Aphrodisiac activity of vigna mungo seeds

Pharmacological Evalution of The Potential Aphrodisiac Activity of Methanolic and Chloroform Extracts of *Vigna mungo S* eeds in Male Albino Rats

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ABSTRACT

The present study was conducted to investigate the aphrodisiac activity of methanolic and chloroform extracts of seeds of *Vigna mungo* (MEVM and CEVM) in male albino rats. The aphrodisiac activity of MEVM and CEVM wasevaluated by observing sexual behavioral parameters including Mount frequency (MF), Mount latency (ML), Ejaculatory latency (EL), Intromission latency (IL), Intromission frequency (IF), Ejaculation frequency (EF) and Post ejaculatory interval (PEI) and biochemical parameter like serum testosterone concentration in male rats. Both extracts were administered orally at doses of 200mg/kg and 400 mg/kg, showed a significant increase (P < 0. 05) in MF, IF, EF as well as a significant decrease (p <0. 05) in ML, IL, EL and PEI was observed when compared to control groups. There was also a significant increase (p <0. 05) in serum testosterone concentration were recorded. The results of the present study demonstrate that MEVM and CEVM enhance sexual activity in male rats. This improvement in sexual function might be due to the presence of phytoconstituents like alkaloids, saponins, steroids and flavonoids found in the methanoic and chloroform seed extracts of *Vigna mungo.*

Keywords: *Vigna mungo,* Aphrodisiac, Flavonoids, Testosterone, Sexual behavior

INTRODUCTION

Male infertility is a world-wide medical and social problem. In *Homo sapiens* , reproduction is initiated by the mating of a male with a female in sexual intercourse which facilitates the coming together of sperm and egg for the purpose of fertilization [1] . For there to be a normal sexual intercourse and sexual fulfillment in males, the male sexual organs (the copulatory organ, the penis) and factors relating to erection must function normally. Inability to perform this function effectively is a major problem facing the reproductive process. This is known as sexual dysfunction [2] . This condition which is of various types can be managed by the use of aphrodisiacs.

An aphrodisiac can therefore be described as any substance that enhances sex drive or sexual pleasure. Aphrodisiac can also be viewed as any food, drug, scent or device that can arouse or increase sexual drive or libido [3] .

Sexual dysfunction in men refers to repeated inability to achieve normal sexual intercourse. It can also be viewed as disorders that interfere with a full sexual response cycle. These disorders make it difficult for a person to enjoy or to have sexual intercourse. While sexual dysfunction rarely threatens physical health, it can take a heavy psychological toll, bringing on depression, anxiety, and debilitating feelings of inadequacy. Unfortunately, it is a problem often neglected by the health care team who strive more with the technical and more medically manageable aspects of the patient’s illness [4] . Sexual dysfunction is more prevalent in males than in females, It has been discovered that men between 17 and 96 years old could suffer sexual dysfunction as a result of psychological or physical health problems [5] .

Male sexual dysfunction (MSD) could be caused by various factors. These include: psychological disorders (anxiety, strained relationship, depression, stress and guilt), androgen deficiencies (testosterone deficiency), chronic medical conditions (diabetes, hypertension, vascular insufficiency (atherosclerosis, venous leakage), neurological disorders (Parkinson's disease, stroke, cerebral trauma, Alzheimer's spinal cord or nerve injury), drugs (side effects) (anti-hypertensive's, central agents, psychiatric medications, antiulcer, antidepressants, and anti-androgens), life style (chronic alcohol abuse, cigarette smoking), aging (decrease in hormonal level with age) and systemic diseases (cardiac, hepatic, renal pulmonary, cancer, metabolic, post-organ transplant) [2, 6, 7] .

Vigna mungo most commonly known as Black gram or urad is an erect, fast-growing annual, herbaceous legume grows in many parts of India. It is extensively cultivated all over the India. The seeds are emollient, astringent, thermogenic, diuretic, aphrodisiac, nutritious, galactogauge, appetizer, laxative, styptic and nervine tonic. They are useful in treating scabies, leucoderma, gonorrhea, pains, epistaxis, piles, asthma, heart trouble, dyspepsia, anorexia, constipation, haemorrhoids, hepatopathy, neuropathy, schizophrenia, hysteria, nervous debility, partial paralysis, facial paralysis and weakness of memory. Seeds are believed as spermatopoetic, and used for treating erectile dysfunction and premature ejaculation [8-11] .

