APHRODISIAC EFFECT OF ETHANOLIC EXTRACT OF MUCUNA PRURIENS SEED IN MALE ALBINO MICE

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ABSTRACT

The seed of Mucuna Pruriens Linn, belonging to the leguminous family (Papilionacae) has been used in Ayurvedic medicine since ancient times for treatment of male sexual disorder. This study was aimed at evaluating the aphrodisiac effect of the ethanolic extract of Mucuna Pruriens seed in normal male albino mice by looking at the general mating behaviour, libido and potency using sildenafil citrate as the standard reference drug and also to investigate the possible mechanism by which the drug enhances sexual function. Animals were divided into five groups. The first group was used as control (received distilled water) and experimental groups 2 - 5 were divided on the basis of the dosage of extract to the animals as follows: 150mg/kg body weight (group 2), 200mg/kg body weight (group 3) and 250mg/kg body weight as standard. Animals were fed per oral (PO) with distilled water or extract or standard once a day for 7days. Female mice with oestrus phase were used for mating behaviour. The acute toxicity test done showed that Mucuna Pruriens seed has LD_{50} > 2000mg/kg. The extract administered significantly ($P<0.05$) increased the mounting frequency, intromission frequency and ejaculation latency and decreased the mounting latency, intromission latency and post-ejaculations intervals. The potency test significantly ($P<0.05$) increased erections, quick flips, long flips and total reflexes. The results indicated that the ethanolic extract of Mucuna Pruriens produced a significant and sustained increase in sexual activity of normal male mice without any conspicuous gastric ulceration and adverse effects.

INTRODUCTION

A medicinal plant can be described as any plant in which one of its organs contains substance that can be used for therapeutic purposes or materials for the synthesis of useful drugs[1]. Medicinal herb and plant extracts are now generally considered as effective medicines to be respected and appreciated and they play a major role in modern pharmacy[2].

The use of herbs is very common in developing countries particularly in rural setting.

However, during the last decade, an increase in the use of plants has been observed in metropolitan areas of developed countries[3]. Plants are extensively used to relive sexual dysfunction for example *Rauwolfia vomitoria, Garcina Kola* etc[4]. In many countries, different varieties of plants have been used as sexual stimulants in traditional medicine, one such plant, which claims various medical properties, is *Mucuna pruriens, Linn,* one of the popular and important medicinal plants of India. Sexual dysfunction is a common problem with increase in prevalence and etiological factors including degeneration disease, increase in injuries and stress associated with industrialized lifestyles. Sexual dysfunction can be treated by both medical and surgical treatment modalities, however, plants 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 treatment[5].

Mucuna pruriens has been recognized as an aphrodisiac agent. The plant and its efficacy in treating sexual disorder have been documented in Ayurveda, but lacks scientific validation.[6] It has been reported that the number of spermatozoa increased when the rats were treated with bark extract of *Mucuna*

pruriens. Further, it was also reported that the sexual and androgenic activities in adult male rats were sustained while improving the mass of the muscles [7] and[8]. The relevant parameters such as sexual behavior potency, libido, acute toxicity and possible mechanism by which this plant produces aphrodisiac effect has not been reported to the best of our knowledge. This present study aimed at finding scientifically the aphrodisiac effect of *Mucuna pruriens,* and determining the possible mechanism through which the plant produces aphrodisiac effect.

MATERIALS AND METHODS

Collecting and Preparation of Plant Materials

Dried velvet bean *(Mucuna pruriens)* fruits were collected locally from Amatutu village, Agulu in Anaocha Local Government Area, Anambra State, Nigeria and were identified by a botanist Mr. Ezioko at the Department of Botany, University of Nigeria, Nsukka. The seeds were removed from their hairy husks of the fruits and stored in a dried plastic container.

The dried seeds were oven dried at 40°c to allow the seed to be removed from their arilluses. The dried seeds were then pulverized into coarse powder using an electric blender (Blender/Miller III, Model MS-223, Taiwan, China) and sieved through No. 20 mesh size. The powder obtained was stocked in a plastic container and was weighed in a weighing balance. The overall weight was 44gm.

Solvent used for Extraction

Ethanol and distilled water were mixed in the ratio of 1:1 (i.e.) 100ml of ethanol and 100ml of distilled water to get 50% absolute ethanol which was used for sequential extraction of the powdered seeds of *Mucuna pruriens*.

Method of Extraction

The dried coarse *Mucuna pruriens* weighing 44gms were used for the extraction. The extraction was carried out by mixing the coarse powdered seeds in 50% ethanol using soxhlet apparatus. The extraction was carried out in cycles in which 15gm of the powdered seed was extracted per sample using 200ml of 50% absolute ethanol at a temperature of 70°c and each cycle lasted for 48hrs. The extract was filtered and the solvent from the filtrate was removed by open air evaporator under reduced pressure and low temperature. The weight of the extract was 5.05gm and the yield was 11.48% in terms of dried starting material. It was blackish and of pleasant smell. The extract was preserved in a refrigerator.

