Original Article

Antifertility effect of methanolic extract of *Butea monosperma* (Lam.) Taub. flower in male albino rats

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Abstract

Background: Search for an effective, feasible, and safe male contraceptive has been one of the major public health challenges. The present contraceptive methods are either permanent or impractical. Herbal methods are considered safe, and thus, their acceptability is higher than other prospective methods. **Aims:** In the present study, oral administration of methanolic extract of *Butea monosperma* (Lam.) Taub. flower was investigated for its potential role in the modulation of fertility in male albino rats. **Materials and methods:** Healthy male albino rats were randomly distributed into three groups, i.e., a control and two groups administered with 50 and 500 mg/kg body weight/day of methanolic extract of *B. monosperma* flower for 30, 90, and 180 days, respectively. Fertility records were maintained throughout the experimental period. At the end of experiment, animals were sacrificed and the weight of reproductive organs, sperm characteristics, and histopathology of testicular and epididymal tissues were evaluated. A 45-day withdrawal period was also investigated for parameters as described above for each group. **Results:** A 40% decline in fertility rate was evident in rats administered with 500 mg/kg of *B. monosperma* flower extract for 180 consecutive days. A significant reduction in testicular and epididymal weight was observed in these animals. Sperm count, motility, and viability were also reduced significantly in animals treated for 180 days. Histological evaluation of testicular cells indicated distortions in germ cell arrangements at various stages of spermatogenesis. Following 45 days of withdrawal, the resumption of normal functional and histological characteristics was apparent. **Conclusion:** Based on the abnormalities present in the sperm characteristics and damages in testicular histology, it was confirmed that methanolic extract of *B. monosperma* flower contain antifertility potential.

Keywords: Antifertility, *Butea monosperma*, herbal method, male contraceptive

Introduction

Population growth is leading the world to an inevitable shortage of resources, increased global temperature, and eventual climate change.[1] Besides, unintended pregnancies are also a global concern. According to a study, more than 40% of pregnancies worldwide are unintended.[2] There is an immediate requirement of safe, feasible, and effective contraceptive. Available contraceptives are either not completely safe or lack acceptability in general masses.[3] Although the use of contraceptives has been increased in recent times,[2,4] currently available contraceptive methods are mostly for females, which can be doubled if males are provided with equally convenient options.

Methods for male contraceptive that is currently in use are condoms[5] and vasectomy.[5] Other methods for males are either not effective or reduce sexual pleasure substantially.[6] However, researchers around the globe are working toward the development of feasible and effective male contraceptives which include both hormonal,[7] occlusion[3] and barrier methods.[6] Despite significant progress in these areas, one of the common drawbacks is potential side effects.[8,9]

Plant-based contraceptive methods have been in use for centuries. There are many plants and plant products that have proven antifertility activities in both males[10,11] and females.[12,13] These methods also have great acceptability considering their prices and accepted notion of least side effects. Regardless, there are many cases of side

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effects reported by herbal male contraceptives such as gossypol[14] and *Tripterygium wilfordii*.[15] The present study has explored the antifertility ability of *Butea monosperma* (Lam.) Taub. (family – Fabaceae), a rather unconventional medicinal plant. *B. monosperma* is commonly known as "*Palasha*," and in Ayurvedic texts, it is sometimes referred to as *Raktapushpa*, *Brahmavriksha*, *Kinshuka,* and *Yajniya*. It is classically used in Ayurveda for the treatment of *Maharoga* or *Mahagada* (diseases of great concern) (described in chapter 13 of *Chikitsa Sthana*).[16] However, earlier reports suggested its use for various other medical conditions (such as diabetes, helminths, stress, cirrhosis and inflammation) by general Ayurvedic practitioners.[17] Arguably, flowers of *B. monosperma* have been noted with antifertility activity.[18] Previous studies have noted alteration in reproductive properties[19] and estrogenic and postcoital anticonceptive activity[20] following the administration of *B. monosperma*. However, available reports are not in great details, as these data were either published based on previous assumptions or interpreted based on its activity in female reproductive system. Rastogi and Mehrotra[21] suggested that *B. monosperma* contains bioactive compounds that play an important role in male steroidogenic activity. Therefore, this study postulated that *B. monosperma* has the ability to reduce fertility in male albino rats. Material and method.

