Butea monosperma (Lam.) induces sexual activity in male albino rat, *Rattus rattus* (Wistar)

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Abstract

In traditional medicine *Butea monosperma* is used as aphrodisiac however its soundness has not been scientifically confirmed. Therefore, the study was aimed to evaluate the effect of methanolic flower extract of *B. monosperma* on male albino rat, *Rattus rattus* (Wistar) of weighing 200-250gm. Doses of 100, 200 and 300 mg/kg body weight were given orally to the three groups of rats respectively and fourth group was maintained as recovery group for fifteen days while control was treated with 15ml distilled water. Each group contains six rats. The copulatory, sexual behaviour and orientation activity were observed. There was significant (***<0.001) increased in mount frequency and intromission frequency. After fifteen days treatment of *B. monosperma* there was also significant (***<0.001) increased in number of mounting ,licking , anogenital sniffing , genital grooming where as significant decreased (***<0.001) in mount latency and intromission latency were observed .The group treated with the dose of 300 mg/kg body weight showed a maximum increase in the orientation toward female rat. Hence it can be concluded that methanolic flower extract of *Butea monosperma* (Lam.) have sex stimulant properties and can improve sexual behaviour , orientation and libido also in male albino rat, *Rattus rattus* (Wistar).

Keywords- Aphrodisiac activity, Albino rat, *Butea monosperma*, Orientation activity.

Introduction

Aphrodisiac are the substances which stimulate sex drive. Due to biochemical nature of sexual activity, herbs and drugs are very important. Large number of plants induces sexual activity (Adimoeljia, 2000).The aphrodisiac property reported in saffron, honey, opium, cinnamon, nutmeg, ginger, cloves, onion, Rhino horn, Spanish fly .The dried and crushed bodies of tiny beetles, oysters also have aphrodisiac properties (Singh and Mukharjee, 1998). In traditional medicine a variety of plants have sex stimulative properties (Islam, *et al*., 1991). Arabs and Chinese also used herbal drugs from ancient time to improve sexual performance and to increase libido (Puri, 1971). Traditional medicines are based on use of plants and their extract (Acarya and Shrivastav, 2008). Male impotence or erectile dysfunction (ED) and sexual dysfunction (SD) are the major problems and may contribute to increase infertility globally (Kaiser, 1988 and Yakubu, *et al*., 2003). Infertility is increasing day by day probably due to aging populations and other risk factors such as the presence of chronic illnesses, smoking, stress, alcohol, drug abuse and sedentary lifestyles. ED and SD in males are inability to achieve an erection sufficient for the purpose of satisfactory sexual intercourse, or to ejaculate and disorder in the normal male sexual response cycle of libido, erection, orgasm, ejaculation respectively (Ogah, 1999). These are the serious medical and social symptoms occur near about 10%-52% in males (Patel, *et al*., 2011). Survival of human race and the "Continuation of progeny" is the basic purpose of sex which is the most intimate part of individual and sexual feeling is the basic tendency of human being (Kothari, 2001).

The plant-based, traditional medicine system acquires an important place in the health care system (Owolabi, *et al*., 2007).*Butea monosperma* (Lam.) is known as flame of the forest, belongs to the family - Fabaceae (Patil, *et al*., 2006). It is locally called as palas, palash,dhak, khakara, bastard teak and is common throughout India (Puri, 1953). *Butea monosperma* is extensively used in Ayurveda , Unani and Homeopathic medicine and even for aphrodisiac purpose (Nadkarni, 2002). The claimed of aphrodisiac properties of *Butea monosperma* is not soundly demonstrated therefore the present work has aimed to confirmed its' validity.

Materials and Methods

The flowers of *B. monosperma* were collected in the month of March in the year 2012 from Pohara jungle near to Amravati, Maharashtra State and authenticated. The flower extract preparation and phytochemical screening were done previously (Thimmaiah, 2004, Kehkashan and Siddiqui, 2011, Deshmukh and Bhagat, 2015) .

