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Research Article

# EVALUATION OF APHRODISIAC ACTIVITY AND SPERMATOGENIC EFFECT OF *VIGNA MUNGO*

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*According to Indian Systems of Medicine, Vigna mungo Linn, belonging to the Fabaceae family, is an important aphrodisiac (Vajikaran drug) as describe in Charak Sanhita. Vigna mungo is used for treating male sexual disorders since ancient times. In this study, the effects of alcoholic extracts of the Vigna mungo Linn. seeds 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. Vigna mungo extract also tested for effect on spermatogenisis. Animals were divided into one control group (Group I—received saline) and two experimental groups (Groups II–III): Sildenafil citrate (4 mg/kg body weight) (Group II) and while Group III received 500 mg/kg body weight. Animals were fed PO with saline or extract or standard drug once in a day for 42 days. To analyse the mating behaviour, female rats with oestrus phase were used.The extract administered PO significantly increased the mounting frequency, intromission frequency and decreased the mounting latency, intromission latency, post ejaculatory interval and inter-intromission interval. The male reproductive organ weight increased, sperm count also increase and it is supported in histopathology slides of testies. Therefore, the results indicated that the alcoholic extracts of Vigna mungo Linn. seed produced a significant and sustained increase in the sexual activity of normal male rats at a particular dose (500 mg/kg).*
*Therefore, the resulting aphrodisiac activity of the extract tends to support to the claim that it has traditionally been used for the treatment of sexual disorders.*

**Key Words:** *Aphrodisiac, Spermatogenesis, Premature ejaculation, copulatory behavior, mounting latency, intromission latency, post ejaculatory interval and inter-intromission interval.*

## 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 [1]. In many countries, different varieties of plants have been used as sexual stimulants in traditional medicine.

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*Vigna mungo* (Linn) Hepper commonly known as Black gram (Mash, Urid), belongs to family Fabaceae. *Vigna mungo* is a diffuse annual herb; stem 30–60 cm long, clothed with brownish silky hairs. Leaves 3 foliolate; leaflets 5–10 cm long, flowers yellow in axillary racemes, pods subcylindric; 3.8–6.3 cm long. It is extensively cultivated all over the India. It has been used for various medicinal purposes in Ayurvedic and Unani systems of medicine. The seeds are sweet, laxative, aphrodisiac, tonic, appetizer, diuretic, galactagogue and styptic; useful in piles, asthma, scabies, leucoderma, gonorrhea, pains, epistaxis, paralysis, rheumatism and affections of the nervous system, liver and cough. It is also prescribed for dropsy and cephalalgia. *Vigna mungo* L. (Fabaceae) has been reported to be used in a variety of disease conditions of

liver in Indian traditional system of medicine [2].

*Vigna mungo* use as Bajikaran Ghrat (aphrodisiac ) in Charak Sanhita .Keeping in mind some of its potential therapeutic applications we will carry out animal experiments to investigate the effects of *Vigna mungo*-extract as aphrodisiac and spermatogenic effects.

**Collection and authentication of *Vigna mungo* (L.):**

The seed of *Vigna mungo (L)* was purchased from Local market of Kota, Rajasthan in the month of Feb. 2013 and were authenticated by Mr. Vinod Meena, scientist at Department of Botany, Botanical Survey of India, Jodhpur and herbarium was deposited with voucher specimen No.: BSI/AZRC/I.12012/TECH/2012-13/181.

**Preparation of aqueous extract of *Vigna mungo* (L.):**

The seeds were powdered and defatted with petroleum ether and then subjected to extraction with methanol (95%) using Soxhlet apparatus for 18 h. The extract was concentrated on water bath to obtain thick pasty mass. The percentage yield was 2.6% w/w. [2]

**Preparation and storage of solutions:**

All solutions were prepared freshly at the time of use and stored in glass bottles till used. The Estradiol valerate were dissolved in distilled water and Hydroxy Progesterone were dissolved in Isopropyl alcohol to prepare appropriate stock. Final dosages were prepared according to body weight of animal.

***Route and dose of administration:***

The *Vigna mungo (L.)* extract was administered per orally (p.o.) using oral feeding needle in a volume of 5 ml/kg and Estradiol valerate 10 µg/kg S.C. and Hydroxy progesterone 1.5mg/kg S.C. injection ,

Preliminary phytochemical analysis of the *Vigna mungo (L.):*

The *Vigna mungo* (L.) extract was subjected to qualitative chemical analysis for the presence of various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids and flavonoids by using standard phytochemical tests [3].

