferous tubules (%)
	Motility (%)	Viability (%)	Count (X 10^6/ml)	
I Distilled water	85.35 ±1.61	83.61 ±1.33	17.35 ±0.74	10.40 ±0.50
II 200 mg/kg	74.25* ±2.37	71.34* ±1.61	12.07* ±0.48	30.54* ±2.54
III 500 mg/kg	49.92[a] ±5.60	65.13[a] ±1.37	8.23[a] ±0.76	45.59[a] ±5.45

Values are Mean ± SEM for five animals;
*significantly different from controls ($p<0.05$);
[a]significantly different from controls and those in group II ($p<0.05$) by ANOVA followed by Newman-Keuls' multiple range test.

In control mice (Groups I), epididymes exhibited normal histological features, *viz*. segment I showed long columnar epithelial cells and a narrow lumen with rare spermatozoa and no PAS-positive material; segment II showed PAS-positive epithelial cells and a few spermatozoa in the lumen; segment III showed a few spermatozoa and PAS-positive material in the lumen; and segments IV and V showed a lumen distended with spermatozoa and a little PAS-positive material and no PAS-positive inclusion was observed in principal cells of segment IV of epididymes in control mice (Figures not given). However, epididymes in *F. bengalensis* leaf extract-treated mice in groups II and III showed presence of relatively less sperm or sperm fragments with accumulation of PAS-positive material in lumen of segments IV and V. of Further, segment IV of epididymes in two mice treated with *F. bengalensis* leaf extract at 500 mg/kg body weight for 35 days showed distinct PAS-positive inclusions in epithelial cells and absence of sperm in the lumen (Figures not given).

Sperm parameters

Significant ($p<0.05$) reductions were noticed in motility, viability, and number of sperm in caudae epididymidis of *F. bengalensis* leaf extract-treated mice (Groups II and III) compared to controls (Table 2). Further, reductions in sperm motility, viability and number were significantly higher in mice treated at 500 mg/kg body weight (Group III) than in those treated at 200 mg/kg body weight (Group II) with *F. bengalensis* leaf extract for 35 days (Table 2).

Table 3 Effect of 50% ethanolic leaf extract of *Ficus bengalensis* on libido of treated males and on pre- and post-implantation losses and number of live implants in impregnated females.

Groups	Number of males			Number of females			Index of libido (%)	Pre-implantation loss (%)	Post-implantation loss (%)	Number of live implants
	T	M	F	T	M	P				
I Distilled water	5	5	5	5	5	5	100	3.63 ± 3.63	7.93 ± 5.58	8.20 ± 0.96
II 200 mg/kg	5	5	0	5	5	0	100	16.00 ± 7.48	14.00 ± 5.78	6.4 ± 0.39
III 500 mg/kg	5	5	4	5	5	4	100	66.00* ± 10.76	6.66 ± 6.66	2.80* ± 0.79

Values are Mean ± SEM for five animals;
*significantly different from controls ($p<0.05$) by ANOVA followed by Newman-Keuls' multiple range test. T- Tested; M-Mated; F- Fertile; and P- Pregnant

Toxicological observations

Treatment with 50% ethanolic leaf extract of *F. bengalensis* did not cause any significant ($p<0.05$) alterations in body weight and in general behavior of extract-treated mice compared to controls (Table 1). No significant differences were found in serum levels of AST, ALT and creatinine in *F. bengalensis* extract-treated mice compared to controls (Table 1). Further, no histopathological alterations were observed in liver, kidney, adrenal gland and spleen in extract-treated mice compared to controls (Figures not given).

Fertility tests

Libido was not affected in *F. bengalensis* leaf extract-treated males (Groups II and III) at 24 hour after cessation of the treatment. However, fertility of males treated with *F. bengalensis* leaf extract at 500 mg/kg body weight for 35 days reduced significantly since impregnated females showed significantly reduced number of live implants at autopsy (Table 3). Pre-implantation loss was significantly high in the females impregnated by males treated with *F. bengalensis* leaf extract at 500 mg/kg body weight for 35 days compared to controls (Table 3). Further, post-implantation loss was not significant in the females impregnated by extract-treated males compared to controls (Table 3).