A total of 30 albino rat, *Rattus rattus* (Wistar), and weighing 220-225 gm were used for the study .The experiments were conducted according to "INSA-Ethical guidelines after permission from ethical committee (registration number [1504/Po/a/11/CPCSE]. The rats were regularly fed on standard pellet diet provided by Trimurti feeds, Nagpur (M. S.) and water was given *ad libitum.* After acclimatization for fore night only healthy rats were used for the experiments.

The experiment were conducted according to the guidelines of Organisation of Economic Co-operation and Development (OECD).The highest dose of 1000 mg/kg body weight was given and were continuously observed for 2 days for any lethality. Later 100, 200, 300 mg/kg body weight dose was determined for the experiment.

Mating Behaviour Test

The test was conducted by the methods of (Dewsbury and Devis, 1970, Szechtman, 1981, Amin, *et al.,*1996). Healthy and sexually experienced male albino rats (200-250 gm) were selected for the study .They were divided into five groups, containing 6 rats each and kept singly in separate cages during the experiments .Group 1, represents the control group, which received 15 ml /kg distilled water orally .Group 2 to 4 received extract of flower of *B.monosperma* orally at the doses of 100, 200 and 300 mg/kg body weight respectively, daily for 15 days at 18:00 hrs. Group 5 served as recovery group and also gave 300mg/kg dose up to15 days later fifteen days only distilled water was given. The male rats tested in familiar circumstances and the albino rats were brought to the laboratory and exposed to the dim light at the stipulated time of testing daily for 6 days before the experiment. The female rats were artificially brought into oestrus (heat) (Sooriya and Dharmasiri 2000).They were administered suspension of ethinyl oestradiol orally at the dose of 100 ug/albino rat and progesterone 1 mg/ albino rat 48 hrs prior to the experiments and the most receptive females were selected for the study. The experiment was performed on 15th day after commencement of the treatment of male rats. The experiment was conducted at 20:00 hrs in the same laboratory and under the light of same intensity. The receptive female was introduced into the cages of male albino rats with 1 male 1 female. The observations for mating behaviour were immediately taken. The test was terminated if the male failed to show sexual interest. If the female didn't show receptivity she was replaced by another artificially warmed female. The occurrence of events and phases of mating were observed and noted.

Later frequencies and phases were determined: No of mounts before ejaculation or mounting frequency (MF), number of intromission before ejaculation or intromission frequency (IF), time from the introduction of female into the cage of the male up to the first mount or mounting latency (ML), time from the introduction of the female up to the first intromission by the male or intromission latency (EL).

Test for Libido

The methanol extract of *B.monosperma* 300 mg/kg body weight was found to be the most active amongst the three treatments in aphrodisiac testing. Hence it was subjected to investigation for the study of the test for libido. The test was carried out by the methods of Davidson (1982), sexually experienced male albino rats were used and divided into 5 groups of 6 male rats each. Group I represents the control group, which received 15 ml/kg of distilled water orally. Group II, III and IV received methanolic flower extract of *B. monosperma* orally at the dose of 100,200, and 300 mg/kg respectively daily for 15 days at 18:00hrs. Group V served as recovery group as above. The female rats made receptive by hormonal treatment and all rats were accustomed to the testing condition as previously mentioned in mating behaviour test and were observed for the mounting frequency (MF) on the evening of 15th day at 20:00 hrs. Each rat was placed individually in a cage and receptive female was placed in the same cage. The number of mountings was noted. The male rats were also observed for intromission and ejaculation.

Orientation activity

For the study of orientation activity the above mentioned doses were given. One hour prior to the commencement of the experiment, the orientation of male rat towards female (licking, a genital sniffing), towards self (non genital grooming) and towards environment (exploration, climbing raring)

were observed at 10, 20 and 30 minutes after treatment to all groups. The intensity of response was counted.

Statistical analysis

The data are expressed as mean ± SE .Statistical analysis was done by student't' test for compare to control.