In this regard, we undertook the present study on *Vigna mungo* which has been in use by the traditional healers. So far there is no systemic pharmacological study were carried out to investigate the aphrodisiac

activity of *vigna mungo* seeds in male rats. Hence, the present study was aimed to demonstrate the aphrodisiac activity of different extracts of *vigna mungo* seeds.

MATERIALS AND METHODS

Collection of Plant material

The dried seeds of *Vigna mungo* plant were procured from the local market of Hyderabad in telangana state. The seeds were authenticated by Dr. S. Madhav chetty, Associate Professor, Department of Botany, Sri Venkateswara University, Tirupathi, Andhra Pradesh, India.

Procurement and Rearing of Experimental animals

Healthy wistar Albino rats of both the sex (200-300 g) were procured from Albino Research Center, hyderabad. They were randomly housed in standard polypropylene cages and maintained under environmentally controlled room provided with a 12: 12 h light/dark cycle approximately 25ºC for 10days before the start of experiment [12, 13] . They were fed with commercially available pellet diet obtained from Amruth foods, Pranav Agro Industries, Sangli, India and water was allowed *ad libitum .* The rats were acclimatized to laboratory conditions minimum one week prior to the experimentation. The study was performed as per the protocols and recommendation of the Institutional Animal Ethics Committee (Reg No: 1662/PO/a/CPCSEA, 2013) of Malla Reddy Institute of Pharmaceutical Sciences, Secunderabad.

Preparation of plant Extract

The dried seeds of *Vigna mungo* was powdered and subjected to soxhlet extraction with methanol, later the dried mark was subjected to soxhlet extraction with chloroform. Both extracts were concentrated by vacuum distillation and subjected to phytochemical screening.

Experimental design

Healthy and sexually experienced male albino rats (200– 300 g) that were showing brisk sexual activity were selected for the study. They were divided into 6 groups of 6 animals each and kept singly 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 was given suspension of sildenafil citrate orally at the dose of 5 mg/kg, 1 h prior to the commencement of the experiment.

Groups 3–6 received suspension of the methanolic and chloroform extracts of *Vigna mungo* seed orally at the doses of 200 and 400 mg/kg, respectively, daily for 21 days at 18: 00 h.

Since the male animals should not be tested in unfamiliar circumstances the animals were brought to the laboratory and exposed to dim light at the stipulated time of testing daily for 6 days before the experiment. The female animals were artificially brought into oestrus phase (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, experimental and standard animals. The most receptive females were selected for the study. The experiment was carried out on the 21st 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 glass cages (40x50x40cm) of male animals with 1 female to 1 male ratio. The observation for sexual behaviour was immediately commenced and continued for first 2 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 following sexual behavioral parameters were recorded on audio video-cassette (Sony Handycam) as soon as they appeared [14-17]

- *Mount latency* : Time duration (in seconds) from the introduction of the female into the cage till the first mount by the male.
- *Intromission latency* : Time duration (in seconds) from the introduction of the female into the cage till the first intromission by the male (vaginal penetration).
- *Ejaculation latency* : Time duration (in seconds) from the first intromission till ejaculation.
- *Mount frequency* : defined as the number of mounts without intromission from the time of

introduction of the female until ejaculation.

- *Intromission frequency* : Total number of intromission preceding ejaculation.
- *Ejaculation frequency* : The number of times there was expulsion semen by males after vaginal penetration –characterized by rhythmic contraction of the posterior abdomen.
- *Post-ejaculatory interval-* is the time interval between ejaculation and the first intromission of the following series

Determination of Serum Cholesterol and Testosterone:

After recording of the sexual behavioral parameters, blood samples were collected from retro orbital plexus, centrifuged and serum was separated, samples stored at-20 ^{0} C which was used for the testosterone level estimation by using ELISA kit. Serum cholesterol concentrations may be determined by the Chod-PAP method $^{18, 19}$. Briefly, 0. 02cm ^{3} of the sample (serum) is mixed with 2. 00cm ^{3} of working reagent and the absorbance of the resulting mixture read after 5min at 546nm wavelength. The blank and standard are composed in a similar way except that they are replaced with 0. 02cm ^{3} each of distilled water and standard solution respectively. The biochemical estimations were done using respective kits.