Stock Solution

1 gm of ethanolic extract of *Mucuna pruriens* was suspended in distilled water using Tween 80 (1%) in the ratio of 2:1; when calculated it gave a stock solution of 50mg/ml as the working stock. Distilled water was used as a vehicle.

Phytochemical Screening

Chemical test was carried out on the ethanolic using stardard procedure to identify the constituents as described by[9], [10] and [11].

Experimental Animals and Drug Preparation

Twelve week old female (body weight 26 - 30gm) and male (body weight 30 - 35gm) albino mice were used for the study. The mice were housed in standard cages and maintained under standard laboratory conditions (temperature 24 - 29°c, relative humidity 60 - 70% and 12h light/dark cycle) with free access to solid pellet diet (Vital Feed Nigeria) and water adlibitum throughout the study except during experiment. The Ethical Committee of the College for Animal Cares and Use, Nnamdi Azikiwe University, Nnewi Campus approved the study design. Animals were randomly divided into five groups with six animals per group.

Drug Preparation

Since *Mucuna pruriens* in Ayuredic medicine is orally administered, therefore, the extract of *Mucuna* seed was suspended in distilled water using Tween 80 (1%) for oral administration.

Sildenafil citrate and ethinyloestradiol were also suspended in distilled water using Tween 80 (1%) separately, for oral use. Progesterone was dissolved in castor oil for subcutaineous injection. All the drug solutions were prepared just before administration. Dosage of *Mucuna pruriens* was selected according to[12] with ± 50mg to confirm effective concentration.

Acute Toxicity Testing

The acute toxicity test of the extract was done by Up and Down Procedure (UDP) in accordance with the organization for Economic Co-operation and Development[13]. Doses were prepared shortly prior to administration. The extract was administered in a single dose. Healthy five male mice were used for the experience. They were fasted prior to dosing by withholding only food not water for 3 - 4 hours. The limit test was done first to know whether the main test should be done. Following the period of fasting, the animals were weighed and the suspension of the extract was administered PO at the dose of 200mg/kg. The animals were observed continuously for the initial 4h for behavioural changes and mortality and intermittently for the next 6h and then again at 24h and 48h after the administration of the dose. The behaviour parameter observed were convulsion, hyperactivity, sedation, grooming and loss of weighting reflex, increased respiration and death. The LD_{50} is greater than 2000mg/kg if three or more animals survive and the experiment is terminated[13].

Determination of the Aphrodisiac Effect of *Mucuna Pruriens* Extract Mating Behaviour Test

The test was carried out by the methods of [14] and[15], modified by [8].

Healthy and sexually experienced thirty male albino mice weighing 30 - 35gm were used. Animals that were showing brisk sexual activity were selected for the study. Female mice showing regular oestrus cycle were used for mating behaviour analysis. The receptivity of the female mice was confirmed before the test by exposing them to male mice. The receptive females were selected for the study. They were divided into 5 groups of 6 animals each and kept singly in separate cages during the experiment. Group 1 represented the control, which received 10mg/kg of distilled water only.

Group 2 - 4 received suspension of the extract of *Mucuna* orally at the doses of 150, 200 and 250(mg/kg) respectively, daily for 7days at 18:00h. Group 5 served as standard and was given suspension of sildenafil citrate orally at the dose of 5mg/kg, 1h prior to the commencement of the experience. Since the male animals should not be tested in unfamiliar circumstance, the animals were brought to the laboratory and exposed to dim light (in 1 watt fluorescent tube) at the stipulated time of testing daily for 6 days before the experiment. The female animals were artificially brought into oestrus (heat)[16] by the[15] method (as the female mice allow mating only during the oestrus phase). They were administered suspension of ethinyloestradiol orally at the dose of l00mg/animal 48h prior to the pairing plus progesterone injection subcutaneously, at the dose of 1mg/animal 6h before the experiment. The experiment was carried out on the 8th day after commencement of the treatment of the male animals. The experiment was conducted at 20:00h in the same laboratory and under the light of same intensity. The receptive female animals were introduced into the cages of male. Mating behaviours were recorded and used for further analysis by giving scores for first four mating series. The test was terminated if the male failed to evince sexual interest. If the female did not show receptivity she was replaced by another artificially warmed female. The occurrence and disappearance of events and phases of mating were recorded as soon as they appeared. Their disappearance was also called out recorded. Later, the frequencies and phase were determined by the recorded transcription: number of mounts before ejaculation or mounting frequency (MF),

number of intromission before ejaculation of 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 up to the next intromission by male or Post Ejaculatory Interval *(PEI)*. The pre-coital sexual behaviours such as chasing, nosing, anogenital sniffing and mounting were observed for up to 2h of pairing. The values of the observed parameters for control and experimental groups were recorded. The values for the observed parameters of controls test and standard animals were statistically analyzed using one-way analysis of variance (ANOVA) method.

