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Anti-spermatogenic Activity of *Butea monosperma* (Lam.) Kuntze Root

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ABSTRACT

Butea monosperma is a commonly used plant in folk medicine in Indian subcontinent. The present investigation was undertaken to examine the anti-spermatogenic activity of the four successive extracts i.e., petroleum ether, chloroform, methanol and aqueous extracts of the root of *B. monosperma*. The *in vitro* studies on human sperms revealed that the petroleum ether and chloroform extracts at the concentration of 15 mg mL^{-1} caused significant immobilisation and reduction of sperm viability. The extracts caused irreversible damage to the sperms. Further, the effect of these extracts was assessed on the testis and epidydmis of the male albino mice at the dose of 200 mg kg^{-1} body weight. A significant decrease in the weight and changes in fructose, alkaline phosphate, total protein and cholesterol level in these organs of the treated mice when compared to control mice was observed. Histological studies of these organs were also performed.

Key words: Anti-fertility, *Butea monosperma*, fabaceae, spermicidal

INTRODUCTION

Butea monosperma (Lam.) kuntze (Fabaceae) commonly known as 'Palasa' has been referred in Brhatrayi (three big compendia) of Ayurveda by Charaka, Shushtra and Vagbhata as a plant of immense medicinal value (Prashad *et al*., 2006). It is a medium sized deciduous tree, common throughout the greater part of India and Burma, up to 3,000 feet and higher in the outer Himalaya. It also grows in the forests of Oudh and Bundelkhand, Chotanagpur, Central and Southern India (Chopra and Chopra, 1955; Kirtikar and Basu, 1991). Traditionally all the parts of the tree are used in treatment of various disorders. The root cures night blindness and useful as an anti-fertility agent (Kirtikar and Basu, 1991). The bark is an appetiser, aphrodisiac, laxative, anthelmintic and useful in treatment of diseases of anus, dysentery (Prashad *et al*., 2006; Khare, 2007). The leaves are used as strong astringent, antibacterial, tonic and cure for pimples (Kirtikar and Basu, 1991; Purohit and Vyas, 2004). A decoction of flowers is given in diarrhoea and haematuria (Purohit and Vyas, 2004; Pulliah, 2006). The stem bark is used as antifungal (Purohit and Vyas, 2004; Prashad *et al*., 2006; Pulliah, 2006; Khare, 2007). The seeds and flowers of *B. monosperma* are reported to posses a number of flavone glycosides and flavonoids (Gupta *et al*., 1970; Wagner *et al*., 1986; Hayashi *et al*., 1996; Yadava and Tiwari, 2005, 2007), alkaloids (Raj and Kurup, 1968; Mehta and Bokadia, 1981), sterols (Chandra *et al*., 1977; Jain *et al*., 1980). The stem is reported to posses sterols (Mishra *et al*., 2000). The plant is reported to posses antibacterial (Zafar *et al*., 1989; Rani and Kullar, 2004) antifungal (Bandara *et al*., 1989),

anticonvulsive (Kasture *et al*., 2000, 2002) antidiarrhoeal (Gunakkunru *et al*., 2005), wound healing (Sumitra *et al*., 2005) and hepatoprotective activity (Sehrawat *et al*., 2006). Extensive studies have been done on the seeds, flowers and fruit, stem and stem bark of *B. monosperma*. Data on clinical efficacy of root is scanty. The present study is aimed to evaluate the spermicidal activity of the successive extracts of *B. monosperma* roots.

MATERIAL AND METHODS

Collection of the plant and preparation of the extracts: *Butea monosperma* (Lam.) Kuntze was collected from Khejuri, Ballia (U.P.), India. The plant was taxonomically identified and authenticated by Dr. H.B. Singh, Head, Raw Materials Herbarium and Museum Division of National Institute of Science Communication and Information Resources. The voucher specimen has been deposited in the herbarium section of the Pharmacognosy Division, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar for further reference. The roots of *Butea monosperma* (Lam.) Kuntze were dried under shade, sliced into small pieces and pulverized. The powdered root (1 kg) was successively extracted with petroleum ether, chloroform, methanol (4 L, each) by continuous soxhlation process and distilled water (5 L, each) for 7 days. The amount of petroleum ether, chloroform, methanol and aqueous extracts was found to be 1.8, 2.2, 2.5 and 2.75 g, respectively. The colour of petroleum ether, chloroform, methanol and aqueous extracts was pale yellow, chocolate brown, dark brown and black brown, respectively. The extracts were filtered and concentrated to dryness using a rotary evaporator. Dried extracts were stored at 4°C till further use.

