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

Sekar Suresh, Elumalai Prithiviraj, Seppan Prakash*

Department of Anatomy, Dr. Arcot Lakshmanasamy Mudaliar Postgraduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai 600 113, India

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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

* Corresponding author. Tel.: +91 44 24547021; fax: +91 44 24540709.
E-mail address: seppanprakash@yahoo.com (S. Prakash).

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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).