Material and methods

Test material

The flower of *B. monosperma* of best quality was obtained commercially (JJWMART, JJW and Brothers Pvt. Ltd, Rajasthan [FSSAI-12217019000070]). Flowers were authenticated at the Department of Botany, Ranchi University, Ranchi. Fresh 250 g of flowers was shade-dried and powdered. The powdered materials were extracted by using methanol as a solvent (5:1, solvent-to-powder ratio). Most of the earlier studies used methanol as a solvent to extract active compounds of *B. monosperma* flowers. The selection of methanol as a solvent was based on a study carried out by Johri *et al*.[22] Methanolic extract of the flower was prepared in Soxhlet apparatus. Fifty grams of powdered flower (whole powder) was taken into thimble of the Soxhlet extractor. Later, the extraction of powdered flowers was conducted at 60°C–80°C. Once extraction was complete, flower extract was separated from methanol in an evaporator. Briefly, the extract was filtered using the Whatman filter paper and concentrated in a rotatory evaporator to obtain the reddish-orange powder. The final yield of reddish-orange powder was 13%. The dried extract was stored in a screwed glass container at −20°C for maximum of 2 weeks.

Test animals

Adult male Wistar albino rats (*Rattus norvegicus*), 3 months old, weighing 150–200 g were used in the present investigation. The animals were maintained in the departmental animal house with 12:12-h light and dark schedule in individual polypropylene cages (size 43 cm × 27 cm × 15 cm). Animals were fed with a rat pellet diet and water *ad libitum*. All animals were maintained under perfect veterinary supervision and in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals.

Ethical clearance

The guidelines of the Indian National Science Academy, New Delhi, for care and the use of animals were followed in all experiments. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC Approval Number – Z/92/February 3, 2015).

Experimental design

The LD_{50} of *B. monosperma* flower (aqueous extract) was estimated to be 3500 mg/kg intraperitoneally, whereas, for acute oral administration, methanolic extract was considered safe up to 6000 mg/kg.[23] According to the Organization for Economic Co-operation and Development (OECD) guideline, the upper oral dose limit of *B. monosperma* (methanolic extract) is 2000 mg/kg. The present study was exclusively carried out on males to investigate the modulation of fertility and spermatogenesis by methanolic extract of *B. monosperma* flower. Adversities at various levels in reproductive system cause infertility; therefore, the selection of doses was based on safety and efficacy. Since disruption of fertility through foreign compound is considered reproductive toxicity, experiments were carried out in accordance with the OECD guidelines for testing chemicals-oral administration-fixed dose procedure-reproductive toxicity.[24,25] Based on the above, considerably safe doses of 50 mg and 500 mg/kg of methanolic extract were used in this study.

Methanolic extract of *B. monosperma* flower was diluted in dimethyl sulfoxide in 1:1 concentration to make stock solution. Experimental design was divided into two phases: (1) treatment phase and (2) recovery phase. During treatment phase, healthy male rats were orally administered (gavage technique) with methanolic extract at corresponding concentrations (i.e., 50 mg and 500 mg/kg body weight/day) using distilled water as vehicle [Table 1]. Investigated doses were administered consecutively for 30 days, 90 days, and 180 days. During recovery phase, animals were withdrawn from daily administration of methanolic extract and kept under observation for 45 days (i.e., 75, 135 and 225 days). At termination of each schedule, a half number of animals from each group were sacrificed for further investigation. The remaining half from each group were examined for another 45 days following withdrawal from treatment and then sacrificed for further study. Animals of the parallel sham control group were administered with distilled water of equal volume. An equivalent number of animals from the control group were sacrificed at termination of each experimental schedule.

Table 1: Experimental groups/subgroups and respective specifications

Experimental groups	Subgroups (n=10)	Specification (a total of 5 animals were sacrificed after termination of days of treatment and the remaining 5 were sacrificed after completion of days of recovery)
Group I	Group Ia	Control-vehicle treated for 30 days + 45 days (normal diet)
	Group Ib	Control-vehicle treated for 90 days + 45 days (normal diet)
	Group Ic	Control-vehicle treated for 180 days + 45 days (normal diet)
Group II	Group IIa	Oral administration of 50 mg/kg of methanolic extract of *B. monosperma* flower for 30 consecutive days + 45 days for recovery (normal diet)
	Group IIb	Oral administration of 50 mg/kg of methanolic extract of *B. monosperma* flower for 90 consecutive days + 45 days for recovery (normal diet)
	Group IIc	Oral administration of 50 mg/kg of methanolic extract of *B. monosperma* flower 180 consecutive days + 45 days for recovery (normal diet)
Group III	Group IIIa	Oral administration of 500 mg/kg of methanolic extract of *B. monosperma* flower for 30 consecutive days + 45 days for recovery (normal diet)
	Group IIIb	Oral administration of 500 mg/kg of methanolic extract of *B. monosperma* flower for 90 consecutive days + 45 days for recovery (normal diet)
	Group IIIc	Oral administration of 500 mg/kg of methanolic extract of *B. monosperma* flower for 180 consecutive days + 45 days for recovery (normal diet)

B. monosperma: Butea monosperma

Parameters

Body and reproductive organs weight

Initial and final body weights were recorded for each group. The final body weight for treatment groups was recorded on the 30th, 90th and 180th days. Whereas, recovery groups were weighed after 45 days of withdrawal (i.e., 75th, 135th and 225th). Likewise, reproductive organs (testes and epididymis) were weighed immediately after euthanization at termination of each experimental schedule.