In vitro spermicidal activity

Preparation of sperm suspension: The experiment was conducted on human sperm. Semen was collected from 15 healthy fertile men after 2-3 days abstinence from sexual activity. The semen samples were stored at 37°C in an incubator for half an hour. The semen analysis was done as per World Health Organization (1999) guidelines. The samples having volume≥2 mL, motility≥50%, sperm concentration 60 to 90×10^6 million and more than 60% with normal morphology were used. For each study five such samples were used.

Immobilization assay and determination of sperm motility: From the stock solution of the extracts, concentrations of 1, 3, 5, 10, 20 mg mL^{-1} were prepared in normal saline and were mixed in the ratio of 1:1 with the sperm suspension. Physiological saline solution (PSS) pH 7.4 was mixed in a ratio of 1:1 with the sperm suspension to be used as a control. A solution of 1% propylene glycol was used as a control in the same ratio. A drop of the mixture was immediately placed on the glass slide, covered with a cover slip and at least five fields were examined 100X under microscope and the motility was noted. The mixtures were incubated at 37°C at 2, 5, 10, 20, 30 min. of incubation and sperm motility was noted (Sander and Cramer, 1941).

Sperm viability test: Sperm viability was determined by Eosin-Nigrosin staining technique (World Health Organization, 1999). One drop of the mixture was mixed with 2 drops of 1% Eosin Y. After 30 sec 3 drops of 10% Nigrosin solution was added and mixed. A drop of the extract treated semen-Eosin-Nigrosin mixture was observed under microscope. Unstained sperms were counted as live and stained sperms were counted as dead.

Sperm morphology: Sperm morphology was observed under microscope by Eosin-Nigrosin staining technique (World Health Organization, 1999). Any change in the morphology was noted.

Sperm revival test: After the completion of the experiment the mixture of the sperm suspension and extract was washed with PSS and incubated at 37°C for 30 min and after some time reversibility of sperm motility was observed.

Hypo Osmotic Swelling test (HOS test): The effect of extracts on sperm membrane integrity was studied by HOS test (Jeyendran *et al.*, 1984, 1992). Sperms were incubated at 37°C for 30 min with 1 mL of HOS solution. The presence of coiling was observed.

In vivo male anti-fertility activity

Animals and treatment: Animals were procured from Disease Free Animal house of Choudhary Charan Singh Haryana Agricultural University, Hisar after recommendation from the Institutional Animal Ethical Committee of Guru Jambheshwar University of Science and Technology, Hisar. Male Swiss albino mice weighing between 25-40 g, of were used for the anti-spermatogenic activity. All animals were acclimatized to laboratory conditions before the beginning of the experiments and were maintained under standard laboratory conditions.

Animal activity was carried on the extracts (petroleum ether and chloroform) that showed significant spermicidal effect *in vitro*. Swiss male albino mice weighing 25-40 g (2-3 months old) of proven fertility were divided into different groups. Each group consisted of six animals. The control group (Group I) received vehicle only (acacia 1%). The test groups (Group II and III) received petroleum ether and chloroform extracts at the doses of 200 mg kg^{-1} body weight orally for 21 days, respectively. The animals were sacrificed by decapitation on 22nd day. The testis and epididymis were dissected out carefully, cleared from adherent tissues and weighed to the nearest mg. For histological observations the testis and epididymis were preserved in Bouin's fluid, sectioned and stained in Harris'haemotoxylin and eosin. These tissues were also preserved in deep freeze for biochemical estimation viz; total protein, alkaline phosphatase, cholesterol and glucose level (Verma *et al.*, 1980). The biochemical analysis was determined as described by Schoenheimer and Sperry (1934).

