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APHRODISIAC ACTIVITY OF VENTHAMARAI MAGARANTHA CHOORANAM (STAMENS OF *NELUMBONUCIFERA* WHITE VARIETY) ON HEALTHY WISTER ALBINO RATS

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ABSTRACT

Nelumbonucifera is often used in siddha medicine as an aphrodisiac and to improve male reproductive functions.Since systematic study on these aspects are few and fare between, we studied its aphrodisiac effect on Normal wister albino rats. Adult wister albino rats were used for aphrodisiac activity protocol. Sexual behavioural parameters were observed on wister albino rats. Blood samples were collected from control and experimental rats to measure hormone testosterone. Testosterone levels showed significant increase in experimental animal compared with control. The test drug may be effective as aphrodisiac through mechanisms such as vasodilation, generation of nitric oxide, elevation of androgens and gonadotropins. It has also been documented that sexual behaviour and erection are dependent on androgen, which may act through central and peripheral mechanisms.Stamens of *Nelumbonucifera* white variety has definite positive effect on male sexual behaviour and increased in hormone profile.

Key words : Nelumbonucifera, stamens, testosterone hormone, sexual behaviour

1. INTRODUTION

*Nelumbonucifera,*which belongs to nelumbonacea family is used in the traditional system of medicine. It is commonly known as lotus or Thamarai. Lotus is a perennial, large and rhizomatous aquatic herb with slender, elongated, branched, creeping stem consisting of nodal roots; Flowers are white to rosy, sweet-scented, solitary, hermaphrodite, 10-25 cm diameter, ripe carpels are 12 mm long, ovoid and glabrous (Sridhar. K.R. 2007).Lotus is the national flower of India. It symbolizes purity, beauty, majesty, grace, fertility, wealth, richness, knowledge and serenity. Lotus means estranged love, the state of spiritual perfection and total mental purity. Traditional knowledge reveals many medicinal uses of lotus. The whole plant serves as astringent, emollient, diuretic, diaphoretic and possesses antifungal, antipyretic and cardiac tonic. Flowers are useful to treat diarrhoea, cholera, fever, hepatopathy, hyperdipsia.lotus stamens having anti-oxidant property (jung et al.2003). It is also considered to be an rejuvenating tonic for overall health. Erectile dysfunction is sexual dysfunction characterized by the inability to develop or maintain an erection of the penis during sexual performance. It is eluding scientific community and medical practitioners since time immemorial (Adimoleilija A. 1997). Generally, a prevalence of about 10% occurs across all ages. A recent

study estimated that 152 million men worldwide experience some degree of impotence. In many countries the stigma of impotence often leads to marital disharmony and divorce.

Research during past two decades has an unfolded focus on impotence**.** There are a number of prescription drugs which may act as sexual stimulant and enhancing the sexual desire and activity, the use of medicines have not shown significant improvement in treating sexual disorders, at the same time there are large number of side effects. These include arrhythmias, suicide tendency, mental confusions and tremors etc. The use of synthetic aphrodisiacs results in the dilatation of blood vessels in other parts of the body causing head ache and fainting. Other side effects include facial flushing, stomach upset and blurred vision. Thus, there is a growing need to look for aphrodisiacs more of natural plant as opposed to synthetic compounds. In this regard, we undertook the present studies on *Nelumbonucifera* which has been known as aphrodisiac. Still there has been no methodical study, to validate this activity. Taking the male sexual dysfunctions in to consideration, the current studies in aphrodisiac activity on *Nelumbonucifera* white variety is intended to look for safe and powerful aphrodisiac. We have studied the crude drug powder for their in vivo aphrodisiac activity on wister albino rats.

2. MATERIALS AND METHODS

2.1 Plant material

The fresh flowers were collected from Kumbakonam, Tamilnadu. The same were botanically identified, confirmed and authenticated by Botanical department, Central research institute of siddha, Chennai-106.The fresh flowers Nelumbo nucifera were peeled out the petals, only the stamens were collected .These were shade dried for one week and the dried materials were powdered and subjected for various studies. The drug was uniformly suspended in 2% Carboxy Methyl Cellulose in water to obtain 100mg/ml concentration as stock solution and Commercially available Sildenafil citrate purchased from Pfizer pharma pvt Ltd used in this study.

