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Dose- and time-dependent effects of ethanolic extract of *Mucuna pruriens* Linn. seed on sexual behaviour of normal male rats

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ABSTRACT

Aim of the study: According to Indian Systems of Medicine, *Mucuna pruriens* Linn., belonging to the leguminous family (Papilionaceae), were used for treating male sexual disorders since ancient times. In this study, the effects of ethanolic extracts of the *Mucuna pruriens* Linn. seed on general mating behaviour, libido and potency of normal male Wister albino rats were investigated and also compared with the standard reference drug, Sildenafil citrate.

Materials and Methods: Animals were divided into one control group (Group I––received saline) and four experimental groups (Groups II–V). Experimental groups were divided on the basis of the dosage of extract to the animals as follows: 150 mg/kg body weight (Group I), 200 mg/kg body weight (Group II) and 250 mg/kg body weight (Group IV) while Group V received Sildenafil citrate (5 mg/kg body weight). Animals were fed PO with saline or extract or standard drug once in a day for 45 days. To analyse the mating behaviour, female rats with oestrus phase were used.

Results: The extract administered PO significantly increased the mounting frequency, intromission frequency and ejaculation latency, and decreased the mounting latency, intromission latency, post-ejaculatory interval and inter-intromission interval. The potency test significantly increased erections, quick flips, long flips and total reflex. Therefore, the results indicated that the ethanolic extracts of *Mucuna pruriens* Linn. seed produced a significant and sustained increase in the sexual activity of normal male rats at a particular dose (200 mg/kg). When compared to control, all the drug-treated groups have shown drug-induced effects for a few parameters. However in Group II, there was an obvious enhancement in all parameters, without affecting the normal behaviour. When compared with the standard drug, the net effect of extract is even less than that in Group II.

Conclusions: Therefore, the resulting aphrodisiac activity of the extract lends support to the claim that it has traditionally been used for the treatment of sexual disorders.

1. Introduction

Sexual dysfunction is a common problem with increase in prevalence and etiological factors, including degenerative diseases, increase in injuries and stress associated with industrialized lifestyles. Sexual dysfunction can be treated by both medical and surgical treatment modalities; however, plant-derived and herbal remedies continue to be a popular alternative for men and women seeking to improve their sexual life despite the availability of effective conventional medical treatments (Rowland and Tai, 2003). In many countries, different varieties of plants have been used as sexual stimulants in traditional medicine.

Indian Systems of Medicine use *Mucuna pruriens* Linn. (MP), a leguminous plant, for improving fertility. The plant is being cultivated in India, Sri Lanka, South East Asia and Malaysia (Kharelep, 2004). The plant is rich in alkaloids such as prurienine, prurienine and prurienidine (Misra and Wagner, 2004). Triterpenes and sterols (β-sitosterol, ursolic acid, etc.) were found in the root and seeds of MP. The seeds also contain proteins, amino acids such as L-DOPA (Siddhuraju et al., 1996), methionine, tyrosine, lysine, glycine, aspartic acid, glutamic acid, leucine and serine along with globulins and albumins (Pant and Joshi, 1970), fatty acids, carbohydrates, and related compounds such as oleic acid, linoleic acid and palmitic acid (Adebowale et al., 2005).

MP has been recognized as an aphrodisiac agent. The plant and its efficacy in treating sexual disorder has been documented in ayurveda, but lacks scientific validation. Saksena and Dixit (1987) have reported that the number of spermatozoa increases when the rats were treated with bark extract of MP. Further, it has been reported that the sexual and androgenic activities in adult male rats were sustained while improving the mass of the muscles (Rao and Parakh, 1978; Amin et al., 1996). Critical parameters such as sexual

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behaviour, potency and libido, acute toxicity and organ morphology were not reported. Therefore, the present study was designed to address these issues to lend support to the existing information pertaining to the beneficial effect of this plant in treating sexual disorder.

