ORIGINAL ARTICLE

Evaluation of Aphrodisiac Activity of Siddha Herbal Formulation Vithu Vagai Chooranam in Experimental Rats

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Abstract

Introduction: Aphrodisiac agents are used to arouse sexual desire, sexual pleasure, or sexual behavior. Many synthetic drugs are available in the market for improving sexual function. Even though modern medicines are widely accessible, it has some ill effects. To combat these side effects, people turn to alternative medical practices to combat these side effects. In this regard, a study was performed to investigate the aphrodisiac potential of herbal formulation *Vithu vagaichooranam* (VVC) by mating behavior study in male Wistar rats. **Materials and Methods:** The test drug VVC at 70 mg/kg and 90 mg/kg, Sildenafil citrate as Standard (5 mg/kg, p.o) and vehicle control (1 mL/kg p.o) were administered orally to rats (n = 6 animals per group) for 14 days. On the 15th day, the sexual behavior of the experimental rats was observed in a dim light in specially designed cages. The mating behavior parameter of male rats in each group was recorded individually. **Results:** Oral administration of VVC at both doses significantly increased the frequencies of mount, intromission, ejaculation frequency, Anogenital sniffing, and genital grooming ($P < 0.001$) when compared to the control and standard drug. The latencies of mount and intromission were significantly reduced and ejaculation latency was prolonged. **Conclusion:** The results of the study strongly suggested that the VVC has significant aphrodisiac potential in the above experimental model in male Wistar rats. Hence, in future, this formulation can be used as a potent stimulator of sexual behavior and an alternative for various sexual dysfunctions. The results thus support the therapeutic activity of this formulation claimed in the literature.

Key words: Aphrodisiac, Sexual dysfunction, Sexual mating behavior, *Vithu vagai chooranam*

INTRODUCTION

Male sexuality is a complex physiological process, which is essential in determining one's quality of life. Normal sexual function is maintained by the coordination of the human multi-system, the coordination of the nervous system, the cardiovascular system, the endocrine system, and the reproductive system. Male sexual dysfunction (SD) is not related to a single phenomenon. It includes the entire process of sexual activity in men, such as male sexual arousal, penis erection, penis inserting into the vagina, and ejaculation. An obstacle in any of these functions results in SD.[1,2] It is also described as a disorder of sexual behavior and sexual sensation that appears in a person, as an abnormality or absence of sexual psychology and physiological reaction.[3] Male SD is one of the most common health problems affecting men and is more common with increasing age. The Massachusetts male aging study found that 52% of men between 40 and 70 years old reported having some form of erectile dysfunction (ED).[4] In the world, about 15% of couples are affected by sexual disharmony among these 40–50% are because of malefactors. In general, the male sexual response cycle is divided into three parts: Libido (desire), erectile function, and sexual activity. Problems in the relationship with the sexual partner, lowered levels of the male hormone such as testosterone, certain drugs, such as antidepressants and blood pressure medications, disorders affecting blood vessels, such as atherosclerosis (hardening of the arteries) and high blood pressure, stroke, cerebral trauma, penile diseases like phimosis and Peyronie's disease, chronic

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renal failure, hepatic failure, multiple sclerosis, Alzheimer's disease, smoking, obesity, kidney problems, depression, anxiety disorders, and alcoholism can cause SD.[5-8]

An aphrodisiac is defined as any substance which may be food or drug that arouses the sexual instinct and increase libido. The drugs that increase the quantity of semen or stimulate the production of semen, purify, and improve the quality of semen, and improve ejaculatory functions, and drugs that delay the time of ejaculation or improve ejaculatory performance are called an aphrodisiac.[9-11] Many synthetic drugs are available on the market to treat SD. However, the high costs, complicacy of infections in surgical procedures, mechanical failure of devices, acceptability, and the serious adverse effects create awareness to limit the usage and search for safe, cost-effective alternatives. Hence, the quest for finding active, natural principles that have been useful in sexual disorders is planned. These drugs should have the potential to improve sexual behavior and performance and increase spermatogenesis and reproduction. The use of plant-based products to stimulate sexual desire and enhance performance and enjoyment is common in humans. Many herbal drugs have been validated for their effect on sexual function and can thus be used to identify new chemical leads useful in sexual and ED.[12,13] Herbs have been used in traditional medicines to support the male reproductive system, stimulate healthy sperm production, maintain hormonal balance, and support stronger erections to enhance male fertility and ensure conception.