Test for Libido

Libido was assessed according to the method described by Davidson[17], later modified by[8]. Sexually experienced male albino mice were divided into 5 groups of 6 animals each and kept singly in separate cages during the experiment.

Group 1 represented the control group, which received l0mg/kg of distilled water orally, group 2 - 4 received suspension of the extract orally at the doses of 150, 200 and 250(mg/kg) respectively, once a day in the evening (18:00) for 7days. Group 5 served as standard and given suspension of sildenafil citrate at the dose of 5mg/kg, 1h prior to the commencement of the experiment. The female mice were made receptive by hormonal treatment and all the animals were accustomed to the testing condition as previously mentioned in mating behavior test.

The animals were observed for the mounting frequency (MF) on the evening of 8th day at 20:00h. The penis was exposed by retracting the sheath and 5% lignocaine ointment was applied 30, 13 and 5 minutes before starting observations. Each animal was placed individually in a cage and the receptive female mouse was placed in the same cage. The number of mountings was noted. The animals were also observed for intromission and ejaculation. The MF in control, test and standard animals was statistically analyzed by employing one-way analysis of various (ANOVA) method.

Test for Potency

The effect of the *Mucuna pruriens* on potency was studied according to the method described by[18] and [19] modified by[8]. The male mice were divided into 5 groups of 6 animals each and kept singly in separate cages during the experiment. Group 1 represented the control group, which received l0ml/kg of distill water orally. Group 2-4 received suspension of the test drug orally at the doses of 150, 200 and 250 (mg/kg) respectively daily for 7 days. Group 5 received a suspension of sildenafil citrate orally at the dose of 5mg/kg. 1h before the commencement of the experiment. On the 8th day the test for penile reflexes was carried out by placing the animals on its back in a glass cylinder partial restraint.

The preputial sheath was pushed behind the glans by means of thumb and index finger and held in this manner for a period of 15mins. Such stimulation elicits a cluster of genital reflexes. The following components were recorded. Erections (E) Quick Flip (QF) and Long Flips (LF) and total penile reflexes (TPR). The frequency of these parameters observed in control, test and standard groups were statistically analyzed by using one-way analysis of variance (ANOWA) method.

Statistical Analysis

Data were presented as the mean ± SD (n = 6). The significant difference between the mean value of control and experimental groups was determined by one-way analysis of variance (ANOVA) with post hoc-test. P value < 0.05 was considered as statistically significant[18].

Results

Weight of ethanolic extraction fraction from *Mucuna pruriens* seeds. The weight of the extract was 5.05gm and that of the dried

powdered drug was 44gm, the percentage yield:

$$\% \text{ yield} = \frac{5.05}{44} \times \frac{100}{1} = 11.48\%$$

% yield was 11.48%

Phytochemical Analysis

Phytochemical screening of the ethanolic extract of *Mucuna pruriens* seeds showed the presence of alkaloids, saponins, glycosides and amino-acids. It is also contain tannins, flavonids, steroids carbohydrate and terpenoids.

Acute Toxicity Test

Acute toxicity studies showed no mortality and normal behaviour was observed in all the treated mice thus the LD_{50} >200mg/kg.

The Aphrodisiac effect of *Mucuna pruriens* seed. Mating behavior. Test for libido and test for potency. The data obtained with the mating behaviour test indicated that *mucuna pruriens* extract at the dose of 150mg/kg did not significantly affect the MF, IF, EL, and PEL; the ML and IL were decreased but not in a significant manner. The dose 200mg/kg increased the mounting frequency (MF) ($P<0.01$), intromission frequency (IF) ($P<0.01$); ejaculatory latency (EL) ($P<0.01$), intromission latency (IL) ($P<0.01$) in a significant manner. However, the standard drug increased the MF ($P<0.01$) IF ($P<0.01$); and IL ($P<0.01$) and PEL ($P<0.01$) in a highly significant manner when compared to control figures 1 to 5 and table 1.

Whereas, the dose of the test drug at 25omg/kg of the extract significant increase the IF ($P<0.01$) but did not significantly affect the post ejaculatory interval (PEL) MF, and IF the ML and IL were decreased but not to significantly manner.

The test for libido showed that the pre-coital sexual behaviours such as chasing, nosing and anogenital sniffing were well performed in the Group 3 (200mg/kg) whereas in control, Groups 2 and 4 the behaviours were not to the extent seen in Group 3 (table2).

