Chapter

Phytochemical Analysis and Comparative Aphrodisiac Activity of Four Species of *Mucuna*

M Karunanithi[1]*, C David Raj[1], M Jegadeesan[2] and S Kavimani[3]

[1]Centre for Advanced Research in Indian System of Medicine, SASTRA University, India
[2]Department of Environmental Sciences and Medicinal Botany, Tamil University, India
[3]Department of Pharmacology, College of Pharmacy, Mother Theresa Post Graduate and Research Institute of Health Sciences, India

***Corresponding Author:** M Karunanithi, Centre for Advanced Research in Indian System of Medicine, SASTRA University, Thanjavur District, Tamil-nadu State, India

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Abstract

This study represents a comparative aphrodisiac activity observed in four species of *Mucuna pruriens, Mucuna deeringiana, Mucuna cochinchinesis* and *Mucuna utilis*. Hydroalcoholic extract of seeds of *Mucuna* species at the dose of 100, 200 and 300 mg/1kg p.o was given to the rats using sildenafil citrate 5mg/kg as standard in male rats. The mounting behavior, intromission frequency and ejaculation frequency were significantly increased by the extract administration and they were compared. The hormonal analysis evidence the increase in testosterone level at the 300mg/kg dose level with no effect in the testis weight. The phytochemical studies show the presence of many other phytochemicals along with L-Dopa and they are contributing to the aphrodisiac activity. The data were analyzed by ANOVA followed by the Tukey test. A probability of $P<0.05$ was considered to show significant differences for all comparisons made.

Introduction

Aphrodisiacs, the substances which increase sexual desire are grouped into two main preparative types, one is psychophysiological stimuli preparations and the other is the internal preparations (food and alcoholic drinks) [1]. Many such natural substances have been historically known as aphrodisiacs in Africa and Europe, such as Yohimbine and the Mandrake plant, as well as grounded Rhinoceros horn in the Chinese culture and the toxic "Spanish fly". Erectile dysfunction(ED) is defined as the persistent inability to obtain and maintain an erection sufficient for naturally satisfactory intercourse. Sexual dysfunction is a serious medical symptom and social issue that occurs in 10%-52% of men and 25%-63% of women. Erectile dysfunction is adversely affected by the other conditions like diabetes mellitus, antihypertensive, antipsychotic, and antidepressant therapeutic drugs. Organic causes of ED include hypogonadism, hyperprolactinaemia, and neurological disorders [2]. Treatment of ED involves

several natural aphrodisiacs. Generally elevated testosterone levels enhance the sexual behavior in humans [3]. Erectile dysfunction (ED) is considered as one of the most important public health problem, since it affects higher percentage of men. Although many conventional medical treatments are available, the increasing availability of effective, plant-derived and herbal remedies continue to provide a popular alternative for men seeking to improve their sexual life. Treatment of sexual dysfunctions may improve not only sexual relationships successfully, but also the overall quality of social life [4].

Dopamine (DA), get released in several major integrative areas before and/or during copulation, facilitates sexual motivation, motor performance, and genital reflexes. Dopamine has long been known to facilitate male sexual function. L-Dopa (the precursor of DA) administered to Parkinsonian patients increases libido and sexual potency, and the nonspecific DA agonist apomorphine has been used to treat sexual dysfunction [5].

A WHO study reveals that male reproductive capacity is found to be deficient in no less than 50% of infertile couples (WHO). The incidence of sexual inadequacy in human males has led to the development of a number of available treatment options. These options are expensive with serious side effects. This problem has necessitated the need for less side effect drugs [6, 7]. Medicinal plants continue to provide valuable therapeutic agents, both in modern and in traditional medicine [8]. The use of herbal medicine has become increasingly popular worldwide especially in the Asian and African countries [9].

Mucuna pruriens (L.) DC., the velvet bean, is one of the important herbal drugs in Siddha, Ayurveda and Unani systems of medicine. Seeds of this plant are rich in L-DOPA content. They have been used both as food and medicine for many other common diseases. The seeds have been sold in the herbal drug stores in many parts of India as 'Atmagupta' or 'Poonaikali'. In our preliminary market survey we found that seeds of many other species other than *M. pruriens* are sold as 'Poonaikali' in Tamil Nadu and 'Atmagupta' or 'Kawanch' in other states of India. It is alarming to note that the crude drug trad-

ers and traditional physicians and pharmaceutical manufactures, who use this seed for preparation of medicine, are unaware of its identity and its adulterants. Being a common drug and great demand in India and abroad, it is essential that a standard is to be established on scientific lines for identifying the authentic drug and to detect its adulterants. Though many pharmacological works on seeds of *M. pruriens* are available, no work on comparative study of *M. pruriens* and adulterants of *M. pruriens* is available.