Spermatozoal characteristics

Following the scheduled termination of experimental groups, spermatozoa were analyzed by chipping off cauda epididymis in 1 ml of normal saline. Sperm count, motility, viability, and abnormality were recorded according to the WHO method manual.[26]

Fertility test

Periodical fertility tests (every 15 days) were carried out throughout the experimental period by cohabitating the male rats with fertile female rats at a 1:2 ratio. The success of mating was confirmed by vaginal plug/appearances of spermatozoa in the vaginal smear. Females were allowed to complete the term and the fertility record was maintained.

Histological analysis

Tissues of epididymis and testis were evaluated for any histological alterations. Tissues were collected after the termination of experimental schedule and fixed in Bouin's fluid for 24 h, dehydrated in ethanol, cleared in xylene, and embedded in paraffin wax. Further 5-µm thin sections were cut and fixed on glass slides followed by staining with Harris's hematoxylin and eosin for light microscopic observations.

Statistical analysis

Results of this study were represented in Mean ± Standard deviation (SD). Student's *t*-test was applied for paired data, whereas analysis of variation among and between multiple data was carried out by one-way analysis of variance in conjunction with Tukey's multiple comparison test. Levels of significance were measured at 0.05 (95% confidence interval [CI]), 0.01 (99% CI) and 0.001 (99.99% CI).

Results

Body and organs weights

Following the administration of 50 mg/kg body weight (group II) of methanolic extract of *B. monosperma* flower, no significant alteration in body weight was observed when compared with control. However, the body weight of animals treated with 500 mg/kg body weight of methanolic extract for 90 days (group IIIb) and 180 days (group IIIc) showed a significant decline. Nonetheless, animals treated with the same dose for 30 days (group IIIa) revealed no alteration in body weight. Following 45 days of recovery, resumption of body weight was recorded in groups IIIb, however, decline in group IIIc remained significant [Table 2].

Organ weight in groups IIIa–IIIc indicated a subsequent decline. Results showed that the administration of 500 mg/kg of methanolic extract significantly reduced the weight of testes and epididymis. However, the same for 50 mg/kg body weight of methanolic extract was nonsignificant. Interestingly, withdrawal from the treatment group indicated the complete resumption of weights of testes and epididymis on the 225th (i.e., 180 + 45 days) day of experiment [Tables 3 and 4].

Cauda epididymis spermatozoal characteristics

Cauda epididymal sperm characteristics indicated no significant alteration in groups IIa and IIb and were found to be within control range. However, group IIc showed a significant decrease in percentage motility and viability in sperm. Percentage abnormality in sperms of group IIc was also increased significantly [Table 4]. Groups IIIa–IIIc showed a significant decline in percentage sperm motility, viability and count, whereas a substantial increase in number of abnormal

Table 2: Body weight (g) of animals treated with methanolic extract of *Butea monosperma* flower extract

Treatment group	Initial	Treatment phase			Recovery phase		
		30 days	90 days	180 days	75 days	135 days	225 days
Group I	175.36±7.51	199.61±14.52	227.87±16.42	255.25±19.25	218.55±14.87	233.18±16.98	291.77±19.63
Group IIa	170.98±6.99	199.15±14.69			215.48±14.73		
Group IIb	179.15±7.45		228.18±16.91			235.09±15.85	
Group IIc	165.21±7.33			251.36±18.13			290.36±19.31
Group IIIa	171.44±6.98	195.11±14.88			219.32±13.54		
Group IIIb	161.73±6.47		210.56±13.45*			228.94±16.31	
Group IIIc	173.59±7.84			235.62±19.36*			265.55±18.42*

*P<0.05 indicates significant variation against Group I. Level of significance was measured against control (Group I)

Table 3: Testis weight (mg/100 g body weight) of animals administered with methanolic extract of *Butea monosperma* flower

Treatment group	Treatment phase					
	30 days		90 days		180 days	
	Left	Right	Left	Right	Left	Right
Group I	422.15±26.95	421.42±26.31	461.28±29.24	461.36±29.89	481.27±32.12	480.59±32.71
Group IIa	419.44±26.12	420.58±25.77				
Group IIb			459.66±29.36	460.47±28.71		
Group IIc					480.59±35.66	480.43±32.88
Group IIIa	401.36±25.48*	401.22±27.15*				
Group IIIb			437±27.19**	438.31±28.33**		
Group IIIc					465.20±31.25**	464.35±32.17**