Statistical analysis

The data obtained from this study were expressed as mean ± SEM, (n= 6). Statistical analysis was done by one way analysis of variance (ANOVA)

followed by Dunnett’s test. P <0. 05 was considered to be statistically significant.

RESULTS

The preliminary phytochemical screening of both the extracts revealed the presence of active constituents which includes alkaloids, flavonoids, tannins, saponins, phenols and steroids (table-1).

The oral administration of two doses (200mg/kg, 400mg/kg) of methanolic and chloroform extracts of *Vigna mungo* seed resulted remarkable increase in sexual activity of the male rats. The results of the sexual behavior test shows that the extracts of *vigna mungo* seed (methanol and chloroform) at the dose of 200mg/kg and 400 mg/kg, significantly increased (p <0. 05) in mounting frequency (MF), intromission frequency (IF) ejaculatory frequency (EF), where as significant reduction (p <0. 05) in mounting latency (ML), intromission latency (IL), ejaculatory latency (EL) and post ejaculatory interval (PEI) when compared to control animals (Table-2 and Fig. 1-7). There was a significant increase (p <0. 05) in serum cholesterol and testosterone levels also observed (Table -3). Similarly, the standard drug increased (P <0. 01) the MF, IF and EF as well as decreased (P <0. 01) the ML IL and PEI in a highly significant manner as compared to control animals. The most appreciable effect was observed in rats treated with dose of 400 mg/kg of *vigna muno* seed.

Table-1: Preliminary phytochemical screening of MEVM and CEVM of *Vigna mungo* seed

Type of phytochemicalconstituents	MethanolicExtract	ChloroformExtract
Alkaloids	++	+
Flavonoids	++	++
Phenols	+	+
Proteins	++	+
Saponins	+	+
Steroids	++	++
Tannins	++	+

+indicates the presence ++ indicates better response

Table 2: Effect of Methanolic and Chloroform seed extracts of *Vigna mungo* on sexual behavior of male rats.

Treatment	ML	MF	IL	IF	EL	EF	PE
Vehicle 1% CMC (ml/kg)	308. 33±14. 18	14. 33±1. 28	558. 33±17. 01	6. 66±0. 84	400. 83±19. 25	1. 5±0. 22	28 66
Sildenafil citrate (4mg/kg)	103. 3	32. 66±2. 41	250±11.	16. 16±2. 44	155±18.	6. 5±0.	90

	±13. 64 [b]	[b]	32 [b]	[b]	39 [b]	42 [b]
MEVM (200mg/kg)	256. 66±19. 60 [a]	18. 66±1. 66 [a]	311. 66±28. 91 [a]	11±1. 31 [a]	395±18. 39 [a]	2. 66±0. 19 21 [a] 52
MEVM (400mg/kg)	168. 33±18. 8 [a]	28. 33±4. 66 [a]	267. 5±14. 41 [a]	13. 83±1. 88 [a]	252. 5±23. 93 [b]	3. 16±0. 15 40 [a] 83 [a]
CEVM (200mg/kg)	269. 16±18. 63 [a]	14±2. 14 [a]	455. 83±21. 6 [a]	8±0. 51 [a]	382±26. 29 [a]	1. 66±0. 26 21 [a] 83 [a]
CEVM (400mg/kg)	241. 66±22. 71 [a]	23. 66±1. 97 [a]	437. 5±22. 72 [a]	9. 5±0. 42 [a]	335. 83±21. 69 [a]	2. 66±0. 20 21 [a] 33 [a]

Values are expressed as mean ± S. E. M. (n= 6).

a= P < 0. 05 as compared to control.

b = P < 0. 01 as compared to control

ML= Mount latency; IL= Intromission latency; EL= Ejaculatory latency; MF= Mount frequency; IF= Intromission frequency; EF= Ejaculatory frequency; PEI= Post ejaculatory interval; MEVM= Methanolic extract of *Vigna mungo,* CEVM= Chloroform extract of *Vigna mungo.*

Table 3: Effect of Methanolic and Chloroform seed extracts of *Vigna mungo* on testosterone concentration in male rats