2.2 Animals selection

Male and female albino rats of average body weight of 190±7g were kept separately in individual polypropylene cages with stainless steel hopper in air-conditioned room (24°C) of the animal house under uniform animal husbandary conditions. The animals were fed basal diet (Sai meera foods. Bangalore) and water *ad libitum*. The animals were acclimatized to temperature and lighting (12 h light/dark) conditions of the animal house. Healthy and sexually experienced male albino rats that show brisk sexual activity selected for the study.

2.3 Preparation of Animals

Female rats were housed in groups of two and males were housed singly in polypropylene cages with free access to standard feed (Hindustan Lever) and tap water. A reversed twelve hour light-dark cycle was employed with fluorescent ceiling light. A dim red light was provided during dark cycle. All the rats were allowed 2 weeks to adjust to the environment prior to the experiment.

2.4 Preparation of females

Female albino rats were overectomized at 80 days of age and allowed 10 days to recover from surgery. After ovariectomy, the animals were in diestrousstage. To bring them to estrous, they were given 2 µg/mkg oestrogen orally 48 hours before test and 500µg/kg of progesterone 4-6 hours before copulatory test.

2.5 Training of males

Males were trained individually with active females in oestrus in a transparent mating arena (60×30×18 cm) for 5 mnts.Amale was considered sexually active when it attempted to mount any active female introduced in to the cage. Only such males were used for subsequent experiments. The average mountings in a normal male found to be about 8-10 in 5 minutes. The males, which mounted 3-5 times in 5 min, were considered as sexually sluggish.

2.6 Experimental details

The experimental protocol for *venthamarai magarantha Chooranam* (XIII/VELS/COL/05/CPCSEA/IAEC/23.09.11)

was approved by the CPCSEA/IAEC of Vel's College of Pharmacy, Vel's University, Pallavaram, Chennai.

2.7 Acute toxicity study

The substance is administered orally to a group of experimental animals at one of the defined doses. The acute oral toxicity study was carried out as per the OECD guidelines-425. The substance is tested using a stepwise up and down procedure, Absence or presence of drug-related mortality of the animals dosed at one step to determine the next step, i.e.; − no further testing is needed. The method will enable a judgement with respect to classifying the test substance to one of a series of toxicity classes. Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. All observations are systematically recorded and Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. One-tenth of the lethal dose was considered as therapeutic dose for further pharmacological study.

2.8 Animals

Female mice were fasted overnight and given single oral dose of *Venthamaraimagarantha chooranam* suspended in 2% Carboxy Methyl Cellulose with water (Starting dose 500mg/kg and upto 5000mg /kg body wt). The animals were fed with *Venthamaraimagarantha chooranam* and then they were observed for 14 days to record signs of toxicity and death if any.

2.9 Materials and methods adopted in this study

The siddha drug *Venthamaraimagarantha chooranam* was uniformly suspended in 2% Carboxy Methyl Cellulose in water to obtain 100mg/ml concentration as stock solution and Commercially available Sildenafil citrate was purchased from Pfizer pharma pvt Ltd, used in this study.

2.10 Evaluation of aphrodisiac activity

Mating behaviour test

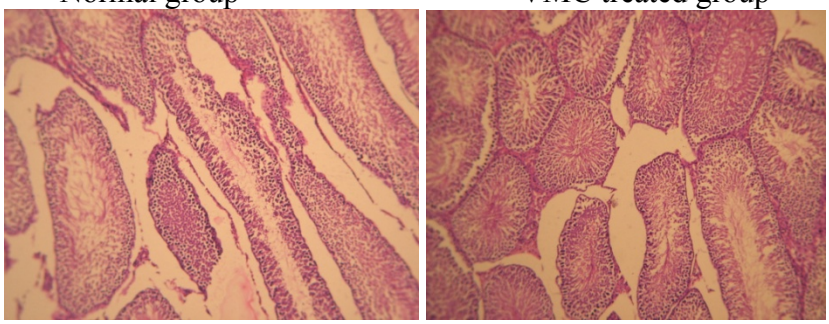
After drug administration to the animals according to the experimental design and objective, the male animals were brought to the laboratory and exposed to dim light at the stipulated time of testing daily (3-6 days) before the experiment. The female animals were artificially brought into oestrus by administering ethinyloestradiol orally at the dose of 100µg/animal 48h prior to the pairing. The receptivity of the female animals was studied before the test by exposing them to male animals. The most receptive females were selected for the study. The experiment was conducted at 20:00 h in the same laboratory and under light of same intensity. The receptive female animals were introduced into the cages of male animals in the ratio 1:1. An initial period of 15 minutes was considered as acclimatization period. After 15 minutes, the drug was administered the activity of male rat in each group was recorded individually for 60 minutes, the parameters of observed were**,** Mount frequency, Intromission frequency, Mount latency, Intromission latency, Anogenital sniffing, Genital grooming.