In the Siddha system, many formulations are available to improve sexual function and performance. Siddha drugs are becoming widely popular because of their effectiveness with minimal side effects. According to *Siddhar Theraiyar*, it is understood that the vital thing that is essential for the origin of life is *vindhu* from the fusion of *vindhu* (sperm) with *nadham* (ovum), life originates. In our Siddha system, spermatogenesis and aphrodisiac activities may be compared to *Thathuviruthi*.[14] In that aspect, *Vithu Vagai chooranam* (VVC) is a classical Siddha compound drug that is mentioned in Siddha literature *Noigaluku Siddha parigaram*- part 1; page no: 325, written by Dr. M. Shanmugavelu. This drug is used for the management of *Neerchurukku* (Oliguria), *Thathuviruthi* (Spermatogenesis), and *Vellai* (Leucorrhea).[15] Moreover, most of the ingredients of VVC have already been proven the aphrodisiac, spermatogenic, anti-fertility, and anti-oxidant activities in various scientific studies. The present study aimed to evaluate the aphrodisiac activity of siddha herbal formulation VVC in male Wistar rats.

MATERIALS AND METHODS

Identification and authentication of the drug

The collected raw materials were identified and authenticated by the Assistant Professor, Department of Medicinal Botany, National Institute of Siddha, Tambaram sanatorium. (Certificate No: NISMB4812021).

Preparation of trial drug: VVC

The ingredients of VVC are shown in Table 1. The ingredients after purification were ground separately to powder. The powder was sieved through a white cloth. All these powdered ingredients were mixed thoroughly in a stone mortar. The prepared test drug was stored in a clean, air-tight glass container.

Drug dosage: *Mooviralalavu* (800–1000mg), twice a day.

Adjuvant: Milk

Indications: *Neerchurukku* (Oliguria), *Thathuviruthi* (Spermatogenesis), *Vellai* (Leucorrhoea).

Evaluation of the aphrodisiac activity by mating behavior study in male Wistar rats animal procurement and maintenance

a) Species/Commonname: Rattus norvegicus/Wistar albino rat
b) Age/weight/size:180–200gms, 8–10weeks
c) Gender: Male
d) AcclimatizationPeriod: 7 days prior to dosing
e) Housing: Polypropylene cages
f) Husbandry: 12-h light/12-h cycle
   Photoperiod 24°c (±2°)
   Relative humidity 30–70%
g) Feed andwater: Rodent pelletedfeed, RO purified water adlibidum

Healthy male Wistar albino rats weighing between 180 g and 200 g were used in the study. The animals were obtained from Kerala Veterinary and Animal Science University, Thiruvazhamkunnu, Kerala. After receiving the animals, they were acclimatized in the animal house condition of Nandha College of Pharmacy, Erode, for 7 days by placing the animals in polypropylene cages with paddy husk as bedding. All the experimental procedures and protocols used in this study were reviewed and permitted by the Institutional Animal Ethics Committee (688/PO/Re/S/02/CPCSEA) and were under the Institutional ethical guidelines (Proposal Number: NCP/IAEC/2021-22/14).

Animal grouping and experimental protocol

The sexually active male rats were selected for testing aphrodisiac activity. The male rat that did not show any sexual interest while exposed to female rats were considered sexually inactive. The sexually active male rats were chosen separately and divided into four groups, each group consisting of six animals. Different groups of animals that received the drug VVC and the control are as follows: Group I served as

control received milk (1 mL/kg, P.O) and 10 mL/kg of 6% ethanol, Group II animals administered with 10 mL/kg of 6% ethanol and Sildenafil citrate (5 mg/kg., P.O.). Group III and IV animals were administered with 70 and 90 mg/kg of VVC, respectively, along with 10 mL/kg of 6% ethanol orally.

All the test compounds were administered orally once daily for 14 days. On the 15th day, the sexual behavior of the experimental rats was observed in dim light in specially designed cages that had glasses on all sides and measured 50×30×30 cm. The male experimental rat was first placed in the cage and then two female rats in the estrous phase by administering estradiol valerate (10 µg/kg., S.C) and hydroxy progesterone (1.5 mg/kg., S.C) were introduced. An initial period of 15 min was considered an acclimatization period. After 15 min, the activity of male rats in each group was recorded individually for 60 min. The aphrodisiac activity of VVC was measured by observing the following parameters such as mount frequency (MF), mount latency (ML), intromission frequency (IF), intromission latency (IL), ejaculation latency (EL), genital grooming (GG), and anogenital sniffing (AS) after exposure to the female animals.[16]

The following mating behavior parameters were recorded:

a. MF: The number of mounts without intromission from the time of introduction of the female until ejaculation.
b. IF: The number of intromissions from the time of introduction of the female until ejaculation.
c. ML: The time interval between the introduction of the female and the first mount by the male.
d. IL: The interval from the time of introduction of the female to the first intromission by the male (characterized by pelvic thrusting and springing dismount)
e. EL: The time interval between the first intromission and ejaculation (characterized by longer, deeper pelvic thrusting, and slow dismount followed by a period of inactivity)
f. AS: Sniffing of the genital part by the experimental male rat after exposure to the female rats.
g. GG: Licking of the genital part by the active male with its tongue.