The seeds of *Mucuna pruriens* are said to have Aphrodisiac activity along with other activities. Many times *Mucuna pruriens* is adulterated with other species of the genus like *Mucuna cochinchinesis, Mucuna utilis, Mucuna deeringiana.* Previously detailed pharmacognostical work has been carried out in this department on seeds of *Mucuna* and its adulterants along with the basic phytochemical and pharmacological works [10]. In the present work, we would study a comparative and detailed phytochemistry and pharmacology of the seeds of *Mucuna pruriens* and its adulterants. Common adulterants of *Mucuna pruriens* are *Mucuna cochinchinesis, Mucuna utilis and Mucuna deeringiana*.

Materials and Methods

Collection of Seed Samples

Seeds of '*Poonaikali*' (minimum 2 kg) were purchased from different drug stores in Madurai, Thanjavur, and Chennai. Some of the seed samples were also grown in Tamil University Herbal Garden.

Preparation of Extract

The collected seed samples were thoroughly dried in the open sunlight for 2 days. Then the dried seeds were cleaned and any foreign matter, broken seeds and immature seeds were removed. The seeds were stored in a plastic container at room temperature. Then the seeds were powdered separately in a mechanical way to 60 mesh size. The seed powder was soaked in 70 % ethanol for 72 hours with occasional

shaking. The solvent was decanted and filtered. The marc was subjected to further extraction by repeating the procedure thrice. The solvent was removed from the extract by vacuum distillation. Previous work on the seeds of *Mucuna spp.* [10] had revealed the presence of most of the active principles in alcoholic and water extracts. Hence, in the present analytical and experimental study, only hydro alcoholic extract of the seeds were used.

Qualitative Phytochemical Studies

Qualitative phytochemical analysis was done using the procedure of Kokate [11]. Alkaloids, carbohydrates, tannins and phenols, fixed oils and fats, saponins and gums and mucilages were qualitatively analyzed [12].

UV Spectral Analysis

Mucuna pruriens seed powder dissolved in 0.1N HCl and used as sample. The marketed tablet containing L-DOPA was dissolved in 0.1N HCl and used as standard. Both the sample and standards were scanned from 230 – 330 nm in a Shimadzu UV spectrometer.

The comparative peak of L-DOPA tablet and *Mucuna pruriens* sample were observed.

Atomic Absorption Spectroscopy

Sample Preparation

About 0.1 – 1.0 gram of powered sample or 1- 20 ml of liquid samples are taken in a known weight silica crucible and it is kept in a muffle furnace at 450-500°C for 4-5 hours till it becomes as ash. Liquid samples having more volume are reduced to 1-2 ml before keeping it for ash. Thus obtained ash is dissolved in suitable acid. If necessary this ash will be subjected for digestion as described below:

Description of Methodology

Samples were digested with 1:3 concentrated Nitric acid and Hydrochloric acid on hot plate for 4-5 hrs. They were kept in open

condition. The losses of acid by vaporizations were adjusted by adding the same acid mixture. After cooling they were made up to 250 ml by adding Distilled water. They were filtered through Whatman No. 1 filter paper before AAS analysis.

Aphrodisiac Activity [13]

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.01/690/02/C/ CPCSEA). 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 fourteen groups with six animals per group. Group I represented the control animal, Groups II rats received Sildenafil citrate (5 mg/kg body weight), while the remaining 12 groups were treated with three doses (100, 200 and 300 mg/kg) of hydro alcoholic extract of MC, MD, MP and MU orally for 28 days as per the animal grouping schedule.

Animal Grouping Schedule

Groups	Treatments	Dose (mg/kg., p.o)	No. of animals
G1	Normal control	Water	6
G2	Standard control Sildenafil citrate	5	6
G3	MC-low	100	6
G4	MC-Middle	200	6
G5	MC-high	300	6
G6	MD-low	100	6
G7	MD-middle	200	6
G8	MD-high	300	6
G9	MP-low	100	6
G10	MP-middle	200	6
G11	MP-high	300	6
G12	MU-low	100	6
G13	MU-middle	200	6
G14	MU-high	300	6

Sexual Behavior

To quantify mounting behavior, non-estrus female rats were paired with males treated with a single dose or repeated doses of the plant drug (water suspension or extract). The control rats received the vehicle in an identical manner. The animals were observed for 3 hr and their mounting behavior was scored blindly as described earlier [14]. Half an hour after drug administration, the males were placed individually in a clean aquarium. After 15 min acclimation, a non–estrus female rat was introduced into the arena. The animals remained paired for 3 hr. The number of mounts was recorded by two observers, who were uninformed about the drug treatment. All experiments were performed from 09.00 to 12.00 hr on sunny days (room tem-

perature 27-28ºC). A mount was operationally defined as the male assuming the copulatory position but failing to achieve intromission. Intromission was defined as the male's penis entering the vagina in association with thrusting behavior.