Treatment	Serum testosterone (ng/ml) levels	Serum cholesterol levels (mg/dl)
Control (1% CMC)	2. 90 ± 0. 072	59. 31 ± 1. 695
Sildenafil citrate (4mg/kg)	3. 24 ± 0. 049 [b]	86. 42 ± 1. 70 [b]
MEVM (200mg/kg)	3. 06 ± 0. 033	69. 47 ± 1. 69 [a]
MEVM (400mg/kg)	3. 17 ± 0. 064 [a]	73. 224 ± 2. 892 [a]
CEVM (200mg/kg)	3. 08 ± 0. 036 [a]	66. 09 ± 1. 69
CEVM (400mg/kg)	3. 14 ± 0. 023 [a]	72. 86 ± 1. 70 [a]

Values are expressed as mean ± S. E. M.(n= 6).

a= P < 0. 05 as compared to control.

b = P < 0. 01 as compared to control.

DISCUSSION

Male impotence or erectile dysfunction (ED) is a significant problem that may contribute to infertility. The incidence of erectile dysfunction, probably due to aging populations and other risk factor such as the presence of chronic illnesses (e. g. heart disease, hypertension and diabetes mellitus), smoking, stress, alcohol, drug abuse and sedentary lifestyles. A number of synthetic drugs are available for treating the infertility problems but because of their unwanted side effects folk remedies are gaining importance. Hence, the present study was carried out to investigate the aphrodisiac effects of methanolic and chloroform extracts of *Vigna mungo* seedin male rats.

The preliminary phytochemical screening study of both the extracts revealed the presence of active constituents which includes alkaloids, flavonoids, tannins, saponins, phenols, and steroids. It has been reported that steroids and saponin constituents found in the many plants possess fertility potentiating properties, and useful in the treatment of impotence [20] . The saponins may therefore boost the level of testosterone in the body as well as trigger libido enhancing effect observed in this study [21] . The presence of flavonoids in the *Vigna mungo* extract which has been implicated to have a role in altering androgen levels may also be responsible for the enhanced male sexual behaviour in this study [22] . The alkaloids can also cause facilitation of sexual behaviour and has effect on sexual behaviour [23] . The improvement in sexual function demonstrated in the current study might be due to the presence of such compounds in *Vigna mungo* seed extracts.

In the present study the sexual behavioral parameters were evaluated to estimate the potency of the *Vigna mungo* extract. In male rats mount latency and intromission latency are considered as indicators of the sexual motivation, where as intromission and ejaculation are considered as behavioural indication of sexual performance and facilitation [24] . There was a significant decrease in the latency for mount and intromission by the administration of various doses of seed extract of *vigna mungo* indicating an enhancing sexual motivation. The methanol and chloroform extracts have shown pronounced effect on sexual behavior by significant increase in mounting frequency (MF), intromission frequency (IF) as compared to control. Mounting frequency (MF) and intromission frequency (IF) are considered the indices of both libido and potency. Increase in these frequencies by the administration of the seed extracts might be due to increase in the concentration of several anterior pituitary hormones and serum testosterone, which in turn stimulated dopamine receptor synthesis and sexual behavior [25] . Such increase in testosterone concentration should normally reflect a corresponding increase in libido [26] . Hence, from these results the aphrodisiac effect of the plant extract may be due to the presence of alkaloids, saponins and flavonoids through a multitude of central and peripheral mechanisms.

In the present study there is a significant increase serum cholesterol concentration which may imply stimulation in the steroid genesis, that leads to increased testosterone concentration [27] . Such increase in testosterone concentration should normally reflect a corresponding increase in libido [26] .

The present finding shows that the methanolic and chloroform seed extracts of *vigna mungo* produces a striking enhancement of over- all sexual performance of male rats. Our findings also showed that the aphrodisiac effect of *vigna mungo* seeds extract, investigated in male rats at 400 mg/kg, which significantly reduced ML, IL, EL and PEI with increased MF, IF and EF.

CONCLUSION

On the basis of our results the present study revealed that the methanolic and chloroform extracts of seeds of *Vigna mungo* showed the aphrodisiac activity and it is dose dependent in male rats. From this we conclude that the *Vigna mungo* seed extract may prove to be an effective and safe alternative remedy in sexual disorders.

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