Statistical analysis

The results were expressed as mean ± standard error mean (SEM). One-way analysis of variance was applied for the statistical analysis, followed by Dunnett's *t*-test. Statistical analyses were performed using GraphPad Prism 5.0 and a $P < 0.05$ was statistically significant.

RESULTS

The drug VVC showed a significant increase in mating behaviour as compared to vehicle control . the results are presented in Tables 2 and 3.

DISCUSSION

The aphrodisiac activity of VVC was studied in male Wistar rats at the dose levels of 70 and 90 mg/kg body weight and the results are shown in Tables 2 and 3, Figures 1–9 show the aphrodisiac activity which was assessed by observing the sexual behaviors (MF, ML, IF, IL, EL, GG, and AS) after oral administration of VVC for 14 days. Sildenafil citrate is a well-known synthetic agent which increases sexual desire and was used as the standard for reference. The mating behavior study revealed that with continuous oral administration for 14 days, both doses of VVC (70 and 90 mg/kg) were able to significantly ($P < 0.001$) decrease sexual behaviors like ML, IL, and EL when compared to vehicle control rats. It further significantly ($P < 0.001$) increases the male sexual behaviors like MF, IF, AS, and GG in both doses of VVC when compared to control animals. Among both doses of VVC, 90 mg/kg was found to be significant ($P < 0.001$) and more effective. There was an overall increase in the sexual behavior parameters in VVC at 90 mg/kg treatment group of rats as reflected in MF, IF, AS, GG, and a reduction in ML, IL, and EL [Tables 2 and 3]. MF and IF are indices of libido and potency, while ML and IL are also indicators of

Figure 1: Ejaculation latency (70 mg/kg *Vithu vagaichooranam*)

Figure 2: Mount latency (70 mg/kg *Vithu vagaichooranam*)

Table 1: Ingredients of *Vithu vagaichooranam*

S. No.	Name of the drug	Scientific name	Common name	Quantity
1	*UlunthuMaavu (Fabaceae)*	*Vigna mungo (L.)*	Black gram	*1 palam* (35g)
2	*Ellu (Pedaliaceae)*	*Sesamum indicum L.*	Sesame	*1 palam* (35g)
3	*Poonaikkalivithu (Fabaceae)*	*Mucuna pruriens (L.)*	Velvet bean	*1 palam* (35g)
4	*Neermullivithu (Acanthaceae)*	*Hygrophila auriculata (schumach.) heine*	Marsh barbel	*1 palam* (35g)
5	*NilappanaiKizhangu (Amaryllidaceae)*	*Curculigo orchioides gaertn*	*Golden Eye Grass*	*1 palam* (35g)
6	*ThanneervittanKizhangu (Lilliaceae)*	*Asparagus racemosus willd*	Wild asparagus	*1 palam* (35g)
7	*Chukku (Zingiberaceae)*	*Zingiber officinale rosc*	Ginger	*½ palam* (17.5g)
8	*Milagu (Piperaceae)*	*Piper nigrum L*	Black pepper	*½ palam* (17.5g)
9	*Thippili (Piperaceae)*	*Piper longum L*	Long pepper	*½ palam* (17.5g)
10	*Vellai sarkkarai (Poaceae)*	*Saccharum officinarum L*	White sugar	*½ palam* (17.5g)

Table 2: Effect of *Vithu VagaiChooranam* on sexual behavior (mount frequency, intromission frequency, anogenital sniffing, and genital grooming) in male rats

Groups	Drug treatment	Mount frequency	Intromission frequency	Ano-genital sniffing	Genital grooming
I	6% ethanol (10 mL/kg)+control milk (1 mL/kg)	2.00±0.26	0.33±0.21	3.67±0.56	3.17±0.48
II	6% ethanol (10 ml/kg)+sildenafil citrate (5 mg/kg)	11.83±0.60***	1.50±0.22***	11.17±0.40***	14.83±0.48***
III	6% ethanol (10 mL/kg)+ *Vithu Vagai Chooranam* (70 mg/kg)	9.83±0.54***	1.33±0.33***	12.00±0.58***	15.33±0.49***
IV	6% ethanol (10 mL/kg)+ *Vithu Vagai Chooranam* (90 mg/kg)	12.50±0.43***	1.67±0.21***	14.67±0.42***	16.17±0.31***