Treatment group	Recovery phase					
	75 days		135 days		225 days	
	Left	Right	Left	Right	Left	Right
Group I	436.78±28.44	436.47±28.91	471.61±30.86	470.93±30.03	535.51±34.56	535.39±35.13
Group IIa	430.16±28.06	431.75±28.32				
Group IIb			468.19±30.92	468.66±30.23		
Group IIc					530.89±31.37	531.73±35.49
Group IIIa	415.55±28.61*	415.99±28.15*				
Group IIIb			444.36±30.24*	443.74±30.44*		
Group IIIc					519.45±32.89	520.26±35.71

*P<0.05, **P<0.01 indicate significant and highly significant variation against Group I. Level of significance was measured against control (Group I)

sperms was evident in all the three subgroups. Following withdrawal from treatment, groups those indicated alterations in sperm motility, count, viability and abnormality resumed back to control range [Table 5].

Fertility record

All animals during pretreatment phase showed 100% fertility. The percentage fertility of control animals remained 100% throughout the duration of investigation. No alteration in fertility of group IIa was evident; however, a reduction in percentage fertility was observed in groups IIb and IIc. Group IIc showed a reduction in fertility from the 60th day onward. The lowest fertility (70%) was recovered on the 180th day of treatment with methanolic extract. All subgroups of group III showed a significant decline in percentage fertility post the 30th day of administration. Minimum as 60% fertility was observed in group IIIc following 180 days of continuous administration of methanolic extract of *B. monosperma* flower [Table 5]. Interestingly, following withdrawal, complete resumption of fertility was witnessed in all subgroups of group III [Table 6].

Histological analysis

Testis

Histological study of testis of animals in Group I (sham-treated control) showed clear round or oval seminiferous tubules (STs) with the epithelium containing Sertoli cells and germ cells of various stages covering the complete spermatogenesis. Germ cell differentiation appeared normal and the spermatocytes and spermatids were prominent with well-defined nuclei and granular cytoplasm. The interstitium occupied distinct Leydig cells and intertubular elements. The Leydig cell was observed to be round and granular and containing prominent nucleus [Figure 1].

Group II histological architecture of testis showed an appreciable difference comparing to group I. The tubules

Table 4: Epididymis weight (mg/100 g body weight) of animals treated with methanolic extract of *Butea monosperma* flower

Treatment group	Treatment phase					
	30 days		90 days		180 days	
	Left	Right	Left	Right	Left	Right
Group I	76.12±7.29	77.48±7.38	127.56±9.77	127.23±9.81	145.99±11.25	145.61±11.88
Group IIa	76.35±7.33	76.24±7.42				
Group IIb			120.45±9.85	120.37±9.96		
Group IIc					141.58±11.23	140.72±11.46
Group IIIa	69.76±7.03*	69.36±7.11*				
Group IIIb			113.29±9.32*	113.81±9.84*		
Group IIIc					129.40±11.58*	130.55±11.03*
Treatment group	**Recovery phase**					
	75 days		135 days		225 days	
	Left	Right	Left	Right	Left	Right
Group I	82.74±8.13	83.41±8.26	133.85±10.91	134.33±10.28	158.66±12.62	157.59±12.45
Group IIa	83.2±7.92	83.57±8.43				
Group IIb			131.56±10.95	132.94±10.46		
Group IIc					157.10±12.62	157.37±12.81
Group IIIa	80.2±7.92	80.57±8.43				
Group IIIb			125.77±10.44	124.81±10.25		
Group IIIc					149.52±11.99*	149.45±12.17*

*P<0.05 indicates significant variation against Group I. Level of significance was measured against control (Group I)

Table 5: Cauda epididymal sperm characteristics of animals administered with methanolic extract of *Butea monosperma* flower

Treatment Groups	Sperm motility (%)	Sperm count (million/mL)	Sperm viability (%)	Sperm abnormality (%)
Treatment phase				
Group I	73.19±2.61	64.01±5.71	73.27±2.41	20.37±1.51
Group IIa	70.22±3.09	61.44±4.83	68.45±2.24	19.61±1.01
Group IIb	68.45±3.69	61.73±5.09	66.24±1.91	20.43±1.97
Group IIc	53.89±3.80*	59.23±4.83	54.16±2.64*	32.15±2.05*
Group IIIa	55.33±3.27*	51.74±3.98*	49.66±1.24**	41.96±1.14**
Group IIIb	42.76±2.91*	40.04±5.11*	40.36±1.26**	42.69±1.09**
Group IIIc	39.08±2.84**	40.01±6.72*	39.47±2.13**	42.19±1.21**
Recovery phase				
Group I	70.25±3.22	61.41±3.94	74.09±3.45	19.71±1.88
Group IIa	69.40±3.12	62.05±3.99	71.88±2.61	20.01±1.19
Group IIb	68.59±3.51	62.15±4.82	68.46±2.05	19.85±2.05
Group IIc	68.19±3.19	60.25±4.04	69.15±2.01	19.01±1.95
Group IIIa	70.18±3.25	62.05±2.95	72.19±2.15	22.45±2.01
Group IIIb	69.33±3.48	61.35±4.29	69.94±3.05	21.51±1.62
Group IIIc	71.13±2.16	63.25±3.55	71.41±2.37	19.94±1.25