2. Materials and methods

2.1. Animals

Twelve-week-old female (body weights around 175–200 gm) and male (body weights around 225–250 gm) albino rats of Wistar strain were used for the present study. The rats were housed singly in separate standard cages and maintained under standard laboratory conditions (temperature 24–28 °C, relative humidity 60–70%, 12 h light–dark cycle) with free access to solid pellet diet and water *ad libitum* throughout the study. The study was approved by Institutional Ethical Committee (IAEC No. 01/031/05). Animals were maintained according to the guidelines of the Canadian Council for Experimental Animal Care and the Laboratory Animal Science Association of India. Animals were randomly divided into five groups with six animals per group. Group I represented the control animal, animals in Groups II, III and IV were given oral suspension of MP extract for 45 days at 18:00 h, at doses of 150, 200 and 250 mg/kg, respectively, and Group V rats received Sildenafil citrate (SC) (5 mg/kg body weight) reference drug, which served as a positive control. Dosage of MP was selected according to Rathi et al. (2002) with ±50 mg to confirm effective concentration.

2.2. Drug preparations

The seeds of MP were procured locally after authentication and the voucher specimen (herbarium voucher no. 6907) was deposited in the Department of Plant Biology and Plant Biotechnology (The Presidency College, Chennai, India). Seeds were washed twice using tap water and then washed again in distilled water to remove the dust. The seeds were dried in the shade for 7–12 days, and then crushed into coarse powder. Later, it was transferred into a container and ethanol was added as a solvent until the coarse particles of the seed were completely soaked. The container was gently shaken for 72 h with every 1-h interval (until the colour of the solvent becomes colourless) and the filtrate was vacuum concentrated to remove the moisture content (Harborne, 1973). Percentage of yield was around 20%, the percentage of L-DOPA was analysed using HPLC and it was found to be 25.80%.

2.3. Mating behaviour test

The test was carried out in accordance with the method of Agmo (1997). Healthy male albino rats showing brisk sexual activity were selected for the study. Female animals showing regular oestrus cycle were used for mating behaviour analysis. The receptiveness of the female rats was confirmed before the test by exposing them to male rats. Female rats with maximum receptivity were selected for the experiment. The tests for sexual desire were carried out on 15th, 30th and 45th day after commencement of the MP treatment. The experiment was conducted at 20:00 h in the same laboratory and under the light of same intensity. The male and receptive female rats were introduced into the mating cages, with one female to one male ratio. The mating behaviours were recorded and used for further analysis by giving scores for first four mating series. Test was terminated if the male rat failed to evince sexual interest. The occurrence and disappearance of events and phases of mating were recorded as soon as they appeared. Later, the frequencies and phases were determined by the recorded transcriptions: number 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 (IL), time from the first intromission of a series up to the ejaculation or ejaculatory latency (EL), number of intromission in a single attempt or number of intromission (NI), number of mount in a single attempt or number of mount (NM), time from the first ejaculation up to the next intromission by the male or post-ejaculatory interval (PEI), and time between two adjacent intromission or inter-intromission interval (III). The pre-coital sexual behaviours such as chasing, nosing, anogenital sniffing and mounting were observed for up to 2 h of pairing. The values of the observed parameters for control and experimental groups were recorded.

2.4. Test for libido

Libido was assessed according to the method described by Davidson (1982), later modified by Amin et al. (1996). This test was done using the MF of the mating behaviour test during 15th, 30th and 45th day. The number of mountings along with intromission and ejaculation were analysed.

2.5. Test for potency

The effect of the MP on potency was studied according to the method described by Hart and Haugen (1968) and Hart (1979) modified by Amin et al. (1996). On the 46th day, the test for penile reflexes was carried out by placing the animal on its back in a glass cylinder partial restraint. The preputial sheath was pushed behind the glands by means of thumb and index finger and held in this manner for a period of 15 min, which elicited a cluster of genital reflexes. The following components were recorded: erections (E), quick flips (QF), long flips (LF) and total reflex (TR).

2.6. Adverse effects

All drug-treated rats were observed at least once daily for any overt sign of toxicity (salivation, rhinorrhoea, lachrymation, ptosis, writhing, convulsions and tremors), stress (erection of fur and exopthalmia) and changes in behaviour (such as spontaneous movement in the cage, climbing and cleaning of face). In addition to food and water intake, animal body weights were also noted every day before drug administration.