However, effect of Group 3 showed less than Group 5 and also increased the MF in a significant manner ($P<0.05$). The extract at the doses of 150mg/kg and 250mg/kg did not significantly alter the MF. The standard drug striking increased the MF.

($P<0.01$), intromission and ejaculation were absent in control, test and standard groups table 1.

The test for potency exhibited that the higher dose (250mg/kg) of the test drug significantly increased the frequency of erections (E) ($P<0.05$), quick flips (QF) ($P<0.01$) long flips (LF) ($P<0.01$) as well as the aggregate of these penile reflexes (APR) ($P<0.01$) the extract at the dose of 200mg/kg significantly increased the E ($P<0.01$) QF ($P<0.001$), LF ($P<0.01$) and TPR ($P<.01$) comparatively less than standard drug, whereas, the test drug at the dose of 150mg/kg did not alter the E, QF and TPR in a significant manner (figure 6).

Table 1: Effect of 50% ethanolic extract *of Mucuna pruriens on* mating behaviour in male mice

Parameters	Control (Group 1) (10ml/kg)	Group 2 (150mg/kg of extract)	Group 3 (200mg/kg of extract)	Group 4 (250mg/kg of extract)	Group 5(5mg/kg of Sildenafil citrate)
Post ejaculatory Interval (PEI, in sec.)	239.4±0,19	248 ± 0.17	212.5 ± 0.14**	241 ± 2.1:2	4.84 ± 1.03***
Number of intromission (M)	2.94±0.34	3 ± 0.37	3.08 ± 0.35	2.85 ± 0.3	3.73 ± 0.08***
Number of Mount (NM)	2.94 ± 0.3	2.72 ± 0.45	2.8 ± 0.25	2.63 ± 0.31	3.5 ± 0.11***

Mean ± SD; $n = 6$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

In table 1, the standard drug increased the NI ($P < 0.001$), NM ($P < 0.001$) as well as decreased PEI ($P<0.001$) in a highly significant manner when compared to control. The dose of the test drug at 250mg/kg of the extract did not significantly affect the post ejaculatory interval (PEI).

Table 2: Effect of 50% ethanolic extract of *Mucuna pruriens* on tests for libido of male mice

Test for libido (sec)	Control (Group 1) (l0ml/kg)	Group 2 (150mg/kg of extract)	Group 3 (200mg/kg of extract)	Group 4 (250mg/kg of extract)	Group 5 (5mg/kg of Sildenafil citrate)
Mounting Frequency	16.17±1 .41	18.33±l.26	23.57±0.30*	11.42±1.24	35.50±0.53**
Intromission Frequency	Nil	Nil	Nil	Nil	Nil
Ejaculation	Absent	Absent	Absent	Absent	Absent

Mean ± SEM; n = 6; *P < 0.05, ** P < 0.01.

In table 2, Group 3 showed less effect than Group 5 and also increased the mounting frequency (MF) in a significant manner ($P<0.05$). The extract at the doses of 150 mg/kg and 250 mg/kg did not significantly alter the MF. The standard drug strikingly increased the MF ($P < 0.01$). Intromission and ejaculation were found absent in control, test and standard groups.

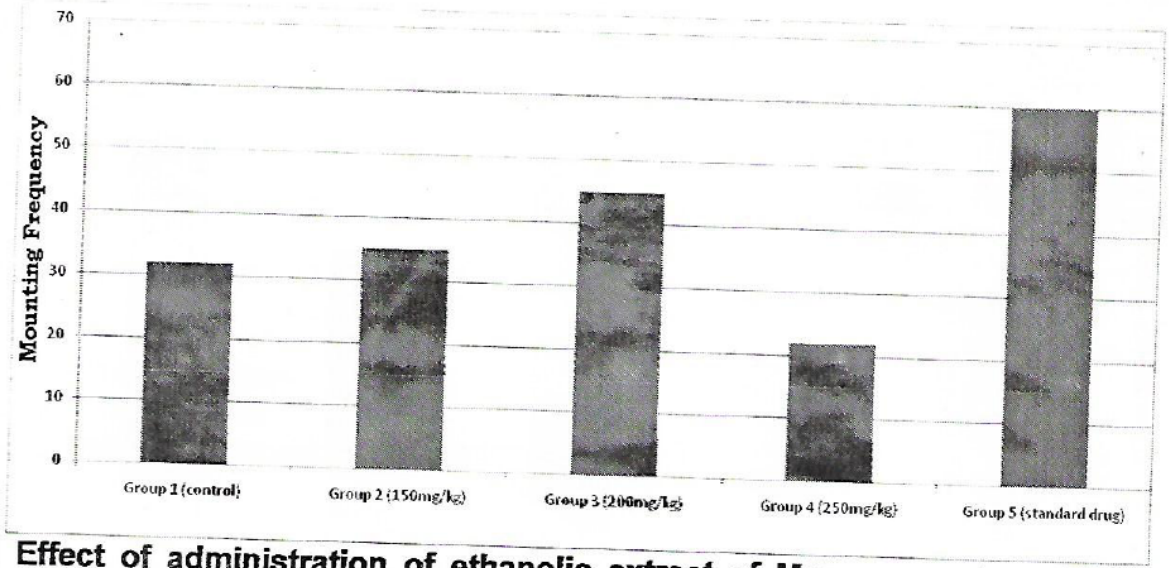

Fig 1: Effect of administration of ethanolic extract of Mucuna pruriens seed on the mounting frequency of male mice