*P<0.05, **P<0.01 indicate significant and highly significant variation against Group I. Level of significance was measured against control (Group I)

showed irregular epithelial characteristics and cytological features of Sertoli cells and germ cells. Spermatids appeared to have disassociated from Sertoli cells, amount of sperm present in the lumen of STs lowered significantly. From 90 days onward, Leydig cell appeared unorganized, showing inconspicuous nuclear and cytoplasmic characteristics [Figure 1]. With increase in the duration of the treatment, the amount of germ line production reduced constantly. Likewise, in group III, histology of testis also revealed a significant difference in comparison to control. The tubules showed greater irregular epithelial characteristics and cytological features of Sertoli cells and germ cells. STs with irregular configuration were inconsistently connected with each other containing thin disoriented basal lamina (BL). Spermatids appeared to have disassociated from Sertoli cells, amount of sperm present in the lumen of STs lowered extensively. In addition, Leydig cells appeared unorganized, showing inconspicuous nuclear and cytoplasmic characteristics [Figure 1]. Longer duration of

Table 6: Percentage fertility of animals administered with methanolic extract of *Butea monosperma* flower

Mating schedule	Percentage fertility						
	Group I	Group IIa	Group IIb	Group IIc	Group IIIa	Group IIIb	Group IIIc
Pretreatment	100	100	100	100	100	100	100
Treatment phase (days)							
15	100	100	100	100	100	100	100
30	100	100	100	100	90	90	90
45	100	-	100	100	-	80	90
60	100	-	100	90	-	70	80
90	100	-	90	80	-	70	70
180	100	-	-	70	-	-	60
Recovery phase (days)							
15	100	100	90	80	90	80	70
30	100	100	100	100	100	90	90
45	100	100	100	100	100	100	100