**Preparation of male rats**

Wister adult male albino rats weighing 150-220 g were given training for sexual experience. To provide sexual experience, each male rats were allowed 30 minutes exposure to a stimulus female in behavioral oestrous, several days before testing for copulatory performance. The animals were tested two times over 10 days period for copulatory behavior and divided into groups demonstrating comparable copulatory performance. Males were trained individually with normal adult female in oestrous in transparent arena. The male rat which did not show any sexual interest during the test period was considered as an inactive male. [5]

**Selection of female rats**

Adult female rats were brought to oestrus phase by the sequential administration of estradiol valerate 10 µg/kg S.C. and hydroxy progesterone 1.5mg/kg S.C., for 48 hours and 5 hours prior to experimentation. These female rats were divided into three groups, each group consisting of six animals. Food and water were provided *ad libitum*. The female rats, which were in oestrous stage, were used for the study.

**Experimental Methods**

Male albino rats were distributed into three groups consisting of six rats per group.

Group I (Control): Normal animals treated (2ml/kg, p.o.) of saline.

Group II (Standard drug): Animals treated with Sildenafil citrate at a dose 4 mg/kg,

Group III: Animals treated with at aqueous extract of ***Vigna mungo* (L.)** of dose 500 mg/kg, p.o. [2]

From 2 weeks prior to the screening tests, until the end of the study, the rats were housed individually at 26°C-28°C. The highly receptive female was introduced into the male's cage and each male rat is observed for 30 minutes for copulatory behavior under dim red light. All the rats were tested for copulatory behavior on 0,7 $^{th}$,14 $^{th}$,21$^{th}$,28 $^{th}$,35$^{rd}$ and 42$^{th}$ days respectively .[4]

**Parameters of the copulatory behavior were recorded:**

(I)Attraction towards female & Determination of Hesitation time**:**

Determination of attraction towards sexually receptive female was done using the methods. A female rat was placed in a cage which had a wooden barrier of 15 cm separating male & female compartments which could be passed by a motivated male rat.

The hesitation time was recorded as the time (in sec) required by the male rat before making an attempt to cross the barrier. In the same way, a scoring for attraction towards female was recorded by a score between 0-5 during an observation period of 15 min. A complete cross of the partition by the male rat each time was given a score of 5 while an attempt to climb was given a score of 2 & disinterest to climb was rated as 0. The readings were recorded on Days 1, 7 & 14 of treatment. This test is useful in determining the willingness of a male rat to cross an aversive or obstructive position, thus indicating the intent of sexual attraction. Male rats of all the groups were subjected to experimentation & their scores for attraction as well as hesitation time were recorded. [6]

(II) Mount latency (ML)**:** Time taken for the first mount following the introduction of females.

(III) Intromission latency (IL)**:** Time taken for first intromission following introduction of the female.

(IV) Mount frequency (MF)**:** No. of mounts observed in 30 min.

(V) Intromission frequency (IF)**:** No. of intromission observed in 30 min.

(VI) Ejaculation frequency (EF): No of ejaculation observed in 30 min.

All animals in each group were administered the requisite dose for 42 days. The weight of the animals was recorded on 0, 7 $^{th}$,14$^{th}$, 21$^{th}$ , 28$^{th}$,35$^{rd}$ and 42$^{th}$ days. After 42days of treatment the body weights of animals were taken after which all animals of saline treated and *Vigna Mungo* treated group were sacrificed by decapitation. Testis, Epididymis , Seminal vesicles and prostate glands were carefully removed and the weight of each organ was determined

(VII) Change in weight of Testis, seminal vesicle, Prostate Glands, epididymus .

(VIII) Effects on epididymal sperm count**.**

(IX) Histopathology study of Testis.

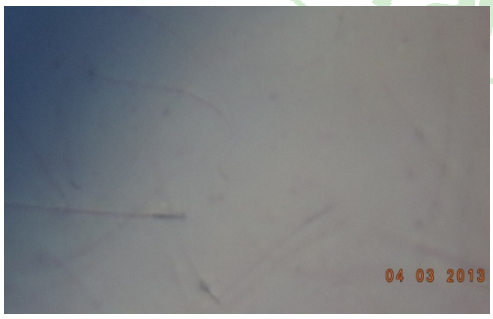


**Fig. 1: Photograph of rat Sperm**

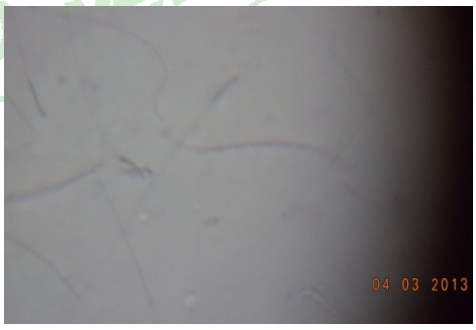


**Fig.2: Photograph of rat Sperm**

### Statistical Analysis

All the results were expressed as Mean ± Standard Error (SEM). Interpretation of the result was supported by statistical analysis. Results of the same group of different days of treatment were analyzed by one way analysis of variance (ANOVA) followed by Dunnett's test to calculate the level of significance. Statistical analysis of data was performed using Graph Pad Prism demo version 6