Statistical analysis: Data are expressed as Mean±SEM and analysis for statistical significance by using one way analysis of variance (ANOVA) followed by Dunnett's test. Results were considered significant at $p<0.05$.

RESULTS

In vitro spermicidal activity

Sperm motility: The results are shown in Table 1. Out of the four successive extracts the petroleum ether and chloroform extracts at the concentration of 15 mg mL^{-1} significantly immobilized all the sperms within 1 min of incubation at 37°C. Within 3 min complete immobilization by these extracts at the concentration of 15 mg mL^{-1} was observed. At the concentration of 3 mg mL^{-1} of petroleum ether and chloroform extracts, 50% and 60% of the sperms were immobilized within 10 min, respectively. At the concentration of 10 mg mL^{-1} of petroleum

Table 1: Effect of different concentrations of petroleum ether and chloroform extract of root of *B. monosperma* on motility of the human sperm

		Motility(%) at different concentration				
		Petroleum ether (mg mL^{-1})				
Time	Control	1	3	5	10	15
0	90.29±1.40	83.11±1.15	81.44±1.56	78.92±1.38	74.65±1.77	68.90±0.73
1	87.28±0.79	82.94±0.69	79.32±0.87	73.46±0.61	68.51±0.52	36.00±1.03**
2	84.56±2.23	79.42±0.96	76.00±1.03	67.33±0.76	65.50±2.11	04.33±0.95**
3	81.85±2.02	73.83±1.25	70.83±1.35	61.83±0.60	57.33±0.67	0
5	78.83±1.13	72.50±1.23	66.33±1.41	58.67±0.88	53.67±0.95**	0
10	77.18±0.45	70.35±0.85	60.67±0.78	50.45±0.65**	44.43±0.85**	0
20	75.38±0.74	60.23±0.76**	55.23±0.54**	49.67±0.98**	30.56±1.08**	0
		Motility(%) at different concentration				
		Chloroform (mg mL^{-1})				
Time	Control	1	3	5	10	15
0	90.29±1.40	87.33±0.60	81.50±0.43	79.67±0.43	76.91±0.58	80.83±0.60
1	87.28±0.79	83.00±0.73	79.83±0.60	74.16±0.47	72.83±0.98	39.17±0.48**
2	84.56±2.23	80.73±0.62	76.05±0.56	72.76±0.59	68.78±0.61	07.28±0.88**
3	81.85±2.02	78.45±0.89	73.49±1.73	69.63±1.47	63.39±1.32	0
5	78.83±1.13	75.85±0.67	72.32±0.61	66.67±0.57	61.85±0.78	0
10	77.18±0.45	67.35±0.89	64.59±0.69	60.82±0.67*	52.00±0.89**	0
20	75.38±0.74	65.78±0.98*	61.36±0.67*	55.09±0.23**	48.32±1.03**	0

Data are expressed as Mean±SEM, n = 15 *p<0.05 **p<0.01 compared with control group

ether and chloroform extracts, 45 and 50% of the sperms were immobilized after 10 min. After 20 min interval all the concentrations of petroleum ether and chloroform extracts significantly immobilized the sperms. The methanol and aqueous extracts did not show any significant effect even after 20 min exposure at incubation at 37°C at all the concentrations.

Sperm morphology: No change in the morphology of sperms treated with the extract was observed as compared with untreated sperm.

Sperm revival test: None of the spermatozoa once immobilized by petroleum ether and chloroform extracts regained their motility after washing it with PSS followed by incubation at 37°C for 30 min.

Hypo-osmotic swelling test: The control group showed the curling where as the sperms treated with petroleum ether and chloroform extracts did not show any curling even at the concentration of 15 mg mL^{-1} immediately after exposure. No curling was observed even after 20 min at this concentration.

Sperm viability: Sperm viability was significantly reduced within 1 min after the treatment with 15 mg mL^{-1} petroleum ether and chloroform extracts. The viable counts further declined with time.