Assessment of Mating Performance

Male rats divided into 14 groups of six each were used in the study. Group 1 represented the control group, which received 10 ml/kg of distilled water orally. Group 2 served as standard and given suspension of sildenafil citrate orally at the dose of 5 mg/kg, 1 h prior to the commencement of the experiment. Groups 3-14 received suspension of the extract orally with hydroalcoholic extract of MC, MD, MP and MU at the doses of 100, 200 and 300 mg/kg, each respectively, once a day for 30 days. The drugs were administered in the evening (17.00 – 18.00 h.) and each male was placed in a separate cage. After 1 hr, five oestrous female were admitted into each cage and they were cohabitated overnight. The stage of the oestrous cycle was determined according to the criteria laid down by Ecksterin *et al.* [15]. The vaginal smear of each female rat was examined under a microscope for the presence of sperm. The number of sperm positive female was recorded in each group.

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 [16].

Histopathological Studies

At the end of 6 weeks, all the animals were euthanized by overdose of anesthesia. Testis was collected, weighed and preserved in 10% neutral buffered formalin and was later processed for microscopic examination and embedded in paraffin. Serial sections of 7 microns thick were obtained using a rotatory microtome. The sections were

deparaffinised and stained with hematoxylin & Eosin. All sections were examined and photographed under Olympus light microscope fitted with Olympus digital camera (Camedia C-7070 wide zoom). Pictures were taken from each specimen at magnification of X10.

Statistical Analysis

Statistical analysis was performed with Graph Pad Prism 5 software (GraphPad Prism Software Inc. San Diego, California, USA). Vacuous chewing movements and tongue protrusions are considered to be nonparametric. Thus, the data were analyzed by Kruskal–Wallis analysis of variance followed by the Dunn's Multiple Comparisons test. Tremor and catalepsy are considered to be parametric, and the data were analyzed by ANOVA followed by the Tukey test. A probability of $P<0.05$ was considered to show significant differences for all comparisons made.

Results

Table 1: Macroscopic details of seeds of *Mucuna pruriens* and its adulterants.

S. No.	Parameters	MP	MC	MD	MU
1	Colour	Black	Dull White	Black	Grey with black spots
2	Weight/100Seeds (gms)	32.72	90.64	162.14	122.36
3	Dimensions of seed LxBxT (mm)	12x9x6	14x10x7	16x11x8	15x11x8
4	Thickness of seed coat (mm)	0.20	0.25	0.17	0.12
5	Dimensions of RaphaeLxB	5x2	7x2	6x2	7x3
6	Thickness of cotyledon (mm)	5.32	6.08	7.61	7.64
7	LOD (%)	5.71	7.13	3.54	10.28

MP - *M. pruriens,* MC - *M. cochinchinensis,* MD - *M. deeringinana,* MU - *M. utilis*

The seeds of *M. pruriens* and its adulterants could be distinguished by their size and shape. Thus seeds of *M. pruriens, M. cochinchinensis* and *M. deeringiana* are oval in shape and smooth and glossy. Seeds of *M. utilis* are angular in shape and smooth and glossy. On the basis of the dimensions, the seeds of *mucuna spp*. could be grouped as medium and small. *M. deeringinana, M. utilis* are medium in size followed by *M. cochinchinensis.* Among all the seed samples, *M. pruriens* seeds are the smallest (Table 1).

Weight of 100 seeds of the four samples has correlation to their size class. Thus among the medium sized class seeds (*M. deeringiana, M. utilis*), *M. deeringiana* has the highest weight. Even though *M. cochinchinensis* is smaller in size the weight of 100 seeds is slightly higher in proportion. *M. pruriens* seeds have lowest weight /100 seeds, among all the samples.

Analytical Values

Analytical values like total ash, acid insoluble ash, acid soluble ash, solubility percentage in water for all the four samples were analysed and their values are given in Table 2. Total ash value is more or less same in all seed samples. *M. utilis* has slightly higher ash value. Acid insoluble ash value is the highest (0.1222) in *M. utilis* and is lowest in *M. deeringiana* (0.0588%), medium values in *M. pruriens* and *M. cochinchinensis* were noted. Value of solubility percentage in water was higher for *M. pruriens* seeds.

Table 2: Analytical values (in percentage) of seed powders.

S. No.	Parameters	MP	MC	MD	MU
1	Total Ash value	3.2732	3.0728	3.0553	3.2908
2	Acid insoluble ash value	0.0854	0.1231	0.0588	0.1222
3	Acid soluble ash value	3.1878	2.9497	2.9965	3.1686
4	Solubility % in alcohol	3.0000	2.9600	6.9200	5.7600
5	Solubility % in water	25.5300	21.2900	19.4700	18.500

Qualitative Phytochemical Studies

Qualitative phytochemical analysis for alkaloids, carbohydrates, tannins, phenols, gums and mucilage, fixed oils and fats, saponins and steroids were screened in the 4 seed samples and were recorded in Table 3. Extractive values in solvents yielded distinct values for the four seed samples. *M. deeringiana* and *M. utilis* did not have any extractive value in pet. ether. All the seed samples had higher extractive values in water followed by in alcohol. Lower values were observed for all the samples in chloroform. The order of extractive values in alcohol is MD > MU > MC > MP; and in water MU > MC > MP > MD; in benzene MD > MU > MC > MP.