### Results

**Table 1: Preliminary phytochemical investigation of *Vigna Mungo***

| Sr. No. | Phytochemical tests | Extract of seed |
|---|---|---|
| 1 | **Test for alkaloids** | |
| | ✓ Hager's Test | - |
| | ✓ Mayer's Test | - |
| | ✓ Dragendroff's Test | - |
| | ✓ Wagner's Test | - |
| 2 | **Test for carbohydrate** | |
| | ✓ Molisch's Test | + |
| | ✓ Fehling's Test | + |
| | ✓ Barfoed's Test | + |
| | ✓ Benedict's Test | + |
| 3 | **Test for steroids** | |
| | ✓ Liebermann-Burchard Test | + |
| | ✓ Salkowski Test | + |
| 4 | **Test for cardiac glycosides** | - |
| | ✓ Baljet Test | - |
| | ✓ Keller Killani's Test | - |
| 5 | **Test for anthraquinone glycosides** | |
| | ✓ Borntrager's Test | + |
| 6 | **Test for saponnins** | |
| | ✓ Froth Test | + |
| 7 | **Test for tannins** | |
| | ✓ Ferric Chloride Test | - |
| | ✓ Lead Acetate Test | - |
| 8 | **Test for proteins and amino acids** | |
| | ✓ Biuret Test | + |
| | ✓ Millons's Test | + |
| | ✓ Ninhydrin Test | + |
| 9 | **Test for flavonoids** | |
| | ✓ Lead Acetate Test | + |
| | ✓ Shinoda Test | + |

**Mount Letancy:**

The experimental data revealed that a significant decrease in mount latency in animals treated with *Vigna Mungo* aqueous extract of 500 mg/kg.

**Table 2: Mount Letancy of *Vigna mungo* treated rat**

| Groups | day0 | day 7 | day 14 | day 21 | day28 | day35 | day42 |
|---|---|---|---|---|---|---|---|
| **Control** | 242.3 ± 27.26 | 230.5 ± 25.27 | 245.8 ± 29.75 | 200.5 ± 21.26 | 264.5 ± 42.43 | 310.5 ± 28.26 | 268 ± 36.4 |
| **Sildenafil citrate** | 188 ± 13.28* | 170.5 ± 17.26* | 154.7 ± 8.827** | 163.16 ± 17.26** | 150 ± 11.52** | 107 ± 27.26*** | 133 ± 14.28*** |
| ***Vigna Mungo*** | 269.7 ± 20.07 | 249.666 ± 23.26 | 230.7 ± 23.27 | 189.166 ± 13.26* | 210.8 ± 23.08* | 178.33 ± 24.26** | 164.3 ± 12.17** |

Administration of (Sildenafil citrate 4 mg/kg p.o.) showed significant ($p<0.05$,$p<0.01$ and $P<0.001$) decrease in Mount latency on 0,7 and 14,21,28 and 35,42 day of observational period respectively as compared with control. Administration of *Vigna Mungo* (500 mg/kg) showed significant ($p<0.05$and $p<0.01$) decrease in Mount latency on 21, 28 day and 35, 42 day of observational period respectively as compared with control.