Table 2: Effect of different concentrations of petroleum ether and chloroform extract of root of *B. monosperma* on viability of the human sperm

		Viability (%) at different concentration				
		Petroleum ether (mg mL^{-1})				
Time	Control	1	3	5	10	15
0	95.29±1.40	93.11±1.15	90.44±1.56	86.92±1.38	82.65±1.77	75.90±0.73
1	92.28±0.79	89.94±0.69	86.32±0.87	83.46±0.61	79.51±0.52	52.00±1.03**
2	90.56±2.23	88.42±0.96	83.00±1.03	80.33±0.76	76.50±2.11	37.67±0.95**
3	88.85±2.02	85.83±1.25	81.83±1.35	78.83±0.60	73.33±0.67	8.33±0.95**
5	87.83±1.13	82.50±1.23	78.33±1.41	74.67±0.88	70.67±0.95	0
10	85.18±0.45	80.35±0.85	75.67±0.78	69.45±0.65**	65.43±0.85**	0
20	82.38±0.74	79.23±0.76	74.23±0.54*	66.67±0.98**	61.56±1.08**	0
		Viability (%) at different concentration				
		Chloroform (mg mL^{-1})				
Time	Control	1	3	5	10	15
0	95.29±1.40	95.09±0.60	91.50±0.43	87.67±0.43	83.9±0.58	80.83±0.60
1	92.28±0.79	91.00±0.73	87.83±0.60	85.16±0.47	79.83±0.98	60.77±0.76**
2	90.56±2.23	87.73±0.62	83.05±0.56	81.76±0.59	78.78±0.61	42.17±0.48**
3	88.85±2.02	85.4±0.89	81.49±1.73	79.63±1.47	76.39±1.32	10.28±0.88**
5	87.83±1.13	83.85±0.67	79.32±0.61	76.67±0.57	71.85±0.78	0
10	85.18±0.45	81.35±0.89	76.59±0.69	72.82±0.67*	68.00±0.89**	0
20	82.38±0.74	79.78±0.98	75.36±0.67	69.09±0.23**	65.32±1.03**	0

Data are expressed as Mean±SEM, n = 6 *p<0.05 **p<0.01 compared with control group

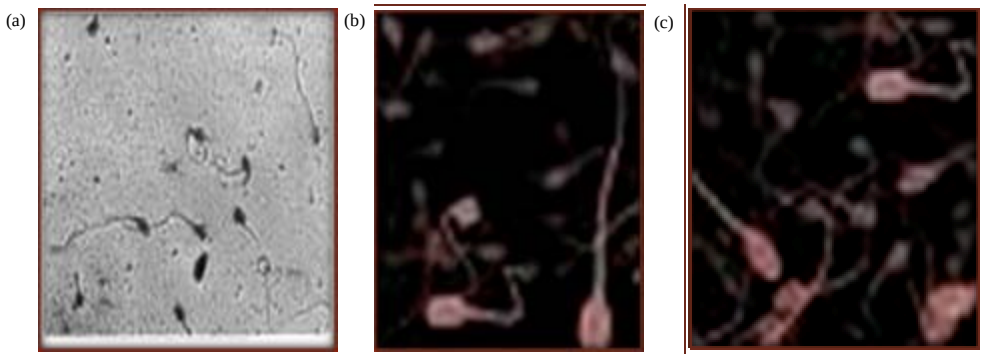

Fig. 1(a-c): Eosin-nigrosin staining of sperm at 10 X (a) Viable sperm (Control) (b) non viable sperms on treatment with petroleum ether extract (c) Non viable sperms on treatment with chloroform extract

All the sperms treated with 15 mg mL^{-1} concentration of petroleum ether and chloroform extracts were found to non-viable after 5 min. At the concentration of 5 mg mL^{-1} and 10 mg mL^{-1} of petroleum ether and chloroform extracts there was significant reduction in viability after 10 min (Table 2 and Fig. 1).