Alkaloids

Presence of alkaloids was noted in all the seed samples in benzene, chloroform, alcohol and aqueous extracts. Out of the four extracts, aqueous extracts of the samples gave more amounts of alkaloids followed by benzene extracts.

Table 3: Successive extractive values of seeds in percentage

S. No.	Name of the samples	Benzene	Pet ether	Chloroform	Alcohol	Water
1	*M. pruriens*	1.932	0.014	0.106	0.857	10.059
2	*M.chochinchinesis*	2.634	0.354	0.226	1.697	11.021
3	*M. deeringiana*	5.086	-	0.569	17.354	7.499
4	*M. utilis*	4.269	-	1.011	5.856	12.555

Preparation of Hydroalcoholic Extract

The collected seed samples were thoroughly dried in the open sunlight for 2 days. Then the dried seeds were cleaned and any foreign matter, broken seeds and immature seeds were removed. The seeds were stored in a plastic container at room temperature. Then the seeds were powdered separately in a mechanical way to 60 mesh size. The seed powder was soaked in 70 % ethanol for 72 hours with occasional shaking. The solvent was decanted and filtered. The marc was subjected to further extraction by repeating the procedure thrice. The solvent was removed from the extract by vacuum distillation and subjected for Qualitative Phytochemical screening (Table 4).

Qualitative Phytochemical Screening

Table 4: Qualitative Phytochemical screening of hydroalcoholic extracts of seeds.

		MP	MC	MD	MU
Alkaloids					
	Mayer's reagent	+	+	+	+
	Dragendroff's reagent	+	+	+	+
	Hager's reagent	+	_	_	_
	Wagner's reagent	+	-	+	-
	Mayer's reagent	+	+	+	+
	Dragendroff's reagent	+	+	+	+
	Hager's reagent	+	+	+	+
	Wagner's reagent	+	+	+	+
Carbohydrate	Molisch's reagent	+	+	+	+
	-	-	-	-	
Fehling's reagent	+	+	+	+	
Benedict's reagent					
Tannins	Ferric chloride	+	+	+	+
	+	-	+	+	
&	+	+	+	+	
Phenols					
Gelatin Solution					
Lead acetate					
Gums and Mucilage	Precipitation test	+	+	+	+
Fixed oils and fats	Paper pressing	+	+	+	+
Saponins	Foam Test	+	+	+	+
Steroids	Libermans Test	-	-	-	-

Carbohydrate

Alcoholic and aqueous extracts of all seed samples of *M. pruriens* and its adulterants gave positive reactions to carbohydrates. Seeds of *Mucuna spp.* have more amounts of carbohydrates. It is significant to note that in Benedict's reagent; more positive reactions were noted in all the samples of *Mucuna spp.*

Fixed Oils and Fats

Fixed oils and fats are present in all the seed samples only in benzene extract. *M. cochinchinensis* seeds have moderate amount of fixed oils compared to other seed samples.

Saponins

Aqueous extracts of all the seed samples showed positive reaction to saponins. High amount of saponins are present in the *M. pruriens* and *M. deeringiana*, and moderate amount in other seed samples.

Steroids

Presence of steroid was not observed in hydroalcoholic extracts of all the seed samples.

Tannins and Phenols

Presence of tannins and phenols was observed in hydroalcoholic extracts of all the seed samples.

All the seed samples gave positive reactions for the presence of tannins and phenols. Seeds of *M. pruriens* sample showed qualitative variations in the presence of tannins and phenols; alcoholic extract of *M. pruriens* have thee lower amount of phenols. Aqueous extract of *M. pruriens* has highest content of tannins and phenols. Alcoholic and aqueous extracts of *M. utilis* have almost similar amount of tannins and phenols as that of *M. pruriens*.

Gums and Mucilage

Hydroalcoholic extracts of all the seed samples revealed positive reactions for gums and mucilage. High amount of gums and mucilage was observed in the extracts of *M. pruriens, M. cochinchinensis* and *M. utilis.*

UV Spectral Analysis

Mucuna pruriens seed powder dissolved in 0.1N HCl and used as sample. The marketed tablet containing L-DOPA was dissolved in 0.1N HCl and used as standard. Both the sample and standards were scanned from 230 – 330 nm in a Shimadzu UV spectrometer. The comparative peak of L-DOPA tablet and *Mucuna pruriens* sample were observed.