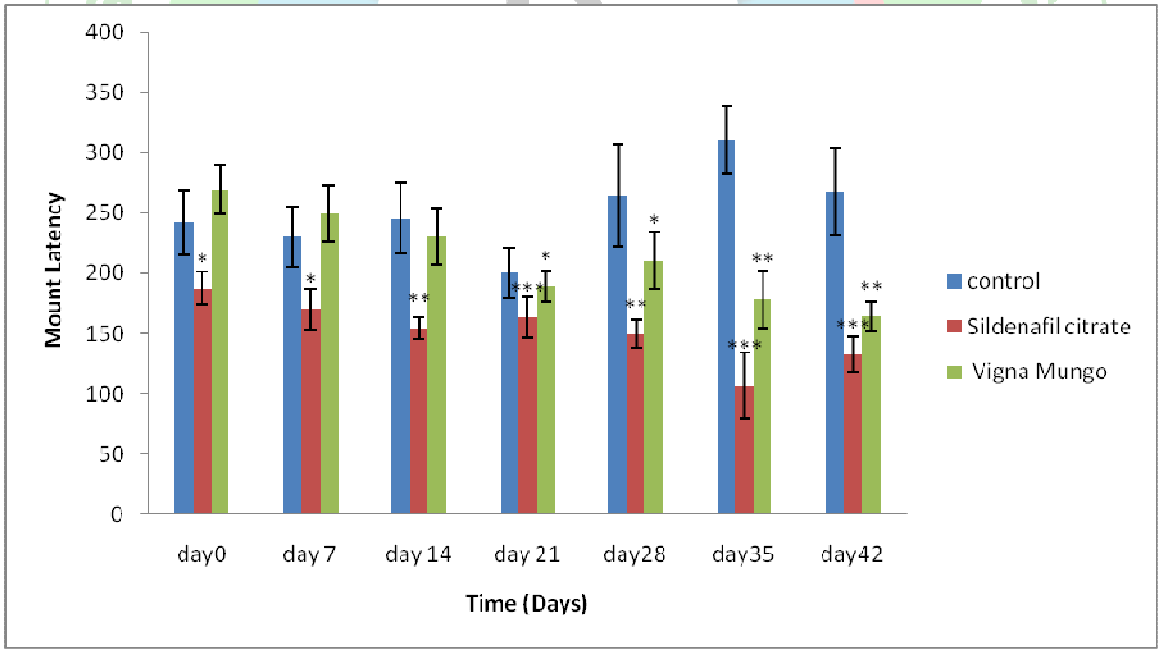


**Fig.2: Mount letancy of *Vigna Mungo***

**Mount Frequency:** The results revealed that a significant increase in mount frequency was observed in animals treated with aqueous extract at a concentration of 500mg/kg body weight

**Table 3: Mount frequency of *Vigna mungo* treated rat**

| Groups | day0 | day 7 | day 14 | day 21 | day28 | day 35 | day 42 |
|---|---|---|---|---|---|---|---|
| Control | 6.833 ± 0.4773 | 7 ± 0.5164 | 6.667 ± 0.6667 | 9 ± 1.033 | 6.833 ± 0.8724 | 7.667 ± 0.6146 | 7 ± 0.5774 |
| *Vigna Mungo* | 6.167 ± 0.4773 | 14 ± 0.7303* | 17.33 ± 0.5578* | 18 ± 0.7746* | 23.5 ± 0.922** | 28.5 ± 0.9916** | 32 ± 1.238*** |
| Sildenafil citrate | 7 ± 0.5774 | 25.17 ± 1.579** | 26.83 ± 1.515** | 31.33 ± 1.579*** | 32 ± 1.291*** | 33.16 ± 2.167*** | 37.5 ± 2.11*** |

Administration of (Sildenafil citrate 4 mg/kg p.o.) showed significant (P<0.01 and P<0.001) increase in Mount Frequency on 7,14 and 21,28 and 35,42 day of observational period respectively as compared with control. Administration of *Vigna Mungo* (500 mg/kg) showed significant (p<0.05, p<0.01and P<0.001) increase in Mount Frequency on 7,14,21 day and 28,35,and 42 day of observational period respectively as compared with control.