Table 3: Body weight, organ weight after 21 days of treatment with petroleum ether and chloroform extract of *B.monosperma* on male mice

Treatment (200 mg kg^{-1}) extract	Initial body weight (g)	Final body weight (g)	Weight of testis (mg)	Weight of epidydmis (mg)
Control	25.00±0.56	27.83±0.48	346.33 ±16.77	143.50±12.55
Petroleum ether	22.33±0.71*	31.83±0.79**	230.50±19.16**	103.33±5.48**
Chloroform	28.17±0.79*	33.00±0.73**	277.00±7.82**	81.17±4.26**

Data are expressed as Mean±SEM, n = 6 *p<0.05, **p<0.01 compared with control group

Table 4: Effect of petroleum ether and chloroform extract of *B. monosperma* on content of glucose, cholesterol, alkaline phosphatase and total protein in male organ after 21 days of treatment

	Control		Petroleum ether		Chloroform	
Treatment (200 mg kg^{-1}) extract	Testis	Epidydmis	Testis	Epidydmis	Testis	Epidydmis
Fructose (µg mg^{-1})	77.82±3.23	84.31±1.53	65.45±8.44**	75.79±14.30**	69.92±7.02**	79.50±2.20*
Cholesterol (µg mg^{-1})	289.54±3.96	280.85±1.75	349.89±2.90**	332.65±5.09**	248.21±2.39**	235.08±1.36**
Alkaline phosphatase (µg mg^{-1})	24.75± 2.36	42.84±0.63	33.89±1.01**	35.93±1.30**	35.47±1.45**	40.13±1.30**
Total protein (µg mg^{-1})	15.21±0.17	15.42±0.19	12.25±0.20**	13.79±0.51**	12.34±0.19**	13.14±0.31**

Data are expressed as Mean±SEM, n = 6 *p<0.05, **p<0.01 compared with control group

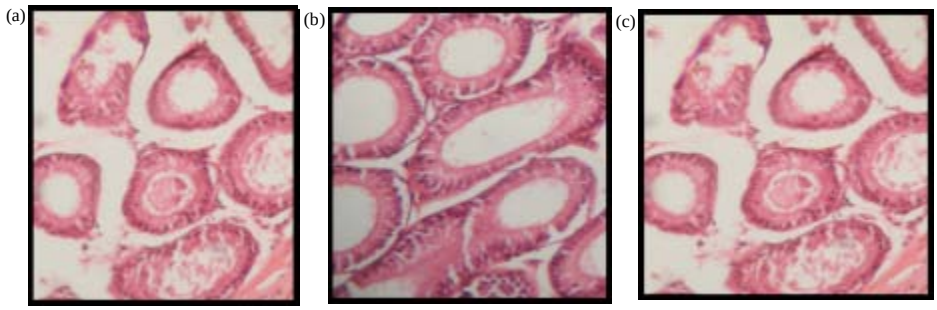

Fig. 2(a-c): Transverse section of epidydmis at 10 X (a) Control (b) Petroleum ether extract (c) Chloroform extract

***In vivo* male anti-fertility activity:** The petroleum ether and chloroform extracts have shown significant *in vitro* spermicidal activity hence their effect on testis and epidydmis was studied on male albino mice. The final body weight of all the groups increased when compared with their respective initial body weights. The weight of testis and epidydmis of petroleum ether and chloroform extract treated groups significantly decreased ($p<0.01$) when compared with the control group (Table 3) which are mostly related to spermatids and spermatozoa present in the tissue.

Fructose, alkaline phosphatase and total protein level in testis and epidydmis of the treated group (petroleum ether and chloroform) decreased significantly when compared with control group ($p<0.01$) whereas the cholesterol level of the petroleum ether extract treated group was increased significantly ($p<0.01$) and significant ($p<0.01$) reduction in the cholesterol level was shown in chloroform treated group (Table 4).

Histological examination showed that treatment of the mice with petroleum ether and chloroform extracts damaged the germinal elements of the mice testis and completely arrested spermatogenesis, in contrast to the control testis where the sperm tails flayed into the lumen. In