2.11 Histopathological study

At the end of the drug treatment and after blood collection the animals from each group was sacrificed with the help of diethyl ether euthanesia method and the abdomen was cut opened and the testis was carefully isolated andfixed in 10% formalin solution. The testis was embedded in paraffin and sectioned and stained with heamtoxylin and eosin and were examined microscopically for histopathological changes. The testis section of control group animals showed normal histological texture. The diameter of seminiferous tubules varied within a range. The tubules having maximum diameter, were not abundant and well within range. The cuboidal germinal epithelium exhibited normal shape and size. Sertoli cells had many cytoplasmic processes, which were normal in size. Spermatozoa were embedded in the sertoli cells and showed normal cytoplasmic

granulation. Leydigs cells had normal nuclear size. Luminal part of the tubule were normal in number with bundles of spermatozoa. Spermatozoa with long tail with small distinct head were more visible. The results shown no other significant changes were noted when compared with the control group (Kanitkar M et al).

Normal group VMC treated group



2.12 Statistical analysis

The obtained data were expressed as mean±standard error of mean (SEM) of six animals in each group. The data from all the groups were analyzed using one way analysis of variance(ANOVA) followed by Dunnett's t-test using Graph pad instate software.

3. RESULTS AND DISCUSSION

In the present investigation, increase in mounting frequency and anogenital sniffing was noticed as compared to control. The attraction towards environment is more in control than drugs treated group. There is also increase in attraction towards female and genital grooming in treated rats, which is comparable with standard but was not statistically significant. An orientation activity reveals that the treatment of test drug causes increase in attraction of male towards femal. Several female proceptive and male precopulatory behaviour parameters were observed from the cage side when the VMC-treated male rats were introduced to the receptive female rats. The proceptive behaviour displayed by the female rats included ear-wiggling characterized by a rapid anteroposterior vibration of the ears, a short run where the female rats suddenly stops and present her posterior to the male rats (darting) and a short jump with stiff legs followed by immobility and presentation (hopping). The male rats, upon introduction, responded with immediate advances towards the females and displayed precopulatory behaviour such as chasing, anogenital sniffing which eventually culminated into mounting.

Lordosis was also displayed by the receptive female rats before, at the beginning and during the mounts. There was genital toileting after every mount that resulted in intromission. The test drug at 500mg/kg body weight, both MF and IF were increased ($P<0.01$) compared to the 2% CMC in saline-administered control and body weight also significantly increased. The MF of the test drug treated animals was remarkably altered and it was statistically significant. Administration of single dose of test drug at 500mg/kg body weight the EF increased significantly ($P<0.01$). There was vaginal plug in the female's vagina after ejaculation was observed. Further administration of the test drug for 14 days increased the EL whereas the PEI decreased. The changes in EL and PEI were statistically significant ($P<0.01$). The male sexual behaviour parameters, which included percentages of index of libido, mounted, intromitted, ejaculated and copulatory efficiency were higher in the test drug treated animals compared to the 2% CMC in saline treated control animals. To understand the scientific reasons behind these traditional claims, an attempt was made to investigate the effects of test drug in this study. In this investigation, treatment of the male rats with the test drug enhanced the sexual behaviour of the male rats with 500mg/kg body weight producing better results. These sexual behaviours were preceded with proceptive and precopulatory behaviours in the animals. (For example, the ear wiggling,

darting, hopping and lordosis by the receptive female rats in this study implied intense proceptivity and receptivity). The pursuit of the female animals (the males running behind the female animals in close contact) suggested imminent copulation. Mount Frequency and Intromission Frequency are useful indices of vigour, libido and potency. While the number of mount (MF) reflects sexual motivation, increase in the number of intromission (IF) shows the efficiency of erection, penile orientation and the ease by which ejaculatory reflexes are activated. Therefore, the increase in MF and IF ($P<0.01$) following the administration of test drug at 500mg/kg body weight observation suggests enhanced libido. Such enhancement of libido might have arisen from increase in the number of concentrations of several anterior pituitary hormones and serum testosterone, which in turn stimulated dopamine receptor synthesis and sexual behaviour.