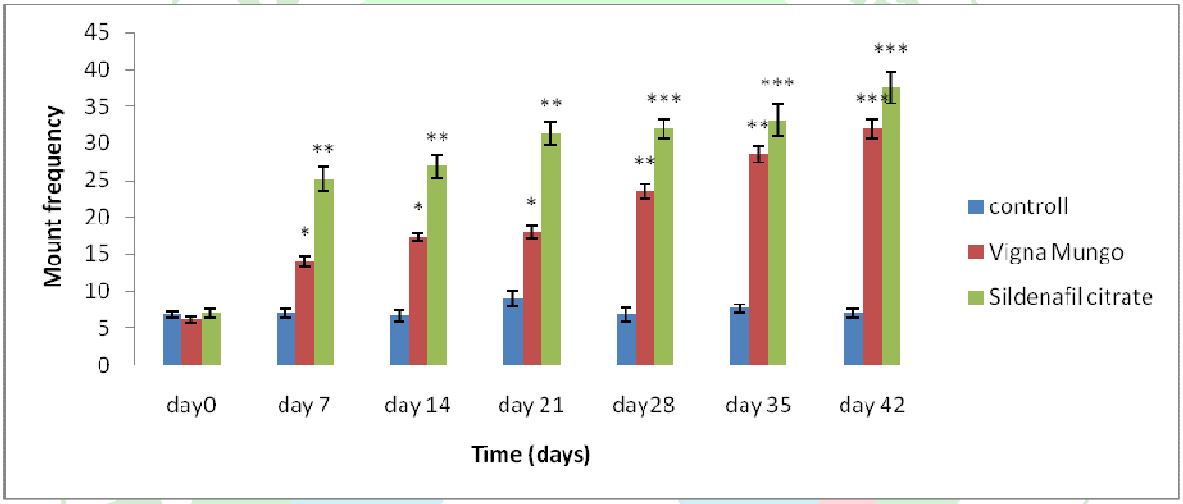


**Fig.3: Mount Frequency of *Vigna Mungo***

**Inter Mission Latency**

**Table 4: Inter Mission Latency of *Vigna mungo* treated rat**

| Groups | day 0 | day 7 | day 14 | day 21 | day28 | day 35 | day 42 |
|---|---|---|---|---|---|---|---|
| Control | 23.33 ± 2.305 | 26.66 ± 2.155 | 18.33 ± 2.39 | 16.67 ± 1.116 | 17 ± 1.693 | 18.5 ± 2.045 | 19.83 ± 3.208 |
| Sildenafil citrate | 23.5 ± 0.9916 | 12.5 ± 1335* | 8.6 ± 0.9189** | 7.6 ± 0.7149** | 7.3 ± 0.7601** | 6.83 ± 0.4773** | 6.167 ± 0.9098*** |
| *Vigna Mungo* | 18.16 ± 1.4 | 14.33 ± 1.116* | 10.66 ± 0.9545* | 10 ± 0.7303* | 9.5 ± 0.7638** | 7.5 ± 0.5627** | 6.66 ± 0.4944*** |

Administration of (Sildenafil citrate 4 mg/kg p.o.) showed significant (p<0.05,P<0.01 and P<0.001) decrease in Intermission Latency on 7 and 14, 21,28,35 and 42 day of observational period respectively as compared with control. Administration of *Vigna Mungo* (500 mg/kg) showed significant (p<0.05, p<0.01and P<0.001) decrease in Intermission Latency on 7,14,21 day and 28,35,and 42 day of observational period respectively as compared with control

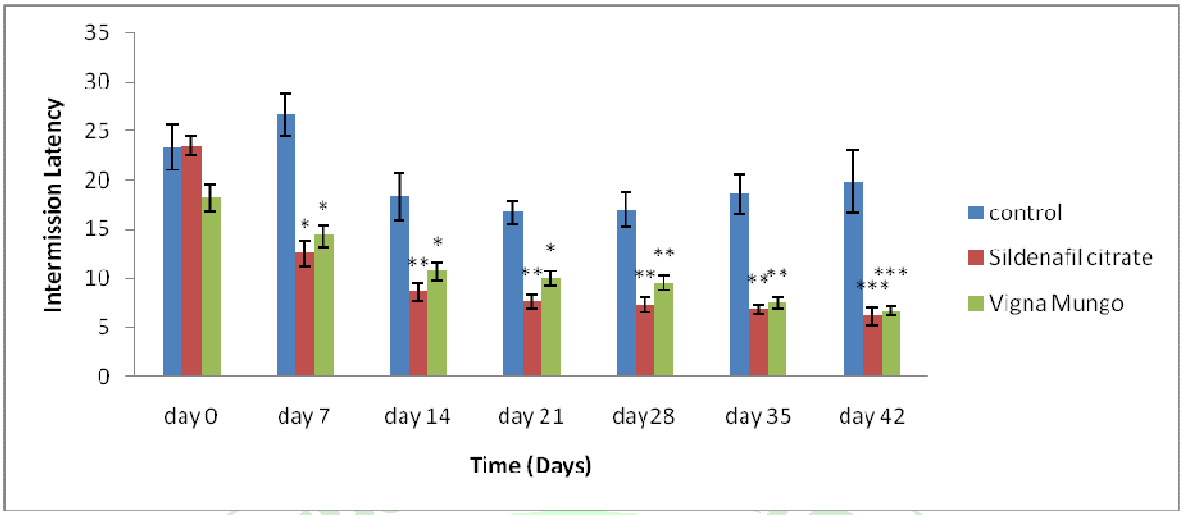


**Fig.4 Inter Mission Latency of *Vigna Mungo***

**Intermission Frequency:** The results revealed that the aqueous extracts at concentration of 500mg/kg body weight are moderate active and possess potent aphrodisiac activity as compared to control animals.