This figure shows that the test drug at the dose of 200mg/kg significantly increased the MF as compared to control but less than that of the standard drug.
Mean ± SD; $n = 6$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
MF: Mounting frequency

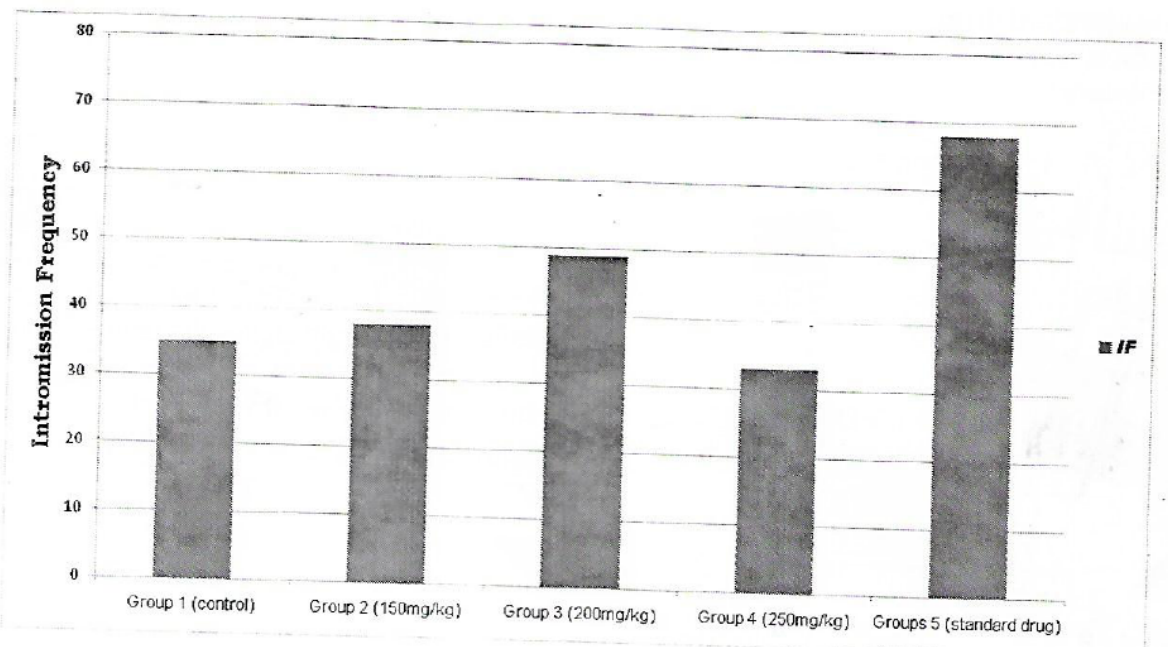

Fig 2: Effect of administration of ethanolic extract of Mucuna pruriens seed on the intromission frequency of male mice

This figure shows that the test drug at the dose of 200mg/kg significantly increased the IF as compared to control but less than that of the standard drug.
Mean ± SD; n = 6; *$p < 0.01$; ***$P < 0.001$.
IF: Intromission Frequency