Results and Discussion

In the present study clinical toxicity symptoms are not found up to 1000 mg/kg dose of *B.monosperma* and similar results were also found in study of *Hibiscus canabinus*(Zade and Dabhadkar , 2013). The administration of methanolic flower extract of *B.monosperma* for 15 days to male rats resulted in remarkable increase in the sexual vigour of the male rats as evidenced by the different parameters studied at different doses. The result of mating behaviour showed that the methanolic flower extract of *B.monosperma* at the dose of 100, 200, and 300mg/kg body weight significantly (P<0.001) increased the mounting frequency (MF) intromission frequency (IF) and caused significant (P<0.001) reduction in mounting latency (ML), intromission latency (IL) and ejaculatory latency (EL) in experimental rat, *Rattus rattus* (Wistar), when compared to control (table 1). Same finding were also reported in the study of *H. canabinus* and stamens of *Nelumbonucifera* white variety and *Chenopodium album* (Vahitabi, *et al.,* 2012 and Zade and Dabhadkar 2013, Baldi and Gupta 2013). Oral administration of the leaves of *Ocimum gratissimum* extract significantly increased the mounting frequency, intromission frequency; intromission latency, erections as well as penile reflexes and caused significant reduction in the mounting latency and post ejaculatory interval (Pande and Pathak 2009).

Results obtained in the test for libido showed that the methanolic flower extract of *B.monosperma* at the dose of 100 and 300mg/kg significantly (P< 0.001) increased the mounting frequency (MF) as compared to control group. Intromission was observed in control and experimental groups while ejaculation was found only at the dose of 200 and 300 mg/kg (table 2). Similar results were obtained when administered 125 mg/kg, of *Chlorophytum borivilianum* and 100 mg /kg *Bombax ceiba* of aqueous extract had a marked aphrodisiac action, such as increased (*P* <0.05) libido, sexual vigour and sexual arousal and all the parameters of sexual behaviour were enhanced (Kenjale, *et al.,* 2008 and Bhargava, *et al.,* 2012) . Oral administration of aqueous, alcohol and chloroform extract of *Psoralea corylifolia* were also significantly increased various sexual behavioural parameters (Dabhdkar and Zade 2013).

The methanolic extract of *B. monosperma* markedly influenced the orientation behaviour of the male rat which demonstrated more attraction towards female rat but enhancement in attraction towards female was noticed at all doses. Behavioural assessment of rats towards environment such as exploration was significantly (P<0.001) decreased at all doses and in recovery group also decreased significantly (p<0.01). Raring decreased significantly (p<0.01) at 100 mg/kg and also decreased at 200mg/kg and 300mg/kg and recovery group (P<0.001). Climbing significantly increased at 100mg dose (P<0.001) but later it is significantly decreased (P<0.001) at 200mg/kg and 300mg/kg dose and in recovery it remains non significant. In experimental male rats a moderate increase (P<0.001) in female anogenital smelling was observed at 100mg/kg and 300 mg/kg dose and also in recovery but significantly decreased (P<0.01) at 200mg dose. However there was significantly increase (P<0.001) in the licking at 100 and 300 mg/kg dose and also in recovery group .There was also significant increase in genital grooming at all doses (P<0.001)and in recovery (P<0.01) (table 3). Pal, *et al*., (2015) found similar results when 50% ethanolic extract of *Mimosa pudica* and roots' extract of *Salvia haematodes (*Islam,

et al., 1991) and reported significant increase in MF, IF, number of mounting, licking, anogenital sniffing, climbing, genital grooming .

In local tribes flowers of *B.monosperma* are used to cure and improve the sexual health as an aphrodisiac (Nadkarni, 2002) .Flowers of *B.monosperma* contains saponins, alkaloids, steroids, flavonoids (Jhade, *et al*., 2009, Sharma and Deshwal, 2011, Deshmukh and Bhagat , 2015) . Enhancement of sexual behaviour and orientation of experimental male rat in present study might be due to flavonoids (Zade and Dabhadkar , 2013) .Singh *et al*., (2012) suggested that latency for mount and intromission are indicators of the sexual motivation, while intromission frequency and ejaculation are behavioural indication of sexual performance and facilititation. A dose dependent improvement in sexual behaviour as well as enhancement in orientation of males towards the female was reported in aqueous extract of the dried fruits of *Tribulus terrestris ,Cocculus hirsutus ,Aframomum melegueta ,Piper guineense* (Singh *et al*., 2012, Patil, *et al*., 2014 ,Kamtchouing, *et al.,* 2002). The results of present observations also demonstrated the dose dependent sexual enhancement behaviour and orientation activity in male albino rat, *Rattus rattus* (Wistar).