This sexual behaviour may also be due to androgenic and gonadotropic activities of test drugin male rats. Furthermore, since intromission is not possible without adequate erection and coordinated activity of penile muscles, the increase in IF by the test drug in this study suggests that the mechanism of penile erection was activated. Therefore, test drug may increase potency by allowing or sustaining erection. The increase in ejaculation frequency by the test drug at 500mg/kg body weight is an indication of enhanced aphrodisiac effect. The presence of plug in the vagina of the female rats indicated that ejaculation occurred. This was further complemented by the genital toileting observed in the male rats. Mount latency and intromission latency are indicators of sexual motivation. ML and IL are inversely proportional to sexual motivation. The decrease in the intromission latencies observed ($P>0.05$) at the dose of 500mg/kg body weight of the test drug in this study might imply stimulation of sexual motivation and arousability but it was not statistically significant when compared to control. Furthermore, the prolonged ejaculation latency by the test drug 500mg/kg body is an indication that copulatory performance in the animals was enhanced. It may also imply prolongation in the duration of coitus. In addition, the display of pelvic thrusting during intromission and ejaculation by the test drug treated animals in this study further indicated that the male copulatory organ was in contact with the vaginal orifice, which might have activated or strengthened lordosis in the female rats. The post ejaculatory interval is considered an index of potency, libido and the rate of recovery from exhaustion after first series of mating. An increased post ejaculatory interval indicates that the male is sexually exhausted and the intensity of sexual behaviour will be reduced in subsequent mating.

Therefore, the significantly decreased post ejaculatory interval at 500 mg/kg body of the test drug may be attributed to enhanced potency and libido or less exhaustion in the first series of mating or both, more so, since the values of PEI obtained in this study are close to the 90 min cut-off. In addition, the higher values of the male rat sexual behaviour parameters following treatment with the test drug when compared with the 2% CMC in saline-administered control animals are indications of significant and sustained increase in sexual activity. As indicated in siddha literature, the test drug may be effective as aphrodisiac through mechanisms such as vasodilation, generation of nitric oxide, elevation of androgens and gonadotropins. It has also been documented that sexual behaviour and erection are dependent on androgen, which may act through central and peripheral mechanisms.

Table 1*: Effect Of test drug On Sexual Behaviour In Rats*

Groups	*Parameters (Duration in Seconds)*					
	Mount frequency	*Intromission frequency*	*Mount latency*	*Intromission latency*	*Anogenital sniffing*	*Genital grooming*
Control	2.24±0.36	298.18±8.14	0.37±0.18	806.13±267.15	3.22±0.63	1.78±0.13
Standard (Sildenafil)	12.41±1.22**	115.19±7.52**	1.48±0.18**	174.99±95.15ns	15.02±1.38**	4.18±0.38**
VMC (500mg/kg)	7.85±0.96**	171.06±4.20**	1.63±0.25**	485.72±188.04ns	10.10±1.24**	2.92±0.50ns

*Values are mean of 6 animals ±S.E.M. (Dunnett's test). nsP>0.05; **P<0.01 compared to control.*

Table 2: Effect of VMC on SERUM TESTOSTERONE LEVELS OF RATS IN ng/ml.

Serum testosterone level (ng/ml)	
Control	*VMC 500mg/kg*
3.44	3.96
3.24	3.72
3.08	3.88
3.80	3.84
3.02	3.80
3.67	3.76

4. CONCLUSION

In conclusion, our results have revealed that the test drug at the dose of 500mg/kg body weight could be used as a stimulator of sexual behaviour in male rats and also indicates the profoundincrease in improvement of sperm health. This study thus supports the acclaimed aphrodisiac use. The data obtained revealed that the action of the test drug was due to the influence on both sexual arousal and performance.

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