2.7. Acute toxicity testing

The acute toxicity test of the extract was done by up and down method [in accordance with the Organization for Economic Co-operation and Development (OECD) guidelines, 2001] using two groups with three animals each as toxicity test groups. The suspension of the extract was administered PO for the doses 250 and 2500 mg/kg. Control animals received 10 ml/kg of distilled water PO. The animals were observed continuously for the initial 4 h for behavioural changes and mortality, and intermittently for the next 6 h and then again at 24 and 48 h after the administration of the dose. The behaviour parameters observed were convulsion, hyperactivity, sedation, grooming, and loss of righting reflex and increased respiration.

2.8. Hormonal analyses

The blood was collected from retro orbital venous plexus of all animals at the 15th, 30th and 45th day of the experiment. The serum was separated, and testosterone and estradiol were measured by using RIA (Anletta, 1974).

2.9. Ulcerogenecity and toxicity

After the treatment, on 46th day, all the animals were killed and the stomach was then incised along the greater curvature and washed carefully with physiological saline. Presence of any gastric lesion was observed using a magnifying glass. The severity of the lesion was assessed according to the scale described by Cioli et al. (1967): 0 = absence of lesion, vasodilatation or up to 3 pinpoint ulcers; 1 = more than 3 pinpoint ulcers; 2 = from 1 to 5 small ulcers (<2 mm); 3 = more than 5 small ulcers (<2 mm); 4 = 1 or more giant ulcers. Two observers, following the same evaluation criteria, carried out evaluation of gastric damage. The histological study of the kidney and liver was carried out for all the groups.

2.10. Morphological studies

At the end of study, the animals were killed by overdose of anesthesia. Immediately after the respiration ceases, the animals were fixed by trans-cardial perfusion with formal saline after flushing the blood using normal saline. Before perfusion, right-hand side of the epididymides was removed and used for sperm analysis; left-hand side was used for morphological study. Main and accessory reproductive organs were dissected and weighed.

2.11. Sperm analysis

Sperm parameters were studied as per WHO laboratory manual (WHO, 1987). Sperm count: Using haemocytometer. Motility—percentage of motile sperm with time and speed of motility of individual sperm was also assessed. The speed of motility was graded semiquantitatively on a scale of 0–5 and the spermatozoa were evaluated for the rate of forward movement and graded accordingly: 0 = no movement, 1 = sluggish or tail movement alone, 2 = intermittent sluggish movement, 3–4 = fair and good movement and 5 = maximum movement in forward direction.

2.12. Statistical analysis

The significant difference between the mean value of control and experimental groups was determined by one-way analysis of variance (ANOVA) with *post hoc t*-test. *P* value < 0.05 was considered as statistically significant.

3. Results

3.1. Mating behaviour, test for libido and test for potency

The test for libido showed that the pre-coital sexual behaviours, such as chasing, nosing and anogenital sniffing, were well performed in the Group III (200 mg/kg) whereas in Groups I, II and IV the behaviours were not to the extent seen in Group III (200 mg/kg) (Table 1). However, effect of Group III showed less effect than Group V. The test for potency has shown that the 200 mg/kg dose of the test drug significantly increased the frequency of erections, quick flip, long flip and total reflex. Nevertheless, the effect was less when compared with Group V (Table 2).

3.2. Adverse effects and acute toxicity

In all the groups, toxic changes, stress and changes in behaviour were not observed. An increase in the body weights of the animals in all the groups was observed, whereas in Group III (200 mg/kg) there was a gradual increase in body weight during the complete course of the study. Hence, weight loss was not reported in any of the group. Food intake and water intake were same in all the groups. When compared to control, marked histological changes were not observed in kidney and liver of the drug-treated groups (Groups II–V). Acute toxicity studies showed no mortality, and normal behaviour was observed in all the treated and control groups.

3.3. Hormonal analysis

Hormonal analysis revealed that the levels of testosterone and estradiol increased gradually in all the experimental groups. Par-

Table 1
Mating behaviour.