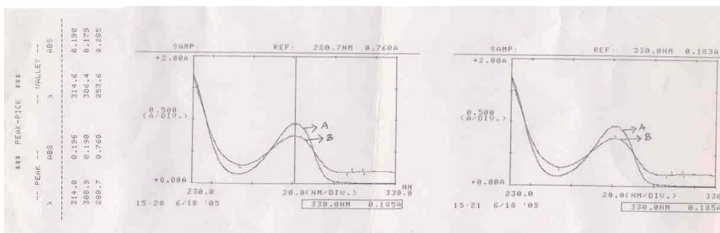

Figure 1: UV Spectrum (A – Standard; B - *Mucuna pruriens* sample).

Atomic Absorption Spectral Analysis

The content of minerals was estimated in hydroalcoholic extract of *Mucuna* seeds. Chromium, zinc, magnesium, copper were estimated in parts per million level. Zinc content was highest in *M. pruriens* and less amounts in other three samples (Table 5). Magnesium content was highest in *M. cochinchinensis*. Heavy metals lead, cadmium and mercury were quantified using respective lamps and they are found within the limit of WHO.

Table 5: Mineral content in hydroalcoholic extract of *Mucuna spp.* Samples.

S. No.	Name of the samples	Cr (ppm)	Zn (ppm)	Mg (ppm)	Cu (ppm)	Pb (ppm)	Cd (ppm)	Hg (ppm)
1	*Mucuna cochinchinensis*	< 1	51.41	171.10	10.23	7.88	0.636	< 10
2	*Mucuna pruriens*	< 1	128.19	154.09	< 0.5	10.93	0.685	< 10
3	*Mucuna deeringiana*	< 1	54.32	155.10	<0.5	4.01	0.372	< 10
4	*Mucuna utilis*	< 1	59.65	159.76	3.93	6.89	0.615	< 10

In-vivo Aphrodisiac Activity

Mounting Behaviour of Male Rats

Mounting behaviour of male rats was studied using different concentrations of extracts of four samples along with standard drug (sildenafil citrate) (Table. 6)

Table 6: Effect of Hydroalcoholic extract of MC, MD, MP and MU on mounting behaviour of male rats.

Treatments	Mounting Latency	Mounting Frequency
NC	37.52±3.173	10.24±1.0476
STD	10.24±0.992**	45.42±3.856**
Mucuna cochinchinensis (100 mg/kg)	35.22±3.024	10.68±0.934
Mucuna cochinchinensis (200 mg/kg)	30.32±2.86*	16.32±1.298
Mucuna cochinchinensis (300 mg/kg)	27.26±2.112	22.14±1.834**
Mucuna deeringiana (100 mg/kg)	34.67±3.224	12.14±1.116
Mucuna deeringiana (200 mg/kg)	29.34±2.221	18.52±1.358*
Mucuna deeringiana (300 mg/kg)	25.64±1.986**	23.46±1.75**
Mucuna pruriens (100 mg/kg)	33.68±2.846	14.86±1.222
Mucuna pruriens (200 mg/kg)	25.52±2.188**	23.54±1.992**
Mucuna pruriens (300 mg/kg)	15.72±1.242**	38.42±2.566**
Mucuna utilis (100 mg/kg)	33.67±3.214	11.68±1.008
Mucuna utilis (200 mg/kg)	29.12±2.146	18.64±1.542*
Mucuna utilis (300 mg/kg)	19.56±0.994**	26.46±2.122**

The values are in Mean ± SEM, n=6, *$p < 0.05$, **$p < 0.01$ and compared with normal control. Values given in parenthesis represent percentage of control values.

The number of mounts was studied for the entire drug treatments and vehicle treated group and it was observed that *Mucuna pruriens* at the dose of 300 mg/kg orally showed significant ($p < 0.05$) increase in number of mounts after extract treatment when compared with normal control. Whereas *Mucuna cochinchinensis* at the highest dose produced significant ($p < 0.05$) changes in number of mounts after treatment. Sildenafil citrate at the dose of 5 mg/kg orally showed significant ($p < 0.01$) increase in number of mounts as compared to normal control.

Test for Libido

The test for libido was carried out by the method of Davidson [17], modified by Amin et al. [18].

Table 7: Effect of Hydroalcoholic extract of MC, MD, MP and MU on sexual function in male rats.

Treatments	Intromission Frequency	Ejaculation Frequency
NC	7.4±0.552	1.4±0.108
STD	22.1±1.886**	7±0.588**
Mucuna cochinchinensis (100 mg/kg)	8.2±0.658	1.8±0.102
Mucuna cochinchinensis (200 mg/kg)	11.22±0.924	2.2±0.118
Mucuna cochinchinensis (300 mg/kg)	12.8±1.168*	2.8±0.196*
Mucuna deeringiana (100 mg/kg)	9.4±0.834	2±0.188
Mucuna deeringiana (200 mg/kg)	13.5±1.268**	2.4±0.194
Mucuna deeringiana (300 mg/kg)	15.4±1.598**	3±0.324**
Mucuna pruriens (100 mg/kg)	10.42±0.992	2.2±0.168
Mucuna pruriens (200 mg/kg)	14.27±1.22**	3.5±0.242**
Mucuna pruriens (300 mg/kg)	18.54±1.0226**	5.4±0.646**
Mucuna utilis (100 mg/kg)	9.46±0.752	1.7±0.122
Mucuna utilis (200 mg/kg)	12.68±1.004*	2.6±0.214
Mucuna utilis (300 mg/kg)	15.6±1.18**	4.4±0.308**