DISCUSSION

The results of the present study show that treatment with 50% ethanolic leaf extract of *F. bengalensis* at 200 and 500 mg/kg body weight for 35 days resulted in degenerative histological alterations in seminiferous tubules in testes of extract-treated mice. The histological alterations were non-uniform as both affected and normal seminiferous tubules were observed in the same testis sections. It has been suggested that focal damages in testis occur because seminiferous tubules in certain stages of spermatogenesis are more prone to damage by various treatments than the other [20]. The affected tubules in testes of extract-treated mice, in general, showed intraepithelial vacuolation, exfoliation of germ cells, phagocytosis of elongated spermatids and presence of spermatids of different stages of spermatogenic cycle in the same tubule. Similar histological alterations, as induced in testes of extract-treated mice in the present study, have also been observed in testes of mice treated with 50% ethanolic root extract of *Martynia annua* [25], hydroalcoholic extract of *Achillea santolina* [26] and ethanolic bark extracts of *Terminalia chebula* [6]. Further, treatment with 50% ethanolic leaf extract of *F. bengalensis* resulted in a dose-dependent degeneration in the seminiferous tubules, since mice treated at 500 mg/kg body weight showed more severe histological alterations in their testes than those treated at 200 mg/kg body weight for 35 days. The seminiferous tubules in testes of mice treated with *F. bengalensis* leaf extract at 500 mg/kg body weight for 35 days often showed formation of multinucleated giant cells containing round or elongated spermatids which has been also observed after treatment with bark extracts of *Terminalia chebula* [6]. It is reported that germ cells in the seminiferous tubules are connected with each-other by intercellular bridges and any alterations in the intercellular bridges results in the formation of multi-nucleated giant cells by fusion of germ cells [27]. Further, formation of multinucleated giant cells in the seminiferous tubules suggests suppression of spermatogenesis in the testis. The frequency of affected seminiferous tubules was significantly higher in testes of mice treated with *F. bengalensis* leaf extract at 500 mg/kg body weight than in those treated at 200 mg/kg body weight for 35 days. Therefore, it can be speculated from the histological observations that treatment with *F. bengalensis* leaf extract causes suppression of spermatogenesis in testis in a dose-dependent manner.

Extract-treated mice showed accumulation of PAS-positive inclusions in the epithelial cells of segment IV of epididymis. It has been described by Abe and his group [28,29] that principal cells in segment II (caput) secrete PAS-positive material in the lumen which is utilized by sperm during maturation in subsequent segments, and that in absence of sperm, such material is reabsorbed by the principal cells of segment IV (corpus) of epididymis. Thus, presence of PAS-positive inclusions in epithelial cells of segment IV might be because of absence or reduced number of sperm in epididymal lumen which is evident from histological observation and reduced sperm count. It is well known that the structure and function of epididymis are dependent on androgens [30] and testicular fluid is thought to influence the function of the epididymal duct [31]. It can be speculated that *F. bengalensis* leaf extract had no direct effect on the epididymal function and histological degeneration in the epididymis might be due to the suppression of androgen production in the testis. Thus, reduced sperm count in *F. bengalensis* leaf extract-treated mice may be due to the suppression spermatogenesis in the testis, while alterations in sperm motility and viability might have resulted from disturbances in epididymal function due to androgen deprivation [32].

The present study shows that treatment with *F. bengalensis* leaf extract had no impact on libido of males, though, fertility of males treated at 500 mg/kg body weight for 35 days reduced significantly since impregnated females showed reduced number of live implants due to significantly increased pre-implantation loss. The increased pre-implantation loss could be probably the result of severely reduced number, motility and viability of sperm in caudae epididymidis of above treated males. Further,

treatment with *F. bengalensis* leaf extract had no effect on body weight and on general behavior of mice. In addition, absence of histopathological alterations in liver, kidney, adrenal gland and spleen, and in serum levels of ALT, AST and creatinine suggests that the treatment with *F. bengalensis* leaf extract was not associated with any toxic effects.

The present results of the present study in albino mice, thus, suggest that treatment with 50% ethanolic leaf extract of *F. bengalensis* causes suppression of the spermatogenesis in the testis and has adverse effects on sperm functions and fertility in a dose-dependent manner without signs of clinical toxicity.

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