	Control			Group I			Group II		
	15 days	30 days	45 days	15 days	30 days	45 days	15 days	30 days	45 days
ML	11.16 ± 1.37	10.65 ± 1.77	9.98 ± 0.65	10.58 ± 0.16	8.42 ± 1.12*	9.7 ± 0.30	2.27 ± 0.24***	3.27 ± 3.10***	2.65 ± 1.09***
IL	10.14 ± 1.31	10.74 ± 1.78	10.13 ± 1.72	10.09 ± 0.07	8.03 ± 1.04*	10.18 ± 4.18	1.73 ± 0.24***	2.11 ± 2.25***	1.86 ± 0.12***
EL	249 ± 0.35	252 ± 1.61	239 ± 0.53	247 ± 0.32	235 ± 3.44	267 ± 3.34*	365 ± 0.33**	1238 ± 1.06***	1265.4 ± 4.56***
PEI	478.8 ± 0.37	464.6 ± 2.75	448 ± 3.57	496 ± 0.33	561 ± 2.27	639.6 ± 2.37	425 ± 0.28**	9.02 ± 0.16***	5.96 ± 0.45***
NI	5.88 ± 0.68	5.03 ± 0.57	5.23 ± 0.48	6 ± 0.73	5.48 ± 0.98	5.75 ± 0.61	6.16 ± 0.69	6.28 ± 0.91	5.93 ± 0.68
III	17.92 ± 0.60	18.03 ± 2.59	17.24 ± 1.58	17.38 ± 0.84	22.31 ± 1.48	18.22 ± 0.38	11.32 ± 0.44***	8.84 ± 0.23***	8.2 ± 0.44***
NM	5.88 ± 0.60	5.85 ± 0.43	5.94 ± 0.27	5.44 ± 0.89	4.81 ± 0.79	5 ± 0.66	5.6 ± 0.50	6.33 ± 0.52	5.33 ± 0.55
MF	67.86 ± 5.64	68.89 ± 5.02	69.02 ± 5.22	72 ± 5.03	74.33 ± 5.47	42.17 ± 0.39	94.33 ± 3.22**	163.17 ± 3.44***	122.5 ± 9.85***
IF	70.57 ± 5.83	70.98 ± 5.80	71.05 ± 5.79	78.14 ± 6.57	79.83 ± 6.37	49.66 ± 5.61	98.33 ± 2.80**	166.16 ± 3.31***	135.33 ± 10.65**

	GroupIII			*GroupIV*		
	15 days	30 days	45 days	15 days	30 days	45 days
ML	10.21 ± 0.16	9.41 ± 1.35	10.17 ± 1.16	2.87 ± 0.21***	2.17 ± 0.13***	2.01 ± 0.09***
IL	9.72 ± 0.24	9.38 ± 2.67	9.9 ± 0.09	0.99 ± 0.12***	1.98 ± 0.21***	1.01 ± 0.05***
EL	282 ± 0.13*	218 ± 4.76	235 ± 2.88	385 ± 1.21***	1301 ± 1.10***	1323 ± 1.65***
PEI	482 ± 4.24	102.32 ± 3.67**	98.1 ± 0.25**	9.68 ± 2.05***	7.66 ± 1.35***	4.65 ± 2.00***
NI	5.91 ± 0.60	6.03 ± 0.87	6 ± 0.74	7.55 ± 0.16***	7.69 ± 0.12***	7.99 ± 1.01***
III	17.06 ± 0.39	36.54 ± 10.30	36.17 ± 12.02	9.17 ± 2.13***	7.12 ± 1.38***	6.98 ± 2.33***
NM	5.26 ± 0.62	5.25 ± 0.45	5.52 ± 0.59	7.01 ± 0.23***	6.99 ± 1.12***	7.25 ± 1.02***
MF	63.71 ± 4.96	61.44 ± 10.2	48 ± 5.42	122 ± 2.31***	185.65 ± 1.65***	201.10 ± 0.15***
IF	69.29 ± 5.88	60.44 ± 10.92	54.71 ± 5.19	135.21 ± 1.18***	191.25 ± 2.01***	215 ± 2.45***

Mating behaviour scores of various groups in 15th, 30th and 45th days of experiment are given in the table (mean ± SD), $n = 6$ (number of animals in each group); significant difference from control, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. MF: mounting latency, IL: intromission latency, EL: ejaculation latency, PEI: post-ejaculation interval, NI: number of intromission, III: inter-intromission interval, NM: number of mount, MF: mounting frequency and IF: intromission frequency.

Table 2
Test of potency.