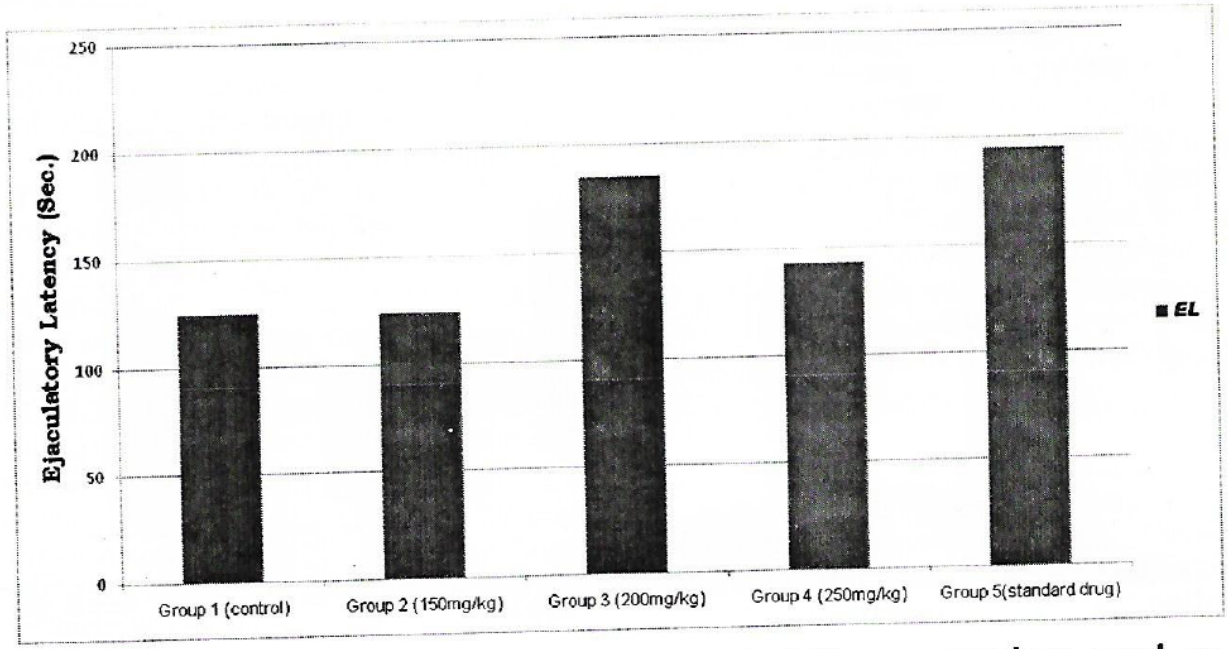

Fig 3: Effect of administration of ethanolic extract of Mucuna pruriens seed on the ejaculatory latency of male mice

This figure shows that the test drug at the doses of 200mg/kg and 250mg/kg significantly increased the EL as compared to control but 200mg/kg is higher in significance; it is also less than that of the standard drug.
Mean ± SD; n = 6; $*p < 0.05$; $**P < 0.01$; $***P < 0.001$.
EL: Ejaculatory latency

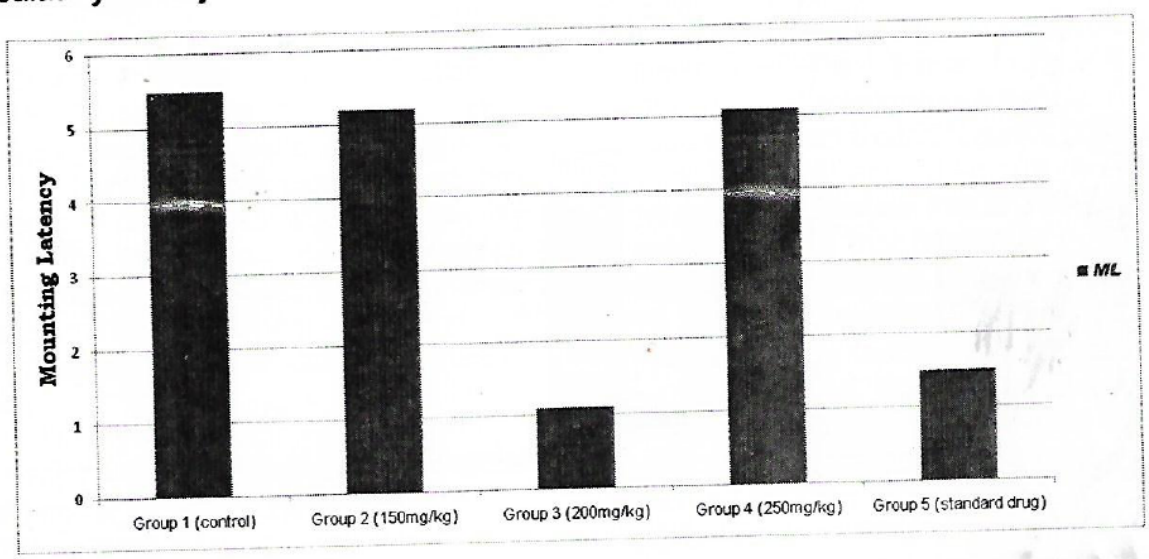

Fig 4: Effect of administration of ethanolic of Mucuna pruriens seed on the mounting latency of male mice

This figure shows that the test drug at the dose of 200mg/kg produced a significant reduction in the ML when compared to control, which indicates the aphrodisiac nature of *mucuna pruriens*.
Mean ± SD; n = 6; $*p < 0.05$; $**P < 0.01$; $***P < 0.001$.
ML: Mounting latency