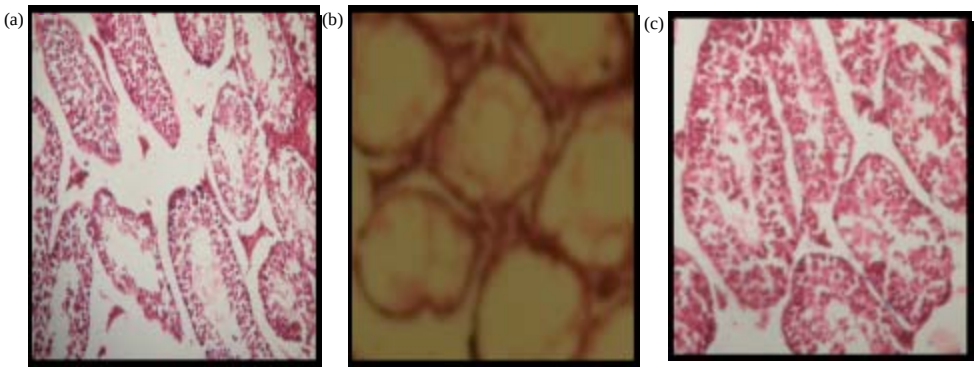

Fig. 3(a-c): Transverse section of testis at 10 X (a) Control (b) Petroleum ether extract (c) Chloroform extract

most of the semeniferous tubules of the treated mice the gonial elements lied intermingled with degenerating sperms. The Leydig cells of the treated mice were delimited; they appeared degenerated with accumulation of oedomatus fluid. Lesions were observed in epidydmis, compared to control (Fig. 2 and 3).

DISCUSSION

***In vitro* spermicidal activity:** Various parameters viz; motility, viability, hypo-osmotic swelling tests are studied to assess the spermicidal activity of the herbal extracts. In the present study it was identified that all the sperms were immobilized by petroleum ether and chloroform extract at concentration of 15 mg mL^{-1} after 3 min. Apart from motility there are other functional characteristics on which the fertilizing ability of sperm is dependent, these parameters include viability (World Health Organization, 1999), hypo-osmotic swelling (Jeyendran *et al*., 1984) and nuclear chromatin decondensation. At 5 mg mL^{-1} concentration the sperm motility was affected but at this concentration total immobilization of sperm motility was not observed even after incubation at 37°C for 30 min. The sperm failed to revive after being washed with PSS thereby the extracts caused irreversible damage to the sperms. There are number of herbal spermicidal agents that have shown the activity by damaging the plasma membrane as they act on the sperm surface (Lohiya *et al*., 2002).

The damage in the sperm membrane integrity by the petroleum ether and chloroform extract is supported by significant reduction in sperm viability and tail curling. Hypo-osmotic swelling responses reflect integrity of the sperm membrane. The intact sperms permit free passage of the fluid into the cell to reach in the osmotic equilibrium on exposure to osmotic environment, thereby increasing the sperm volume and bulging of plasma membrane (Jeyendran *et al*., 1984, 1992). The sperms treated with extracts did not show any curling of tail. This observation suggests that the functional integrity of sperm was lost following exposure to extracts. This result was further supported by differential reaction of the normal and extracts exposed sperms to Eosin Y-Nigrosin. Functionally intact membrane in live sperm offers selective permeability and, therefore, debars entry of colorants like Eosoin Y. The control spermatozoa showed no Eosin staining, whereas almost all the sperm in the treated groups positively stained with Eosin Y. Hence the extracts led to loss of functional integrity of the sperm membrane.

***In vivo* male anti-fertility activity:** Oral administration of the petroleum ether and chloroform extracts at the dose of 200 mg kg^{-1} body weight to the male mice for 21 days bought about significant loss in testis weight, which is related to the number of spermatids and spermatozoa present in the tissue. The reduced weight and shrunken somniferous tubules indicate damage of these tissues (Keel and Abney, 1980). The tubules and the geminal elements account for approximately 98% of testicular mass, hence testicular weight determination is primary assessment of spermatogenesis (Sherins and Hawards, 1978). The total volume of leydig cells is correlated with the onset of spermatogenesis. The degeneration of the testis cells in treated mice suggests reduction in fertility (Monet-Kuntz *et al*., 1984).