The values are in Mean ± SEM, n=6, $^{**}p < 0.01$ and compared with normal control

Sexually experienced male albino rats were divided into 14 groups of 6 animals each and kept individually in separate cages during the experiment. Group 1 represented the control group, which received 10 ml/kg of distilled water orally. Group 2 served as standard and given suspension of sildenafil citrate orally at the dose of 5 mg/kg, 1 h prior to the commencement of the experiment. Groups 3-14 received suspension of the extract orally with hydroalcoholic extract of MC, MD, MP and MU at the doses of 100, 200 and 300 mg/kg, each respectively, once a day for 30 days.

Since the male animals should not be tested in unfamiliar circumstances the animals were brought to the laboratory and exposed to dim light (in 1 W fluorescent tube in a laboratory area of 14' × 14') at the stipulated time of testing daily for 6 days before the experiment. The female animals were artificially brought into oestrus (heat) [19] by the Szechtman et al. method [20] (as the female rats allow mating only during the estrus phase) They were administered suspension of ethinyl oestradiol orally at the dose of 100 µg/animal 48 h prior to the pairing plus progesterone injected subcutaneously, at the dose of 1 mg/animal 6 h before the experiment. The receptivity of the female animals was confirmed before the test by exposing them to male animals, other than the control, test and standard animals. The most receptive females were selected for the study. The experiment was carried out on the 7th day after commencement of the treatment of the male animals. The experiment was conducted at 20:00 h in the same laboratory and under the light of same intensity. The receptive female animals were introduced into the cages of male animals with 1 female to 1 male. The animals were observed for the Mounting Frequency (MF) on the evening of 30th day at 20:00 h. The penis was exposed by retracting the sheath and 5% xylocaine ointment was applied 30, 15 and 5 min before starting observations. After genital anaesthetization, this does away with the reinforcing effect of genital sensation thus, affording the study of pure libido or intrinsic sexual desire. Each animal was placed individually in a cage and the receptive female rat was placed in the same cage. The number of mountings was noted. The animals were also observed for intromission and ejaculation.

From the Table 7, it was clearly prominent that *Mucuna* deeringiana, *Mucuna* pruriens, *Mucuna* utilis at the dose of 300 mg/kg and sildenafil citrate (5 mg/kg, p.o.) showed a significant increase in sexual function when compared with normal control rats.

Hormonal Analysis

By the end of 30 days, rats were anesthetized and the blood was collected and serum was separated after centrifugation (4500 rpm, 20 min, 4°C) and frozen at -20°C for the measurement of serum estradiol and testosterone. Serum estradiol and testosterone were measured by radioimmunoassay using commercial kits (Diagnostic Products Co., Los Angeles, USA). All the samples were run in the same assay to avoid variations between assays. Sensitivity of testosterone assay was 40 ng/dl and that for estradiol assay was 8 pg/ml.

Table 8: Effect of Hydroalcoholic extract of MC, MD, MP and MU on Serum estradiol and Testosterone in male rats.

Treatments	ESTRADIOL pg/ml	TESTOSTERONE ng/ml
Normal Control	1.36±0.5173	0.34±0.06782
Sildenafil citrate (5 mg/kg; p.o.)	7.78±2.464**	1±0.1414**
Mucuna cochinchinensis (100 mg/kg)	1.4±0.04472	0.31±0.01
Mucuna cochinchinensis (200 mg/kg)	1.39±0.01	0.32±0.02236
Mucuna cochinchinensis (300 mg/kg)	1.84±0.06708	0.34±0.06782
Mucuna deeringiana (100 mg/kg)	1.15±0.02236	0.35±0.02236
Mucuna deeringiana (200 mg/kg)	1.81±0.04472	0.65±0.06708
Mucuna deeringiana (300 mg/kg)	3.64±0.5899	0.8±0.08944**
Mucuna pruriens (100 mg/kg)	1.45±0.02236	0.41±0.02236
Mucuna pruriens (200 mg/kg)	4.35±0.06708	0.49±0.01
Mucuna pruriens (300 mg/kg)	10.84±1.779**	1.38±0.2245**
Mucuna utilis (100 mg/kg)	1.47±0.01225	0.37±0.01225
Mucuna utilis (200 mg/kg)	2.77±0.01225	0.47±0.01225
Mucuna utilis (300 mg/kg)	4.26±0.02449	0.96±0.02449**

The values are in Mean ± SEM, n=6, $^{**}p < 0.01$, $^{***}p < 0.001$ and compared with normal control.