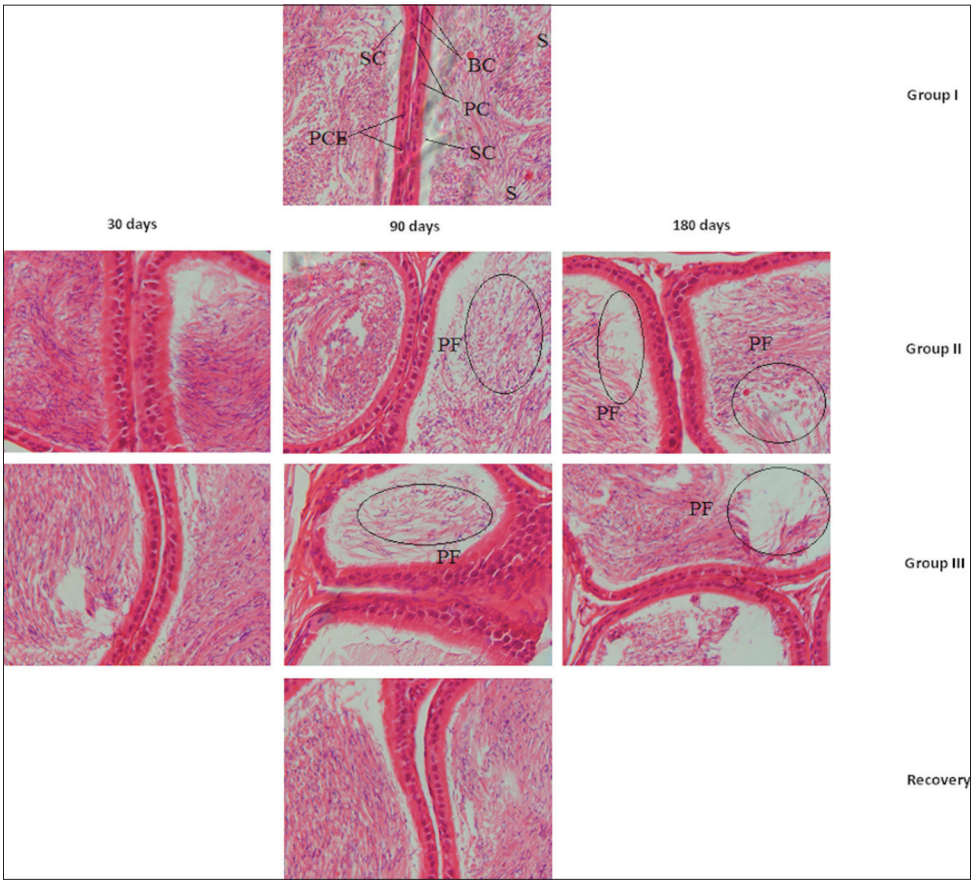

Figure 1: Histological changes in testes of different treatment groups. Group I animals showed round or oval STs with the epithelium containing Sertoli cells (S) and germ cells of various stages covering the complete spermatogenesis. The BL was thick showing closer association with SG and Sertoli cells. Structures of SG were oval in shape, resting on the basement membrane. Both Type A (SG[A]) and Type B (SG[B]) SG were distinguished in pattern. Sertoli cell cytoplasm showed closer association with germ cells and the elongated spermatids (SD), and the lumen contained mature spermatozoa. CT. In Group II and III animals, most of the damages in testis occurred at the 90th and 180th days of administration of *Butea monosperma* flower extracts. Testicular histology showed round or oval STs with the IE containing DSCs and germ cells. Lack of and disorientation of Type A and B SG indicative of incomplete spermatogenesis. The dilated basal lamina (DBL) showing clear disassociation with SG and Sertoli cells. Spermatids appeared to have disassociated from Sertoli cells, amount of sperm present in the lumen of STs lowered extensively. In addition, Leydig cells appeared UL, showing inconspicuous nuclear and cytoplasmic vacuolization (V) characteristics. The amount of damages was higher in Group IIIb (90 days) and IIIc (180 days) animals comparing to other groups. Following withdrawal from treatment, histological architecture resumed back to normal. Nonetheless, the lumens of STs were still partially filled. ST: Seminiferous tubule, BL: Basal lamina, SG: Spermatogonia, CT: Connective tissue, DSC: disoriented Sertoli cell, UL: Unavailable Leydig cells, V: Vacuolization, IE: Irregular epithelium

administration of methanolic extract of *B. monosperma* flower resulted in lower germ line production.

Following withdrawal from treatment, testis depicted normal histological architecture in all test groups (II and III). The epithelium of the STs contained spermatogonia (SG), closely associated with BL, primary and secondary spermatocytes, round and elongated spermatids, and distinct Sertoli cells, lumen contained packed spermatozoa. Leydig cells in the interstitium were conspicuous with distinct nucleus and granular cytoplasm [Figure 1].

Epididymis

Epididymal histological characteristics in group I revealed round, elongated tubules and abundant intertubular elements. The epithelium of the epididymis showed a pseudostratified configuration with prominent basal and principal cells (PCs). The basal cells were short cuboidal relatively less prominent, less granular and also showed distinct elongated nuclei. The PCs were tall columnar and pseudostratified, the nuclei were located at the base, and the cytoplasm appeared granular. The apical cells were characterized by the presence of typical stereocilia and the lumen was packed with spermatozoa [Figure 2].

However, group II indicated no treatment-related variations in epididymal histology. Nonetheless, intertubular elements were abundant and the lumen was partially filled (PF) with spermatozoa. The load of spermatozoa in the lumen appeared to have gradually declined as the duration of administration of extract increased. Similarly, no alteration in epididymal histology was evident in group III. Although the lumen was only partially filled with spermatozoa, it was not related to dose-dependent adverse effect. Some of the lumens were completely vacated, and only traces of sperm were found. Based on the duration of administration of methanolic extract of *B. monosperma* flower, the pool of spermatozoa in the lumen appeared to have significantly declined comparing to control. Following recovery phase (45 days after withdrawal), epididymal histology in test groups (II and III) revealed normal architecture. The lumen was completely filled with spermatozoa signifying normal production of sperm [Figure 2].

Discussion

The use of herbal contraceptive is ancient and the method is considered to be less reactive, thus causing lower side effects comparing to most of the hormonal or obstructive contraceptives. In the last few decades, many herbal male contraceptives have been explored, such as gossypol,[27] *Carica papaya*,[28] *Gendarussa*,[29] and *Daucus carota*.[30] *B. monosperma* (Lam.) Taub. is already being used for the treatment of various ailments and health conditions. The elusive idea of its specific effects on male reproductive organs led us to investigate it further.

Body weight and organ weight can provide substantial information on gross toxicity caused by substances administered in the body. Results of this study showed a significant decline in body weight of rats, administered with 500 mg/kg body weight/day of *B. monosperma* flower extract. This study also showed that the decline in body weight was dependent on the period of administration of *B. monosperma* flower extract. A study by Dixit *et al*.[31] reported similar results. This study also reported a dose-dependent decline in body weight of obese Wistar rats following the administration of bark of *B. monosperma* (Lam.) at doses of concentration 200, 400, and 800 mg/kg body weight.