A significant decrease in the level of protein in the testis is due to loss of germ cells as well as depletion in membrane protein contents (Barenton *et al*., 1982). A significant change in cholesterol level has been observed on treatment with petroleum ether and chloroform extract. Cholesterol is reported to be physiologically important as it inhibits/stimulates spermatogenesis and acts as the precursor for androgen synthesis. The synthesis of fructose in the sex accessories of mammalian male is androgen dependent. The testicular atrophy causes diminished androgen production which leads to reduced fructose level in testis and epidydmis. The fructose has been suggested to arise from glucose via glycogen-phosphohexose pathway in the presence of phosphohexoisomerase and alkaline phosphatase (Mero, 1968). The alkaline phosphatase secretory activity of the seminal epithelium was impaired as shown by decreased level of alkaline phosphatase in the treated animals which is confirmed by decreased fructose level (Verma *et al*., 1980). The anti-spermatogenic activity is also supported by the histological changes observed in testis and epididymis.

The present investigation established the anti-spermatogenic activity of the petroleum ether and chloroform extracts of *B. monosperma*, supported by changes in both histology and biochemical parameters of male reproductive organs.

REFERENCES

Bandara, B.M., N.S. Kumar and K.M. Samaranayake, 1989. Anti-fungal constituents from the stem bark of *Butea monosperma*. J. Ethnopharmacol., 25: 73-75.

Barenton, B., M.R. Blanc, A. Caraty, M.T.H. Reviers, C. Perreau and J. Saumande, 1982. Effect of cryptorchidism in the ram: changes in the concentrations of testosterone and estradiol and receptors for LH and FSH in the testis and its histology. Mol. Cell. Endocrinol., 28: 13-25.

Chandra, S., J. Lal and M. Sabir, 1977. Phytochemical investigation of *Butea frondosa* seeds. Indian J. Pharm., 39: 79-80.

Chopra, R.N. and I.C. Chopra, 1955. Supplement to the Glossary of Indian Medicinal Plants. CSIR, New Delhi, India, Pages: 90.

Gunakkunru, A., K. Padmanaban, P. Thirumal, J. Pritila and G. Parimala *et al*., 2005. Antidiarrhoeal activity of *Butea monosperma* in experimental animals. J. Ethnopharmacol., 98: 241-244.

Gupta, S.R., B. Ravindranath and T.R. Seshadri, 1970. The glucosides of *Butea monosperma*. Phytochemistry, 9: 2231-2235.

Hayashi, K., T. Nagamatsu, S. Honda and Y. Suzuki, 1996. Butein (3, 4, 2', 4'-tetrahydroxychalcone) ameliorates experimental anti-glomerular basement membrane antibody-associated glomerulonephritis. Eur. J. Pharmacol., 316: 297-306.

Jain, S.C., R. Kamal, A.K. Rathore, 1980. A note on phytosterols in some species. Indian Drugs, 17: 145-145.

Jeyendran, R.S., H.H. van der Ven and L.J.D. Zaneveld, 1992. The hypoosmotic swelling test: An update. Arch. Androl., 29: 105-116.

Jeyendran, R.S., H.H. van der Ven, M. Perez-Pelaez, B.G. Crabo and L.J.D. Zaneveld, 1984. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. J. Reprod. Fert., 70: 219-228.

Kasture, V.S., C.T. Chopde and V.K. Deshmukh, 2000. Anticonvulsive activity of *Albizzia lebbeck*, *Hibiscus rosa sinensis* and *Butea monosperma* in experimental animals. J. Ethnopharmacol., 71: 65-75.

Kasture, V.S., S.B. Kasture and C.T. Chopde, 2002. Anticonvulsive activity of *Butea monosperma* flowers in laboratory animals. Pharmacol. Biochem. Behav., 72: 965-972.

Keel, B.A. and T.O. Abney, 1980. Influence of bilateral cryptorchidism in the mature rat: alterations in testicular function and serum hormone levels. Endocrinology, 107: 1226-1233.

Khare, C.P., 2007. Indian Medicinal Plants. 1st Edn., Springer, India, ISBN: 9780387706375, pp: 105-106.

Kirtikar, K.R. and B.D. Basu, 1991. Indian Medicinal Plants. Vol. 1, Lalit Mohan Basu and Co., Allahabad, pp: 785-789.

Lohiya, N.K., L.K. Kothari, B. Manivannan, P.K. Mishra and N. Pathak, 2002. Human sperm immobilizing effect of *Carrica papaya* seed extracts as *in vitro* study. Asian J. Androl., 2: 103-109.