**Table 5: Intermission Frequency of *Vigna mungo* treated rat**

| Groups | day 0 | day 7 | day 14 | day 21 | day28 | day 35 | day 42 |
|---|---|---|---|---|---|---|---|
| Control | 0.6667 ± 0.2108 | 0.6667 ± 0.2108 | 0.6667 ± 0.2108 | 0.5 ± 0.2236 | 0.6667 ± 0.2108 | 0.6667 ± 0.2108 | 0.8333 ± 0.1667 |
| Sildenafil citrate | 0.8333 ± 0.1667 | 6.5 ± 1.118* | 8.833 ± 1.078* | 11 ± 0.5164** | 12.67 ± 0.9189*** | 11.67 ± 0.3333** | 14 ± 0.3651*** |
| *Vigna Mungo* | 0.6667 ± 0.2108 | 1 ± 0 | 1.5 ± 0.2236* | 2 ± 0* | 2.83 ± 0.1667* | 5.5 ± 0.4282* | 7 ± 0.7746* |

Administration of (Sildenafil citrate 4 mg/kg p.o.) showed significant ($p<0.05$,$P<0.01$ and $P<0.001$) decrease in Intermission Frequency on 7 , 14 and 21 ,35 and 28,42 day of observational period respectively as compared with control. Administration of *Vigna Mungo* (500 mg/kg) showed significant ($p<0.05$) decrease in Intermission Frequency on 14,21, 28,35, 42 day of observational period respectively as compared with control

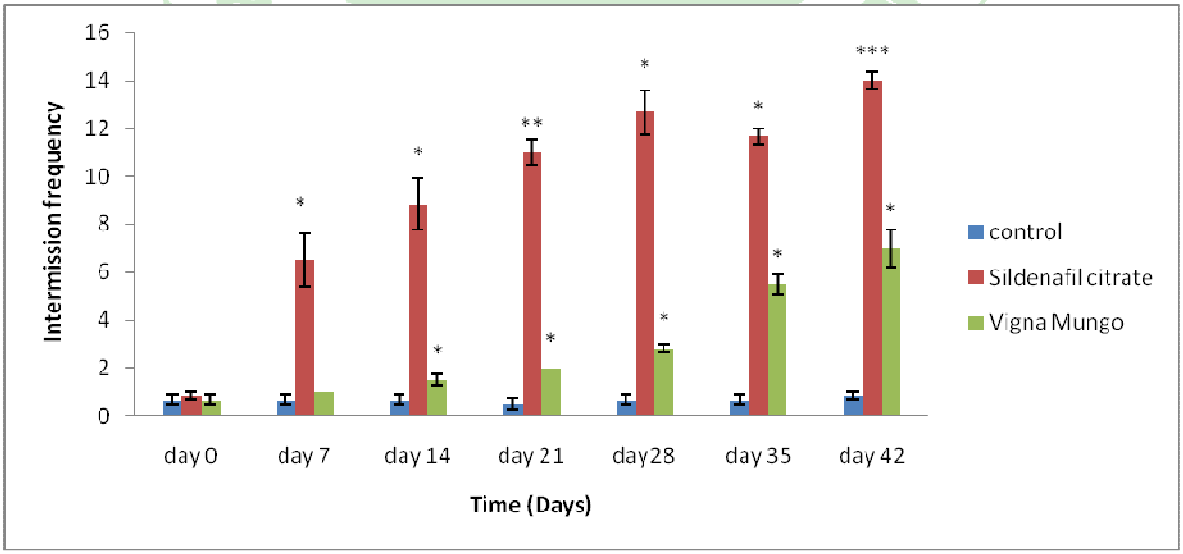


**Fig 5: Intermission Frequency of *Vigna Mungo***

## Ejaculation Frequency

**Table 6: Ejaculation Frequency of *Vigna mungo* treated rat**

| Groups | day0 | day 7 | day 14 | day 21 | day28 | day 35 | day 42 |
|---|---|---|---|---|---|---|---|
| Control | 0.33 ± 0.2108 | 0.5 ± 0.2236 | 0.5 ± 0.2236 | 0.5 ± 0.2236 | 0.6667 ± 0.2108 | 0.6667 ± 0.2108 | 0.8333 ± 0.1667 |
| *Vigna Mungo* | 0.5 ± 0.2236 | 0.83 ± 0.1667 | 1.5 ± 0.2236* | 1.83 ± 0.1667* | 2.66 ± 0.2108* | 4.5 ± 0.4282** | 5.16 ± 0.654** |
| Sildenafil citrate | 0.8333 ± 0.1667 | 3.8 ± 0.6009* | 6.33 ± 0.6667*** | 6.5 ± 0.7638*** | 6 ± 0.6325** | 6 ± 0.5774** | 6.16 ± 0.4773*** |

Administration of (Sildenafil citrate 4 mg/kg p.o.) showed significant (P<0.05 and P<0.001) increase in Ejaculation Frequency on 7 ,28,35 and 14, 21,28 42 day of observational period respectively as compared with control. Administration of *Vigna Mungo* (500 mg/kg) showed significant (p<0.05 and p<0.01) increase in Ejaculation Frequency on 14,21,28 day and 35,and 42 day of observational period respectively as compared with control