Values were in Mean±SEM (n=6); *P<0.05, **P<0.01 and ***P<0.001 compared to control

Table 3: Effect of *Vithu VagaiChooranam* on sexual behavior (mount latency, intromission latency, and ejaculation latency) in male rats

Groups	Drug treatment	Mount latency (s)	Intromission latency (s)	Ejaculation latency (s)
I	6% ethanol (10 mL/kg)+control milk (1 mL/kg)	269.83±2.64	824.50±11.11	628.83±9.33
II	6% ethanol (10 mL/kg)+sildenafil citrate (5 mg/kg)	109.33±2.39***	165.50±5.76***	265.50±5.72***
III	6% ethanol (10 mL/kg)+*Vithu Vagai Chooranam* (70 mg/kg)	90.17±2.00***	173.67±4.98***	260.33±6.33***
IV	6% Ethanol (10 mL/kg)+*Vithu Vagai Chooranam* (90 mg/kg)	88.50±1.84***	155.33±4.76***	245.33±2.13***

Values were in Mean±SEM (n=6); *P<0.05, **P<0.01 and ***P<0.001 compared to control

sexual arousal.[17-19] The significant increases in MF, IF, AG, GG, and the decreases in ML, and IL indicate that libido and potency were enhanced by the trial drug VVC. Furthermore, the prolongation of EL is an indicator of the prolonged duration of coitus. These observations all further support the role of VVC in improving sexual function.

This study described the effect of the Herbal formulation VVC sexual behavior in male rats, with sildenafil citrate as the Standard drug. Sildenafil is a popular drug, which is used to treat male sexual function problems (impotence or ED). In combination with sexual stimulation, sildenafil works by increasing blood flow to the penis to help a man to keep an erection. Sildenafil belongs to a group of medicines called phosphodiesterase 5 (PDE5) inhibitors. These medicines prevent an enzyme called PDE5 from working swiftly. The penis is one of the areas where this enzyme acts. In addition, Sildenafil can cause mild to serious side effects such as headache, flushing, visual disturbances, dizziness, nasal congestion, and rashes which may go away within a few days or a couple of weeks. The drug can cause serious side effects such as allergic reactions, priapism (long-lasting and sometimes painful erection), low blood pressure, and cardiovascular problems, such as heart attack, irregular heartbeat, or stroke.[20]

In the present study, it is known that the trial drug VVC enhanced the sexual behavior of male Wistar rats with

Figure 3: Intromission latency (70 mg/kg *Vithu vagaichooranam*)

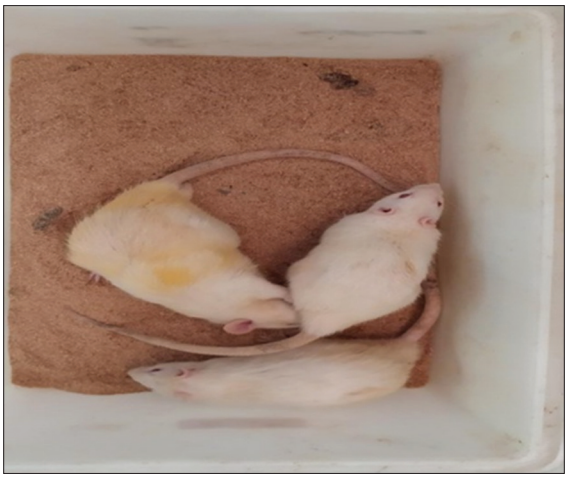

Figure 4: Ano-genital sniffing (70 mg/kg *Vithu vagaichooranam*)

Figure 5: Pouncing behavior (70 mg/kg *Vithu vagaichooranam*)

Figure 6: Anogenital sniffing (90 mg/kg *Vithu vagaichooranam*)

Figure 7: Mount latency (90 mg/kg *Vithu vagaichooranam*)