Serum Testosterone Level

There was no significant difference in the serum levels of Estradiol and testosterone in rats treated with 100 mg/kg and 200 mg/kg of the extracts for 45 days when compared with their control animals. The serum estradiol and testosterone concentration was found to be 1.36±0.5173 and 0.34±0.06782 ng/ml in the control rats while it was 10.84±1.779 and 1.38±0.2245 ng/ml respectively in those treated with 300 mg/kg of hydroalcoholic extract of *Mucuna pruriens*. But the rats treated with *Mucuna deeringiana* and *Mucuna utilis* at the dose of 300 mg/kg showed significant increase in serum testosterone level when compared with that of control rats, whereas there was no significant increase in serum estradiol level (Table 8).

There was no significant change in testis weight of all the treated groups when compared with the normal control (Table 9).

Histopathology

The testis of the control rat showed the presence of seminiferous tubules embedded in interstitial connective tissue. Spermatogenetic cells forming a stratified epithelial and sperms are often found in clusters embedded in cytoplasm of sertoli cells. The Leydig cells are occasionally found in each seminiferous tubule. Spermatogenesis also observed (Plate 1). The cross section of the testis in the animals treated with Sildenafil citrate at the dose of 5 mg/kg, p.o. shows the presence of good seminiferous tubules with uniform arrangement of numerous sertoli cells. There is no evidence of testicular cell inflammation (Plate 2). A group of sperms thread like on the center of seminiferous tubules is observed clearly in all the slides.

Weight of the Testis

The effect of hydroalcoholic extract of four samples on weight if testis was studied.

Table 9: Effect of Hydroalcoholic extract of MC, MD, MP and MU on weight of the testis in male rats.

Treatments	Weight of the testis in gm
Normal Control	1.69±0.05
Sildenafil citrate (5 mg/kg; p.o.)	1.68±0.07
Mucuna cochinchinensis (100 mg/kg)	1.72±0.03
Mucuna cochinchinensis (200 mg/kg)	1.69±0.05
Mucuna cochinchinensis (300 mg/kg)	1.71±0.08
Mucuna deeringiana (100 mg/kg)	1.80±0.04
Mucuna deeringiana (200 mg/kg)	1.78±0.06
Mucuna deeringiana (300 mg/kg)	1.81±0.07
Mucuna pruriens (100 mg/kg)	1.70±0.09
Mucuna pruriens (200 mg/kg)	1.68±0.03
Mucuna pruriens (300 mg/kg)	1.69±0.06
Mucuna utilis (100 mg/kg)	1.68±0.05
Mucuna utilis (200 mg/kg)	1.70±0.04
Mucuna utilis (300 mg/kg)	1.71±0.07

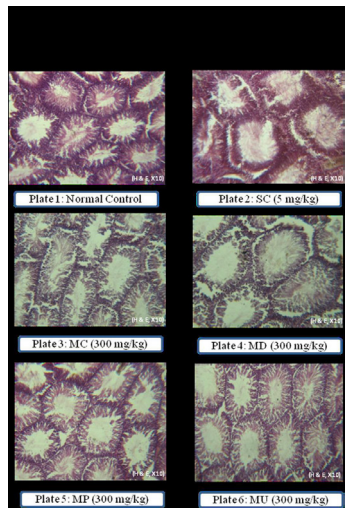

Figure 2: Histopathology analysis of Testis.

Discussion

Atomic Absorption Spectral study of hydroalcoholic extract of *Mucuna spp.* reveals the presence of minerals Zn, Cr, Mg and Cu. The heavy metals content in the extracts are within the WHO admissible limit. Zinc is an aphrodisiac and important in the production of testosterone [21]. The content of zinc in *M. pruriens* is remarkably high when compared with other three species. This higher content would be supporting the *M. pruriens* to have highest aphrodisiac activity among all four species in our study. Magnesium is one of the principal cations of soft tissues and present inside the red blood cells. Chief function of copper is haemopoiesis. Copper is an essential constituent of enzymes like cytochrome oxidase, catalase, tyrosinase, manoamino oxidase, ascorbic acid oxidase and uricase [22]. If haemopoiesis is maintained well, it will support the aphrodisiac activity.

The term aphrodisiac originated from the Greek word Aphrodite, eulogizing the Greek goddess of love and romance. In modern times, this term has been used for substances that enhance sexual activity and are helpful in treating sexual dysfunction [23].

Increase in testosterone level has been associated with a moderate but significant increase in sexual desire as well. Clinical data on testosterone also suggest that a slightly increased level of testosterone in adult males results in an increased sexual desire and arousability. There is also sufficient evidence that for peripheral responses in nervous system an increased testosterone level is a must which is not the case, in case of CNS activity [24]. Therefore, an improved serum testosterone level after administration of extracts could be considered as one of the contributing factors responsible for an overall incremented sexual performance in treated groups. Generally elevated testosterone level also enhanced the sexual behaviour in humans. Moreover, drugs induced changes in neurotransmitter levels or their action at cellular level could also change sexual behaviour [25]. The standard drug Sildenafil citrate was used as a reference only for quantitative comparison and not for mechanistic purpose.