Changes in organ weight have long been accepted as a sensitive marker for chemically induced changes.[32,33] The size and weight of testis are dependent on sperm production, interstitial fluid, and Sertoli cell.[34] The testicular size is an important marker for semen quality and fertility.[35] Results of this study exclusively revealed that the administration of methanolic extract of *B. monosperma* flower led to a significant decline in testicular weight of animals. Both investigated doses, i.e., 50 mg/kg and 500 mg/kg of methanolic extract of *B. monosperma* flower, appeared to have adverse effect on testes.

There are very limited studies available that have asserted a negative effect of *B. monosperma*. Most of these studies are limited to leaves and seeds of *B. monosperma*. A previous study reported that a high dose of crude *B. monosperma* seed can produce toxic effects.[36] This study showed that 90 days of continuous administration of 800 mg/kg/day of *B. monosperma* seed revealed significant pathophysiological alterations such as fatty acid deposition, tubular hemorrhage, decrease in cellularity, and cellular proliferation in various organs. Interestingly, this study also mentioned a brief reduction of spermatogenesis in the testis, following the administration of *B. monosperma* seed. The weight and size of testes are directly proportional to spermatogenesis.

Epididymis is a highly convoluted duct, and it supports passage to the newly produced spermatozoa from testis to vas deferens. The sperm maturation and storage are considered to be a primary function of epididymis. It is believed that epididymis plays other important roles besides storage and passage to newly synthesized sperms. Earlier studies suggested that the functional activity of sperm is gained during epididymal stay of sperm.[37]

Significant decline in weight of epididymis was observed in animals administered with 500 mg/kg of methanolic extract of *B. monosperma* flower. A previous study by Donga *et al*.[36] reported a reduction in spermatogenesis by administration *B. monosperma* seed. A reduction in weight of epididymis greatly depends on the amount of presence of sperm in the lumen. The present study indicated no alteration in the weight of epididymis. However, a reduction in weight of testes reflects adversities at base level. It was assumed that besides seed of *B. monosperma*, methanolic extract of flower also contains bioactive compounds that interfere with spermatogenesis. Therefore, it could be anticipated that if the study was investigated for a longer duration, a likely decline in the weight of epididymis is inevitable.