	Control			Group I			Group II		
	15th day	30th day	45th day	15th day	30th day	45th day	15th day	30th day	45th day
E	7.22 ± 1.52	7.02 ± 1.01	8.33 ± 1.21	8.40 ± 1.33	9.03 ± 1.22	10.50 ± 1.76	12.04 ± 1.64***	17.02 ± 1.46***	19.00 ± 0.64***
QF	5.10 ± 0.73	6.22 ± 1.13	6.33 ± 1.21	5.98 ± .34	6.42 ± 1.03	7.00 ± .22	11.30 ± 0.43***	15.32 ± 1.23***	17.30 ± 1.13***
LF	2.16 ± 1.02	3.30 ± 1.49	3.17 ± 1.50	3.02 ± .03	3.48 ± 2.62	4.20 ± .55	8.43 ± 0.03***	11.41 ± 1.04***	12.00 ± 2.26***
TR	14.48 ± 3.07	17.54 ± 3.63	16.83 ± 3.92	17.40 ± 4.70	18.93 ± 4.31	21.70 ± .53	31.77 ± 2.10***	43.75 ± 3.73***	48.30 ± 4.03***

	GroupIII			*GroupIV*		
	15th day	30th day	45th day	15th day	30th day	45th day
E	11.22 ± 2.02*	12.24 ± 2.02**	13.80 ± 1.98**	19.00 ± 2.64***	20.52 ± 1.03***	22.32 ± 2.01***
QF	10.00 ± 1.06**	11.68 ± 1.02**	14.67 ± 1.37**	17.30 ± 3.24***	17.65 ± 2.01***	20.45 ± 1.02***
LF	7.05 ± 2.36**	9.03 ± 0.22**	7.50 ± 1.22**	12.00 ± 2.26***	16.78 ± 1.05***	19.65 ± 1.00***
TR	22.25 ± 6.44**	32.95 ± 3.36**	30.97 ± 4.50**	48.30 ± 6.03***	54.95 ± 4.09***	62.42 ± 4.03***

Test of potency of the various groups in 15th, 30th and 45th days of experiment are given in the table (mean ± SD) $n = 6$ (number of animals in each group); significant difference from control, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. E: erection, QF: quick flip, LF: long flip and TR: total reflex.

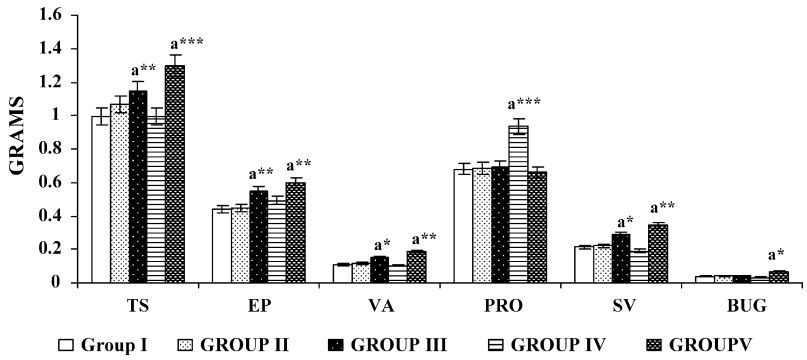

Fig. 1. Effect of drug on organs weight of TS: testis; EP: epididymis; VA: vas deferens; PRO: prostate; SV: seminal vesicle; BUG: bulbourethral gland, each bar depicts mean ± SEM, $n = 6$ (number of animals in each group). (a) Group I, $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

ticularly on the 15th day, the levels of both hormones increased in a significant manner. However, the increase in testosterone and estradiol (Groups II–IV) was lower when compared to the standard reference drug group (Group V).

3.4. Ulcerogenecity

The observation of the stomach showed normal mucosa for doses of 150 and 200 mg/kg (Groups II and III). However, the animals administered with 250 mg/kg MP (Group IV) showed slight erosion in mucosal layer (i.e., scale 0 or 3 pinpoint ulcers) in the course of greater curvature.

3.5. Morphological study

The morphological examination revealed that the organ weights of all the experimental groups increased when compared to the control group. However, a significant increase in organ weight was seen in Group III, but was less than the standard reference drug group (Group V) (Fig. 1).