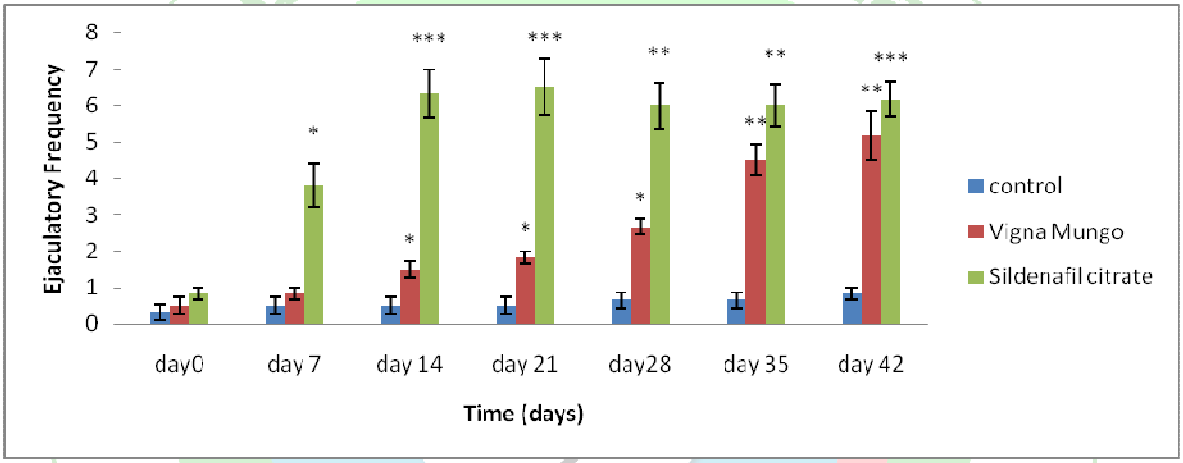


**Fig.6: Ejaculation Frequency of *Vigna Munga***

**Change in Organ weight:** The aqueous extract of *Vigna Mungo* resulted in an increase in organ weight in a dose- dependent manner. The *Vigna Mungo* 500 mg group showed a 26.43% increase in Seminal vesicle weight, a 11.38% increase in weight of Epididymis , a 13.12% in prostate gland weight, and a increase in 15.89% testies weight.

**Table 7: Change in sex organ weight of *Vigna mungo* treated rat**

| Group | Seminal Vesicle | Testies | Prosted galnd | Epididymis |
|---|---|---|---|---|
| Control | 457±17.58 | 979.3±7.196 | 101.66±1.085 | 547±1.238 |
| *Vigna Mungo* | 577.8±10.05 | 1135±13.17 | 115±2.017 | 609.3±2.654 |

## Epididymal sperm count

**Table 7: Epididymal Sperm Count of *Vigna mungo* treated rat**

| Sperm count Group | Million/cauda | Increase in sperm count Compared with control (%) |
|---|---|---|
| Control | 9.1 | 0 |
| *Vigna Mungo* | 11.1 | 14.2 |

**Histopathology of Testies:**

Transverse sections of testes of control group animals showed normal histo-architecture. The Sertoli and Leydigs cells of normal size were present. The somniferous tubules were normal in number with bundles of spermatozoa. In the transverse sections of extract-treated group animals, increase in diameter of seminiferous tubules was observed. Extract treatment also improved spermatogenesis in all groups as compared with control.

Large numbers of different cells at different stages of spermatogenesis were evident. The lumen of every seminiferous tubule had an enormous number of spermato- zoa. Sertoli cells were enlarged, highly pro- cessed, and rich in nutrients as shown by highly granulated cytoplasm. The increment in the volume of both cells and nuclei was strongly suggestive of steroid synthesis under the direct or indirect influence of the extract. Almost all of the tubules were overcrowded with sperm bundles. In some tubules, sperma- tids were found scattered amidst spermatozoa. The blood vessels of testis were slightly dilated. Increased spermatogenesis was evident from the large number of spermatozoa in the seminiferous tubules and was also shown by the increase in spermatogenic elements compared to controls.