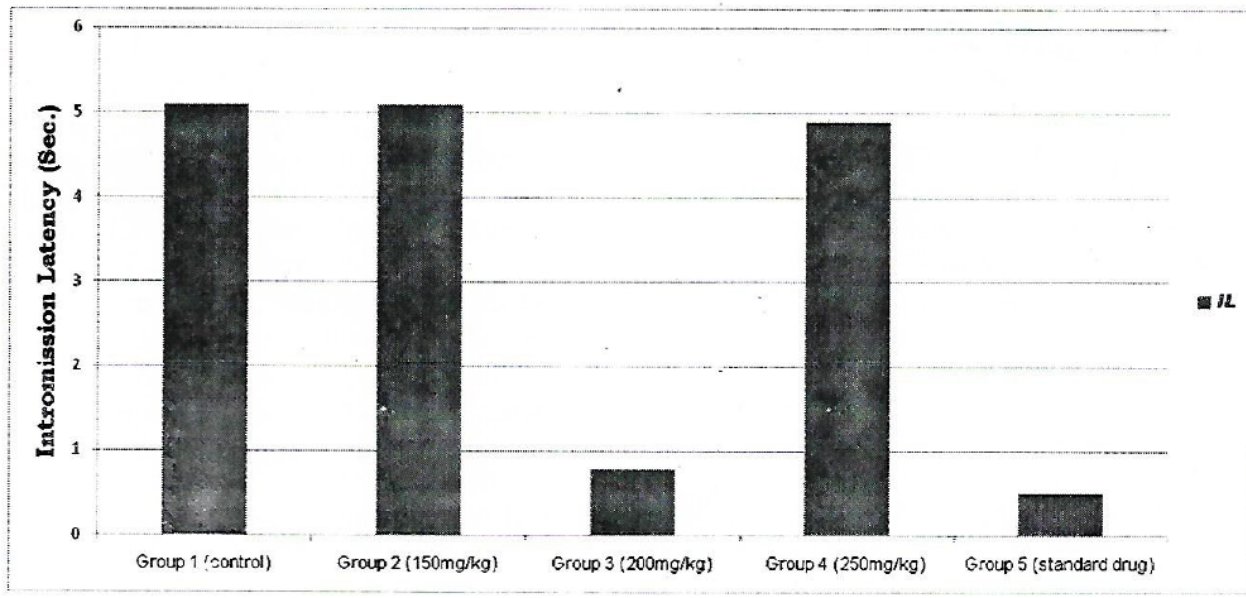

Fig: 5 Effect of administration of ethanolic extract of Mucuna pruriens seed on the intromission latency of male mice

This figure shows that the test drug at the dose of 200mg/kg produced a significant reduction in IL when compared to control, which indicates the aphrodisiac nature of *Mucuna pruriens*.
Mean ± SD; n = 6; *$p < 0.05$; **$P < 0.01$; ***$P < 0.001$.
IL: Intromission latency

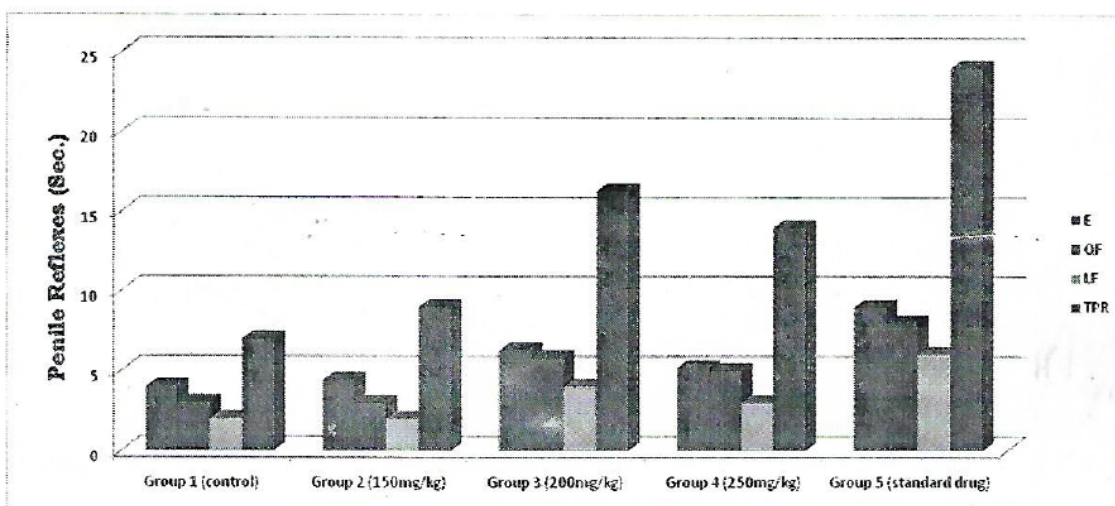

Fig 6: Effect of 50% ethanolic of Mucuna pruriens on penile reflexes (test for potency)

This figure shows that the test drug significantly increased the frequency of all components of penile reflexes (E, QF, & LF) in the test animals as compared to control group but comparatively lesser than the standard drug. Thus, this figure revealed that the test drug produced a marked increase in potency in all experimental groups with a profound increase seen in Group 3 (200mg/kg).