Mehta, B. and M. Bokadia, 1981. Monospermin: A new alkaloid of *Butea monosperma* seeds. Chem. Ind., 3: 98-98.

Mero, R., 1968. Nocenti. In: Medical Physiology, Vernon, B.M. (Ed.) Vol. 1. The C.V. Mosby Co., St Louis, MO., USA.

Mishra, M., Y.N. Shukla and S. Kumar, 2000. Euphane triterpenoids and lipid constituents from *Butea monosperma*. Phytochemistry, 54: 835-838.

Monet-Kuntz, C., M.T. Hochereau-de Reviers and M. Terqui, 1984. Variations in testicular androgen receptors and histology of the lamb testis from birth to puberty. J. Reprod. Fertil., 70: 203-210.

Prashad, P.V.V., P.K.J.P. Subhaktha, A. Narayana and M.M. Rao, 2006. Palasa (*Butea monosperma* (Lamk.) Taub.) and its medico-historical study. Bull. Ind. Inst. Hist. Med., 36: 117-132.

Pulliah, T., 2006. Encyclopaedia of World Medicinal Plants Vol 1. Regency Publications, New Delhi, pp: 376-379.

Purohit, S.S. and S.P. Vyas, 2004. Medicinal Plant Cultivation A Scientific Approach. 1st Edn. Agrobios, Jodhpur, India, pp: 336-339.

Raj, R.K. and P.A. Kurup, 1968. Isolation and characterisation of palasonin an anthelmintic principle of the seeds of *Butea frondosa*. Indian J. Chem., 5: 86-87.

Rani, P. and N. Khullar, 2004. Antimicrobial evaluation of some medicinal plants for their anti-enteric potential against multi-drug resistant *Salmonella typhi*. Phytother. Res., 18: 670-673.

Sander, F.V. and S.D. Cramer, 1941. A practical method of testing the spermicidal action of chemical contraceptives. Indian J. Exp. Biol., 35: 1170-1174.

Schoenheimer, R. and W.M.J. Sperry, 1934. Application of the schoenheimer-sperry method to the determination of cholesterol and cholesterol esters in tissues. J. Biol. Chem., 106: 745-745.

Sehrawat, A., T.H. Khan, L. Prasad, S. Sultana, 2006. *Butea monosperma* and chemomodulation: Protective role against thioacetamide-mediated hepatic alterations in Wistar rats. Phytomed., 13: 157-163.

Sherins, R.J. and S.S. Hawards, 1978. Male Infertility. In: Campbell's Urology, Harrison, J.H., R.F. Gaites, A.D. Pertmuteror, T.A. Stamey and P.L. Walsh (Eds.). 4th Edn., W.B. Saunders Co., Philadelphia, pp: 715.

Sumitra, M., P. Manikandan and L. Suguna, 2005. Efficacy of *Butea monosperma* on dermal wound healing in rats. Int. J. Biochem. Cell Biol., 37: 566-573.

Verma, O.P., B.C. Joshi, S. Kumar and S.N. Chatterjee, 1980. Antifertility effects of *Malva viscus conzattii* Greenm flower extract (SC) on male albino mice. Indian J. Exp. Biol., 18: 561-564.

Wagner, H., B. Geyer, M. Fiebig, Y. Kiso and H. Hikino, 1986. Isobutrin and butrin the antihepatotoxic principles of *Butea monosperma* flowers. Planta Med., 2: 77-79.

World Healh Organisation, 1999. Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. 4th Edn., Cambridge University Press, New York, pp: 68-70.

Yadava, R.N. and L. Tiwari, 2005. A potential antiviral flavone glycosides from the seeds of *Butea monosperma O. Kuntze*. J. Asian Nat. Prod. Res., 7 : 185-188.

Yadava, R.N. and L. Tiwari, 2007. New antifungal flavone glycoside from *Butea monosperma O. Kuntze*. J. Enzyme Inhib. Med. Chem., 22: 497-500.

Zafar, R., P. Singh and A.A. Siddiqui, 1989. Antimicrobial and preliminary studies on leaves of *Butea monosperma* Linn. Indian J. For., 12: 328-329.