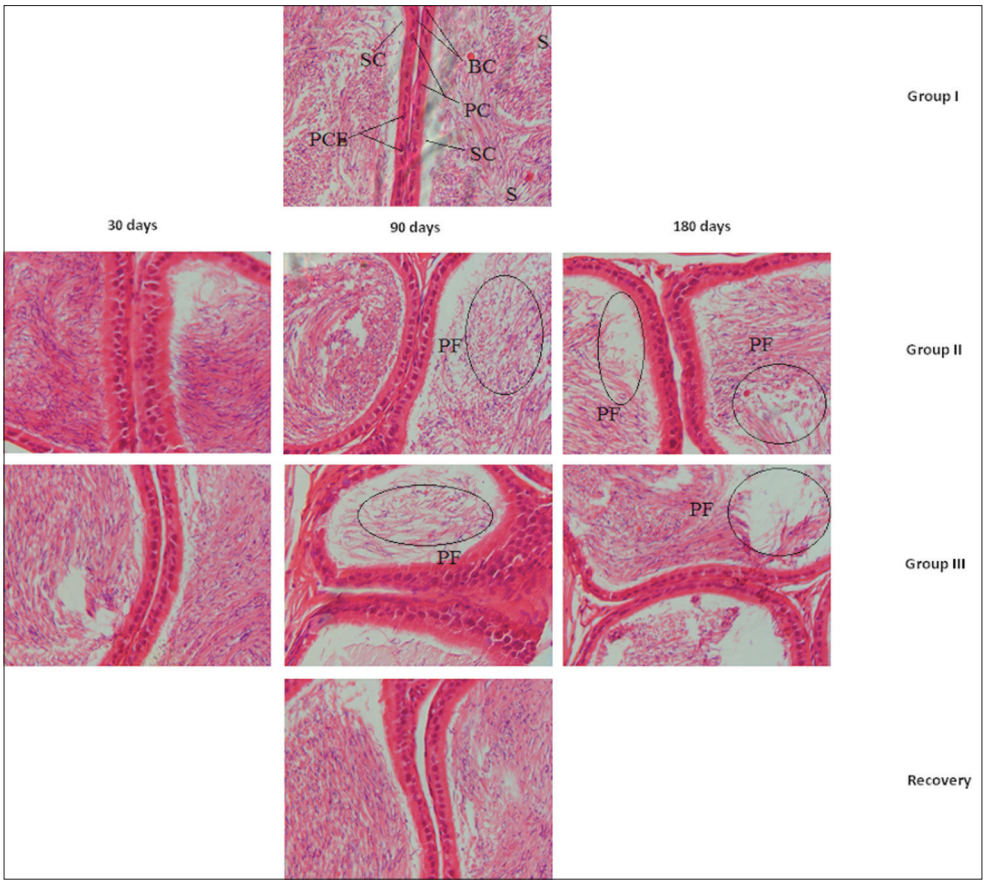

Figure 2: Changes in epididymis of different treatment groups. Group I animals showed round, elongated tubules, abundant intertubular elements, and stereocilia. The epithelium of the epididymis showed pseudostratified columnar epithelium (PCE) configuration with prominent BCs and PCs. The BCs were short cuboidal relatively less prominent, less granular and also showed distinct elongated nuclei. The PCs were tall columnar and pseudostratified, the nuclei were located at the base, and the cytoplasm appeared granular. In Groups II and III, no significant alteration in histological architecture was observed in comparison to control. Nonetheless, the amount of sperm (S) in lumen gradually declined based on duration of treatment with *Butea monosperma* flower extracts. Partially filled lumens were observed in groups of animals treated with maximum doses or longer duration. Normal amount of sperms was present in lumen following withdrawal from treatment. BC: Basal cell, PC: Principal cell

Semen quality is one of the most important markers in male reproductive system to diagnose infertility and associated causes of infertility.[38] In general, the characteristic of a sperm is evaluated based on its count in one single ejaculate, motility, viability, and abnormality. The present study showed dose-dependent effects on sperm characteristics following the administration of methanolic extract of *B. monosperma* flower. Results indicated no adverse effect following the administration of 50 mg/kg methanolic extract of *B. monosperma* flower for 30 days, on sperm characteristics. Remarkably, following 90 and 180 days, a significant decline in sperm characteristics was evident with 50 mg/kg methanolic extract. This observation went in accordance with a study carried out by Vasudeva *et al*.[39] The authors of this study confirmed that administration of 200 mg/kg *B. monosperma* root extract for 21 days, led to irreversible damages in sperm, and considerable reduction in the viability of sperm. Results of the present study affirmatively showed an adverse effect on the sperm characteristics following the administration of 500 mg/kg of methanolic extract of *B. monosperma* flower. A reduction in motility, viability, and the overall count of sperms was also significantly reduced by 500 mg/kg of methanolic extract of *B. monosperma* flower.

Percentage fertility record confirmed observations of sperm characteristics. Low-dose administration of *B. monosperma* flower extract (i.e., 50 mg/kg body weight) had no significant alteration in fertility. However, from the 90th day onward, fertility reduced gradually. High doses (i.e., 500 mg/kg body weight) of flower extract indicated a sustained decline in percentage fertility from the 30th day onward. Earlier studies have reported the antifertility effects of *B. monosperma* extracts. These studies have revealed that 100 mg/kg body weight of *B. monosperma* seed extract suppressed fertility incompletely and without clear dose dependency.[40,41] Based on the present results, it was evident that higher doses of methanolic extract of *B. monosperma* flower are likely to impose an early antifertility response, whereas low dose may take a longer duration to achieve substantial reduction in fertility.

Administration of methanolic extract of *B. monosperma* flower exerted vacuolization and exfoliation of Leydig cells,

specifically when administered for duration longer than 90 days. Severe reduction of sperm in the seminiferous tubules was also evident indicating interference in spermatogenesis at multiple stages. Higher dose for longer duration induced more adverse histo-architectural effects than low dose for shorter duration. A significant decline in spermatogonia and spermatids was evident in animals administered with 500 mg/kg of methanolic extract of *B. monosperma* flower. A study by Donga *et al*.[36] also revealed similar responses with seeds of *B. monosperma*. It reflects that parts of *B. monosperma* contain specific bioactive compounds which have abilities to modulated testicular functions. Overall, the administration of methanolic extract of *B. monosperma* flower may have a significant role in male reproductive system, and 500 mg/kg of methanolic extract can interfere spermatogenesis resulting in high numbers of abnormal sperms and substantially lower sperm count.

Conclusion

Administration of methanolic extract of *B. monosperma* flower has a graded dose-dependent effect on the fertility of male albino rats. Administration of 500 mg/kg of methanolic extract of *B. monosperma* flower indicated a significant reduction in sperm count, viability, and motility. Number of abnormal sperms and disorientation in seminiferous tubules indicated strong adverse effects on spermatogenesis. Based on the results, it was speculated that with higher doses and longer duration, complete sterility is probable. Following recovery phase, the resumption of normal sperm characteristics and histological architecture of testis indicated the reversible antifertility potential of methanolic extract of *B. monosperma* flower. However, more work is required to ascertain this claim.

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Conflicts of interest

There are no conflicts of interest.

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