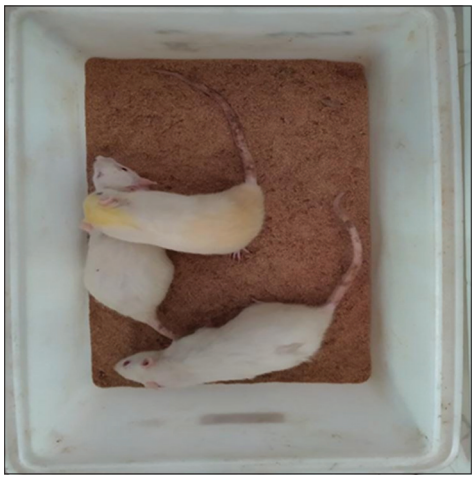

Figure 8: Pouncing behavior (90 mg/kg *Vithu vagaichooranam*)

no adverse effects when compared with the control and standard drug. This result is in agreement with Sahoo *et al.*, who studied the Aphrodisiac activity of polyherbal formulation (PHF) in experimental models on male rats. The study examined the effect of PHF on male sexual competence in rats, with sildenafil citrate as a positive reference drug. A mating behavior study revealed that with continuous administration for 3 weeks, all the doses of PHF were able to significantly decrease mount and intromission latencies when compared to vehicle control and standard drug-treated rats.

Figure 9: Ejaculation latency (90 mg/kg *Vithu vagaichooranam*)

Among the key ingredients, rhizomes of *Curculigo orchioides*, seeds of *Hygrophila auriculata*, roots of *Asparagus racemosus*, *Sesamum indicum*, Seeds of *Vigna mungo* and *Mucuna pruriens* are popular for their aphrodisiac, anti-oxidant, and immunostimulatory properties. These herbs are used to cure impotency and sterility and enhance male sexual potency ultimately for the treatment of certain forms of sexual inadequacies, such as premature ejaculation and oligospermia in various scientific studies.[21,22] *C. orchioides* is a rich source of phytochemicals like flavonoids and polyphenols. This plant is known for adaptogen and aphrodisiac activity due to the presence of flavonoids and polyphenols.[23] Chauhan *et al*. evaluated the ethanolic extract of rhizomes of *C. orchioides* for its effect on sexual behavior in rats. Administration of 100 mg/kg of extract change significantly the sexual behavior as assessed by determining parameters such as penile erection, mating performance, MF, and ML. Ghosh *et al*. investigated the protective effect of ethanolic extract of *H. auriculata* seeds in cyproterone acetate (CPA)-induced SD in male albino rats. Count, motility, and viability of spermatozoa, the number of hypo-osmotic tail-swelled spermatozoa, and serum testosterone level were significantly decreased in CPA treated rats. All parameters were significantly restored after the treatment of *H. auriculata* extract in the CPA-treated rats.[24]

According to Indian systems of medicine, *V. mungo* has been used for treating male sexual disorders since ancient times.[25] The effects of alcoholic extracts of the *V. mungo* Linn. Seeds on general mating behavior, libido, and potency of normal male Wister albino rats were investigated and compared with the standard reference drug, sildenafil citrate. The results indicated that the alcoholic extracts of *V. mungo* Linn. Seed produced a significant and sustained increase in the sexual activity of normal male rats at a particular dose (500 mg/kg).[26] The hydro-alcoholic and aqueous extracts of the roots of *A. racemosus* were subjected to preliminary phytochemical screening which showed the presence of saponins, carbohydrates, glycosides, and mucilages. The hydro-alcoholic extract of *A. racemosus* root at higher concentration (400 mg/kg body weight) showed significant aphrodisiac activity on male Wistar albino rats as evidenced by an increase in the number of mounts and mating performance. Oral administration of 5 g of *Mucuna* seed powder once a day for men with decreased sperm count and motility ameliorated psychological stress and seminal plasma liquid peroxide levels along with improved sperm count and motility.[26] *M. pruriens* seed powder when administered in a dose of 75 mg/kg body weight daily as an aqueous suspension increased the sexual activity of male albino rats considerably.[27] *S.indicum* is a good source of natural antioxidants due to the presence of alkaloids, saponins, flavonoids, tannins, steroids, terpenoids, anthraquinones, and phenols.[28] It improves sexual performance and vitality. The phytochemicals present in the ingredients of VVC might be responsible for the spermatogenic, aphrodisiac, anti-fertility, and anti-oxidant activities which support the therapeutic claim mentioned in the literature. Therefore, the drug VVC could be a potent stimulator of Sexual behavior, especially because it enhances sexual arousal in male rats.

CONCLUSION

The observed results of sexual behavior parameters such as MF, IF, ano-genital grooming, and GG confirmed that the trial drug VVC can be very well used for sexual improvement. This aphrodisiac property of VVC may be due to the possible synergistic action of selected plants used in VVC. The cumulative effect of VVC could enhance overall sexual function and it can be an effective and safe aphrodisiac Siddha herbal combination, which can be used in infertility treatment. This research work throws light to carry out further extensive clinical trials to prove the therapeutic potential of this formulation in humans.

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ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee (Proposal Number: NCP/IAEC/2021-22/14).

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