3.6. Sperm analysis

The epididymal sperm parameters revealed an increase in the number of spermatozoa and motility in all the experimental (MP and SC) groups, i.e. 185, 200, 250, 210 and 270 (million/ml) and 5, 4, 5, 3 and 5 (grade) in Groups I, II, III, IV and V, respectively.

4. Discussion and conclusion

In the present study, sexual behaviour tests showed that the ethanolic seed extract of MP possesses significant sexual function enhancing activity. Mating behaviour test revealed that the test drug at a dose of 200 mg/kg significantly increased the MF, IF and EL in all the experimental days when compared to control. The test drug (200 mg/kg) not only significantly increased the EL but also significantly reducted the ML and IL compared to control, which indicates the aphrodisiac nature of MP. A significant decrease in PEI (the potency and libido) was observed with the administration of MP extract at a dose of 200 mg/kg in all three testing days. The test drug decreased PEI by enhancing either the potency or the libido. ML, IL and PEI were considered inversely proportional to arousal or motivational effects, while III considered inversely proportional to the performance or potency (Beach, 1956). III was found to decrease significantly with a dose of 200 mg/kg MP extract in all three testing days. Similarly, all treated rats copulated more vigorously than controls, especially Group III was very good in overall performance. These results thus provide experimental support to the folk reputation of MP as sexual stimulating drugs without any toxic effect.

The effect on potency was evaluated by testing the effect of the drug on the frequency of penile reflexes such as E, QF and LF. For penile erection, a well-coordinated system of vascular, endocrine and neural networks are required. Hence, a drug that brings about changes in erection and sexual behaviour would induce changes in neurotransmitter levels or at cellular levels (Suresh Kumar et

al., 2000). Penile reflex experiment revealed that the test drug produced a marked increase in potency in all experimental groups with a profound increase seen in Group III (200 mg/kg).

With regard to the efficacy of the MP and SC drugs, SC was predominately used for erectile dysfunction, sexual dysfunction of psychogenic nature, and reported to increase sperm number and functions (Emmanuele et al., 2004); however, exact actions still not clear. In this study, MP showed relatively good result in terms of sexual behaviour, libido, potency and spermatogenic potential. However, it is difficult to interpret the mechanism of MP on potentiation of sexual function from the present study. With studies confirming the action of MP on brain cells, especially dopaminergic neurons (Nagashayana et al., 2000) and dopaminergic pathway controlling sexual activities (Elaine et al., 2004), these correlations strongly suggest aphrodisiac activity through dopaminergic pathway with the presence of high level of L-DOPA in MP. Apart from this, there was an increase in the level of testosterone in MP- and SC-treated animals, which could result in improving total sexual behaviour (McGinnis et al., 1989).

Further, the morphological study revealed an increase in testicular and epididymal weights, along with an increase in sperm count and motility. It was clear that the administration of MP not only increases aphrodisiac activity but also enhances the spermatogenic potential, as the action may be in the hormonal level. With Group III (200 mg/kg) showing best results, it was concluded that changes were dose dependent. The result was close to the effects produced by SC (Group V), which was used as the standard reference drug in the experiment.

Moreover, research should aim at isolating the active principle(s) responsible for aphrodisiac activity and the mechanism by which the drug enhances sexual function. From the present investigation, we conclude that the ethanolic extract of MP 200 mg/kg body weight possesses potent aphrodisiac activity in normal male albino rats without any gastric ulceration and adverse effects. This result is the scientific evidence in favour of the claims made in Indian Systems of Medicine that the MP is clinically useful as sexual invigorator in males.

References

Adebowale, Y.A., Adeyemi, A., Oshodi, A.A., 2005. Variability in the physicochemical, nutritional and antinutritional attributes of six *Mucuna* species. Food Chemistry 89, 37–48.

Agmo, A., 1997. Male rat sexual behaviour. Brain Research Protocols 1, 203–209.

Amin, K.M.Y., Khan, M.N., Rahman, S.Z., Khan, N.A., 1996. Sexual function improving effect of *Mucuna pruriens* in sexually normal male rats. Fitoterapia 67, 53–58.

Anletta, F.J., 1974. In: Jaffe, B.M., Behram, N.R. (Eds.), Methods of Hormone Radio Immunoassay. Academic Press, New York, p. 520.