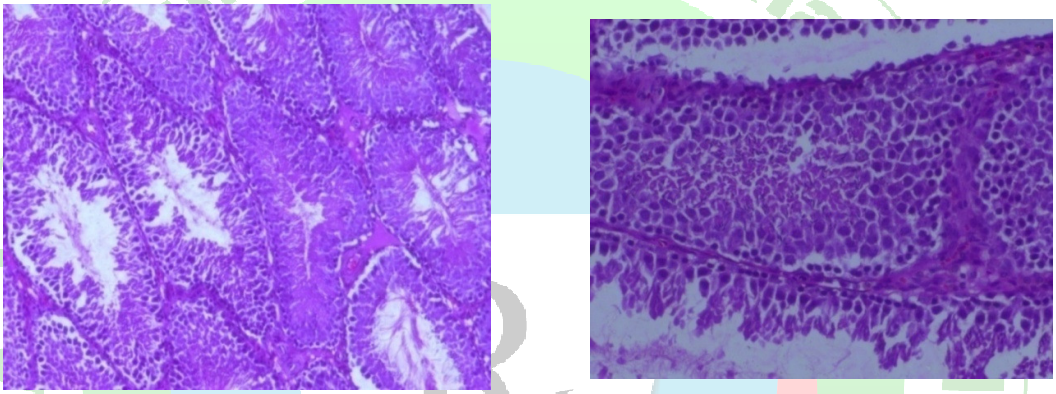

**Fig.7: Histopathology slide *Vigna Mungo* treared rat**

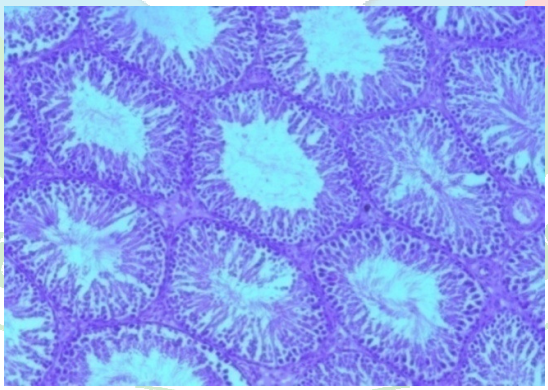

**Fig.8: Histopathology slide Saline treated rat**

## DISCUSSION

The data presented here provides evidence about the ability of the seed extracts of *Vigna mungo* to enhance male sexual behavior expression in sexually active rats and to promote sexual desire in sexually inactive male animals. The data obtained reveal that an oral administration of different doses of *Vigna mungo* extracts effectively facilitate several aspects of copulatory behavior. In the experimental analysis of male sexual activity, the concept of the existence of two different physiological mechanisms responsible for sexual behavior expression was introduced in the early 50s by Frank Beach. This notion

holds that one of these mechanisms is responsible for sexual arousal and the other for sexual performance. This concept has been central for the neurobiology of sexual behavior. [7]

The plant extracts were subjected for preliminary photochemical studies and aphrodisiac activity. The reports of photochemical studies showed the presence of steroids, carbohydrates, glycosides, proteins, saponins, gums and mucilages. Amount these compounds; some of the compounds definitely possess aphrodisiac activity. It was found that an increased copulatory sexual behavior and mounting were observed in animals treated with plant extracts. Among the two extracts, as clearly indicated, aqueous extracts of *Vigna mungo* possess potent aphrodisiac activity as evidenced by an increase in number of mounts, intermission frequency, ejaculatory behavior and mating performance. [8]

Finally, based on this preliminary data, it can be concluded that the herb *Vigna mungo* is a safe drug without any known adverse effects and can be very useful in enhancing the male sexual activity and treating various sexual disorders like erectile failure, premature ejaculation, lack of sexual desire and ejaculatory incompetence. However, further detailed studies are needed to confirm the usefulness this plant extract in treating sexual disorders. This includes separation, purification, and characterization of different chemical constituents of these extracts and testing the aphrodisiac activity of purified compounds.[9]

A histological comparison of the testes of the control group with those of the treated groups revealed a marked effect of both extract and testosterone on spermatogenesis. Sperma-togenesis is a cumulative process that culminates in the production of adult sperm. During this process, different cellular types can be observed, namely spermatogonia, spermatocytes and spermatides, which will ultimately give rise to the spermatozoa. The photomicro- graphs revealed better and non-striated vesicles, compared to those in the control group, suggesting better spermatogenetic activity in the extract-treated groups. Therefore, the results suggest that the extract causes an increase in testosterone level or has a testosterone-type action in the body. In the present investigation, use of the extract showed an improvement in spermatogenesis in the testis.[10]

The results of the present study suggested that *Vigna mungo* have a beneficial effect on male reproductive functions in rats. These data are confirmed by our observation on the increased sperm counts, motility. The increased sperm count and motility thereby shows that treatment with *Vigna mungo* improves and enhances the fertilizing capacity of the Semen. These qualities were often used as a measure of sperm production, testicular function and/ or male fertility.[11]

## CONCLUSION

Hence, the results of present study revealed that, Aqueous extract of seed of *Vigna mungo* improved sexual performance and sperm count. The effectiveness of the *Vigna mungo* in multiple preclinical models with desire mechanism of action might be due to the presence of flavonoids, proteins and tannins or its synergistic action of these phytoconstituents. However, the exact role of phytochemicals and their mechanism of action need future investigation.

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