Mean ± SD; n = 6; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
E: Erection, QF: Quick Flip, LF: Long Flip and TPR: Total Penile Reflex.

Discussion

Phytochemical screening can help to reveal the plant extract. It may also be used to search for bioactive agents for starting products used in the partial synthesis of some useful drugs[9]. Phytochemical screening of the powder, and ethanolic extract of *Mucuna pruriens* seed showed the presence of alkaloids and saponins. It also contains glycosides, ammo-acids, tannins, flavonoids, steroids carbohydrate and terpenoids. Saponins have been implicated as the possible bioactive agent responsible for the aphrodisiac effect in *Tribulus terrestris* extract[20].

The realization that the acute toxicity test showed no mortality, and normal behaviour was observed in all the treated and control groups is probably an indication that the extract is relatively safe.

In this study, seed (M. *pruriens)* was tested in animal experimentation for its effect on sexual behaviour, and sildenafil citrate was used as the standard reference. The study showed that the 50% ethanolic seed extract of *Mucuna pruriens* possesses significant sexual function, enhancing activity as observed in sexual behaviour tests. Mating behaviour test revealed that the test drug at the dose of 200mg/kg significantly increased MF, IF, and EL as compared to control but less than that of the standard drug. The mounting frequency (MF) and intromission frequency (IF) are considered as the indices of both libido and potency. So, this is an indication that test drug possesses a sexual function improving effect.

The test drug (200mg/kg) not only significantly increased the ejaculatory latency (EL) but also was found to produce a significant reduction in the mounting latency (ML) and intromission latency (IL) when compared to control, which indicates the aphrodisiac nature of *Mucuna prurients.*

The effect of the test drug on libido was evaluated by the mounting frequency (MF) after genital anaesthetization which does away with the reinforcing effect of genital sensation thus affording the study of pure libido or intrinsic sexual desire. The effect on potency was also evaluated by testing the effect of the drug on the frequency of penile reflexes namely erections (E), quick flips (QF), and long flips (LF). The test drug significantly increased the frequency of all the components of penile reflexes (E, QF and LF) in the test animals as compared to control group but comparatively lesser than the standard drug. 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 neutransmitter levels or at cellular levels [21]. Penile reflex experience revealed that the test drug produced a marked increased in potency in all experimental groups with a profound increase seen in Group 3 (200mg/kg). The vascular event governing penile erection relies on parasympathetic neural input derived from cholinergic preganglonic neurons residing within the sacral spinal cord.

The cavernous nerves arise from the pelvic nerves that exit sacral cord which supplies autonomic input to the penis. These nerves release at least three neurotransmitters that are capable of relaxing the cavernous smooth muscle. These transmitters include nitric oxide, acetylcholine and vasoactive intestinal polypeptide of which nitric oxide is the most important. Acetylcholine activates endothelium via muscarinic receptors of the MS subtypes. Binding to receptors on endothelium leads to production of nitric oxide which is synthesized by endothdial nitric oxide synthetase.

Vasoactive intestinal peptide as well as forskolin and prostaglandian E acts through adenylate cyclase to trigger a rise in cyclic adensine monophasphate (CAMP). A rise in cAMP results in a fall in cytosolic Ca^{2+} in cavernous smooth muscles which eventually lead to relaxation of cavernous smooth

muscle in the penis. This relaxation of cavernous muscles will allow the blood to flow in the penis which results in erection of the penis.

With regard to the efficacy of the *Mucuna pruriens* and sildenafil citrate drugs, sildenafil citrate was predominatly used for erectile dysfunction, sexual dysfunction of psychologenic nature and reported to increase sperm count and functions[12]. However, extract actions are still not clear. In this study, *Mucuna pruriens* showed relatively good result in terms of sexual behaviour, libido potency and spermatogenic potential. With studies confirming the action *of Mucuna pruriens* on brain cells especially dopaminergic neurous [22], and dopaminergic pathway controlling sexual activities [23] these correlations strongly suggest aphrodisiac activity through dopaminergiic pathway with the presence of high level of L-DOPA in *Mucuna pruriens*. In addition, to discover the applied effective concentration or dosages of the extract, more studies are also required to fully elucidate the mechanism through which *Mucuna pruriens* produces aphrodisiac effect.

CONCLUSION

From the present investigation, we conclude that the ethanolic extract of *Mucuna pruriens* seed (200mg/kg) body weight possesses potent aphrodisiac activity in normal albino mice and may exert its activity through the activation of the cholinergic receptors. This result is the scientific evidence in favour of the claims in Indian system of medicine that the *Mucuna pruriens* is clinically useful as sexual invigorator in males.

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