Beach, F.A., 1956. Characteristics of masculine. Sex drive. In: Jones, M.R. (Ed.), Nebraska Symposium on Motivation. University of Nebraska Press, Lincoln, pp. 1–31.

Cioli, V., Silvestrini, B., Dordoni, F., 1967. Evaluation of potential of gastric ulceration after administration of certain drugs. Experimental Molecular Pathology 6, 68–75.

Davidson, J.M., 1982. In: Zewi, H. (Ed.), Sexology, sexual biology, behaviour and therapy: selected papers of Fifth World Congress of sexology Jerusalem 1981. Amsterdam, Holland. Excerpta Medica, Princeton, Oxford, pp. 42–47.

Elaine, M.H., John, W.M., Satoru, S., 2004. Dopamine and serotonin: influences on male sexual behavior. Physiology and Behavior 83, 291–307.

Emmanuele, A.J., Francesco, L., Pietro, S., Loredana, G., Andrea, L., 2004. Treatment of sexual dysfunctions secondary to male infertility with sildenafil citrate. Fertility and Sterility 81, 705–707.

Harborne, J.B., 1973. Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. Chapman and Hall International, London, pp. 105–185.

Hart, B.L., 1979. Activation of sexual reflexes in male rats by dihydrotestosterone but not estrogen. Physiology and Behaviour 23, 107–109.

Hart, B.L., Haugen, C.M., 1968. Activation of sexual reflexes in male rats by spinal implementation of testosterone. Physiology and Behaviour 3, 735–738.

Kharelep, C.P., 2004. Encyclopedia of Indian Medicinal Plants (Regional Western Therapy Ayurvedic and Other Traditional Usage, Botany). Springer-Verlag, Berlin Heidelberg, New York, pp. 318–319.

Misra, L., Wagner, H., 2004. Alkaloidal constituents of Mucuna pruriens seeds. Phytochemistry 65, 2565–2567.

McGinnis, M.Y., Mirth, M.C., Zebrowski, A.F., Dreifuss, R.M., 1989. Critical exposure time for androgen activation of male sexual behavior in rats. Physiology and Behavior 46, 159–165.

Nagashayana, N., Sankarankutty, P., Nampoothiri, M.R.V., Mohan, P.K., Mohanakumar, K.P., 2000. Association of L-DOPA with recovery following ayurveda medication in Parkinson's disease. Journal of the Neurological Sciences 176, 124–127.

Pant, M.C., Joshi, L.D., 1970. Identification of pharmacologically active substances in the seeds of *Mucuna pruriens* (Abs). Proceedings of the Annual Conference of Indian Pharmacological Society, Lucknow, India. Indian Journal of Pharmacology 2, 24.

Rao, M.R., Parakh, S.R., 1978. Effect of some indigenous drugs on the sexual behaviour of male rats. Indian Journal of Pharmacological Sciences 40, 236.

Rathi, S.S., Grover, J.K., Vats, V., 2002. The effect of *Momordica charantia* and *Mucuna pruriens* in experimental diabetes and their effect on key metabolic enzymes involved in carbohydrate metabolism. Phytotherapy Research 163, 236–243.

Rowland, D.L., Tai, W., 2003. A review of plant-derived and herbal approaches to the treatment of sexual dysfunctions. Journal Sex and Marital Therapy 29, 185–205.

Saksena, S., Dixit, V.K., 1987. Role of total alkaloids of *Mucucna pruriens* bark in spermatogenesis in albino rats. Indian Journal of Natural Products 3, 3–37.

Siddhuraju, P., Vijayakumari, K., Janardhanan, K., 1996. Chemical composition and protein quality of the little known legume, velvet bean (*Mucuna pruriens* (L.) DC.). Journal Agriculture Food Chemistry 44, 2636–2641.

Suresh Kumar, P.K., Subramoniam, A., Pushpangadan, P., 2000. Aphrodiasiac activity of *Vanda tessellata* (ROXB.) HOOK. EXDON. extract in male mice. Indian Journal of Pharmacology 32, 300–304.

WHO, 1987. Laboratory Manual for Examination of Human Semen and Semen-cervical Mucus Interaction, 2nd ed. World Health Organization by Cambridge University Press, pp. 9–10.