Conclusion

Thus, the present observations concluded that methanolic extract of flower of *Butea monosperma* at 300mg dose is highly significant ($p<0.001$) for orientation activity, mating performance and libido and not toxic . Hence, we can say that the flower of *Butea monosperma* have sex stimulant properties and can improve sexual behaviour in male albino rat, *Rattus rattus* (Wistar).

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Table 1. Effect of methanolic flower extract of *Butea monosperma* on sexual behaviour of male albino rat on 15th day

Treatment Group	Dose (mg/kg body weight)	Mountlatency (time in sec.)	Mount frequency (No.)	Intromission Latency(time in sec.)	Intromission frequency (No)	Ejaculatory latency (time in sec)
Group –I		247.91 ±1.5	4.16 ±0.20	340.95 ±2.69	3.49 ± 0.07	262.45 ±2.98
Group –II	100	217.27 ±1.2***	5.36 ±0.08 ***	297.91 ± 6.07***	4.23 ±0.03 ***	320.43 ± 2.20***
Group-III	200	204.85 ± 2.11***	8.4 ± 0.01***	284.06 ±2.38***	5.15 ±0.01***	410.41 ±1.07***
Group –IV	300	185.05 ± 3.5 ***	8.73 ± 0.2 ***	280.21 ±2.17***	6.21 ± 0.04 ***	449.51 ± 6.24***
Recovery		190.25 ±2.6 ***	7.83 ±0.13 ***	302.78 ± 1.85***	3.63 ±0.19***	290.93 ±3.98***
Values are mean ±SE from 6 animals in each group ,p values :*<0.05,**<0.01,***<0.001,when compared with control group						

Table 2. Effect of methanolic flower extract of *B.monosperma* on libido in male rat on 15th day

Treatment Group	Doses (mg/kg body weight)	Mounting frequency(MF)	Intromission frequency (IF)	Ejaculation (EJ)
Group –I		1.28 ±0.05	2.18 ±0.009	Absent
Group –II	100	4.35 ±0.19 ***	7.18 ±0.01***	Absent
Group-III	200	7.005 ±0.24**	12.87 ±0.01***	Present
Group –IV	300	8.2 ±0.17***	31.61 ±0.30 ***	Present
Recovery		4.63 ±0.14***	14.08 ±0.4***	Present
Values are mean ±SE from 6 animals in each group ,p values :*<0.05,**<0.01,***<0.001,when compared with control group				

Table 3 . Effect of methanolic extract of *Butea monosperma* on orientation activities in male rats

On 15th day

Orientation Towards		Group I	Group II	Group III	Group IV	Group V
		Dist. water 15 ml/kg	100mg/kg	200mg/kg	300mg/kg	Recovery
Female	**licking**	11.33 ±0.3	13.5 ±0.2***	17.16 ±0.3**	18 ±0.3***	12.33 ±0.33***
	Anogenital smelling	8.16 ±0.33	14.33 ±0.42***	5.33 ±0.33**	12.33 ±0.42***	10.31 ±0.61***
Environment	**Exploration**	24.33 ±0.33	19.33 ±0.33***	17.66 ±0.42***	10.16 ±0.65***	16.83 ±0.40**
	Raring	19 ±0.36	16.5 ±0.42**	7 ±0.5***	15.33 ±0.49***	11.66 ±0.55***
	Climbing	2.3 ±0.33	2.6 ±2.21***	1.8 ±0.16***	1.3 ±0.21***	02 0.34 ns
Self	**Non genital grooming**	31 ±0.44	9.33 ±0.33***	10.83 ±0.40***	21.16 ±0.40***	17.66 ±0.21***
	Genital grooming	31 ±0.44	24.16 ±0.30***	32.66 ±0.61***	36.16 ±0.30***	21.83 0.30**
Values are mean ±SE from 6 animals in each group ,p values :*<0.05,**<0.01,***<0.001,when compared with control group, ns=non significant