It is likely that the extracts help in the secretion of testosterone and make it better available to gonads. Testosterone production could be a result of gonadotropic activity as well as an increased availability of precursors in the form of steroidal components [26].

The production of testosterone is responsible for androgenic activity as well as development of male accessory sexual organs viz. prostate gland, seminal vesicles, vas deferens, and epididymis [27]. The mechanism of action of testosterone spans from an increased rate of protein formation in target cells. Within a few minutes, testosterone is converted to dihydrotestosterone (DHT) with the help of enzyme 5 a reductase. DHT binds with the cytoplasm receptor proteins and migrate to the nucleus stimulating DNA and RNA transcription process [28]. The activation of RNA polymerase occurs and finally production of protein is enhanced which results in an increase in body mass as well as weight of secondary sexual organs [29].

L-DOPA is the remarkable precursor for the needed and beneficial neurotransmitter dopamine. Clinically proven, it is one of the few substances that cross the blood brain barrier, converting into Dopamine, and thereby stimulating the hypothalamus and pituitary to release and increase the level of growth hormone in the body. Dopamine also regulates motor control, sex drive, immune function, fat gain and loss, lean muscle gain, bone density, energy levels, and the ability to sleep soundly. The high concentrations of L-DOPA contained, are converted to dopamine which acts in a way to release of growth hormone via the pituitary gland. L-DOPA and dopamine also provide another extra benefit they are both inhibitors of prolactin. High levels of prolactin are thought to be the reason that 70% of males fail to get an erection.

Zinc plays a major role in the production of testosterone. Zinc not only helps produce testosterone but also assists to maintain semen volume and keeps sperm healthy. The increase in the level of zinc was seen in *Mucuna pruriens* and this will be one of the contributing factors for the extract to have aphrodisiac property.

The significant increase in the indices of sexual vigor and the significant decrease in mount and intromission latencies are indications of the aphrodisiac potential of the extract. In this study, the marked effects on the sexual behaviour parameters, compared with the control, are indications of stimulation in the desire component of sexuality. Apart from the desire that is essential for initiation of sex, penile tumescence and rigidity as well as the accessory muscles that help in providing additional penile rigidity and ejaculation are dependent on testosterone for normal sexual activity [30]. Such increase in the frequency of mount and intromission suggests that libido, sexual vigor and sexual performance were unimpaired [19]. The prolonged ejaculatory latency indicates enhancement of sexual function and suggests an aphrodisiac action.

It has been documented previously that sexual behaviour and erection are dependent on an androgen that may be acting both centrally and peripherally [31]. Testosterone supplementation has previously been shown to improve sexual function and libido [32], in addition to the intensity of orgasm and ejaculations which might also be expected to improve [33]. The continued administration of the plant extract for five days at various doses which led to the significant increase in serum testosterone may be responsible for the marked effect on sexual behaviour indices of the male rats. Increase in testosterone levels in the present study may thus account for the observed masculine behaviour.

Studies have implicated the saponin component of plants in enhancing aphrodisiac properties due to its androgen increasing property [30]. Saponins present in the hydroalcoholic extract of this plant might have assisted in stimulating an increase in the body natural endogenous testosterone levels by raising the level of leutinizing *hormones* (LH). This LH released normally by the pituitary gland helps to maintain testosterone levels; as LH increases, so does the testosterone [30]. The increase in testosterone seemed to have translated into the male sexual competence observed in this study.

The sexual behaviour was studied with hydroalcoholic extract of all four seed samples *Mucuna spp.* The mounting frequency is increased in all seed extracts at the dose of 300 mg/kg. *Mucuna pruriens* shows highest activity among all samples. Intromission frequency and ejaculation frequency are also improved by the *Mucuna* seed extracts. *M. pruriens* shows higher activity even at the 200mg/kg dose when other species exhibit significant activity at the 300mg/kg dose. *Mucuna* cochinchinensis shows moderate activity in intromission frequency and ejaculation frequency. Furthermore, this study suggests that the aphrodisiac action may be mediated through a change in the blood testosterone level; increase in L-DOPA concentration, increased zinc level and due to the presence of saponin content.

Conclusions

From the present investigation, it is concluded that out of four samples of 'Poonaikali', *M.pruriens* is the authentic and effective drug. Though *M. cochinchienensis, M. deeringiana* and *M.utilis* are adulterants, they could be used as substitutes for *M. pruriens*. *M. pruriens* is the authentic and effective aphrodisiac drug. The L-DOPA content is more in *M. pruriens*. Further clinical trial is needed to support this conclusion. As on offshoot of this work, activity guided fraction of *M. pruriens* seeds, isolations, purification and characterization of compounds in the extracts could be carried out for future work.

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