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APHRODISIAC ACTIVITY OF ETHANOLIC EXTRACT OF *VITEX NEGUNDO* FRUIT IN MALE WISTAR ALBINO RATS

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ABSTRACT

Vitex negundo was a hardy plant belonging to the family Verbenaceae, traditionally used as an aphrodisiac in male. The objective of the present study is to evaluate the aphrodisiac activity of ethanolic fruit extract of *Vitex negundo* on male albino rats and the effects were compared with the normal control and reference control sildenafil citrate. Male Wistar albino rats were divided in to 4 groups of 6 animals each. Ethanolic fruit extract of *Vitex negundo* (200 & 400mg/kg body weight) were administered orally and its sexual performance was compared to the normal and reference control animals (Sildenafil citrate 4.5mg/kg body weight). The aphrodisiac activities of rats due to the effect of the herb were observed by various parameters such as mount frequency, mount latency, intromission frequency, intromission latency, ejaculation latency, post ejaculatory Interval and total sexual behavior. The data's were analyzed using ANOVA followed by dunnetts't' test. *Vitex negundo* showed dose dependent increase in mounting frequency, intromission frequency, ano-genital sniffing and genital grooming and decrease in mounting latency and intromission frequency as compared to control. The ethanolic fruit extract of *Vitex negundo* showed comparable aphrodisiac effect with the reference control sildenafil citrate. From the result it was concluded that, ethanolic fruit extract of *Vitex negundo* posses aphrodisiac activity in male rats.

INTRODUCTION

Erectile dysfunction is sexual dysfunction characterized by the inability to develop or maintain an erection of the penis during sexual performance. An aphrodisiac is defined as an agent that arouses sexual desire. Sexual dysfunction is a repeated inability to achieve normal sexual intercourse, which includes various forms like premature ejaculation, retrograded, or retarded ejaculation, erectile dysfunction, arousal difficulties etc [1].

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Aphrodisiacs are the substances which are used to increase sexual activity and help in fertility. Sexual feelings are an inevitable part of life. The basic and fundamental purpose of sex and sexuality is the "continuation of progeny" and the survival of human race [2]. The search for natural supplement from medicinal plants is being intensified, probably because of reduced side effect, its ready availability and reduced cost [3]. Therefore, it is necessary to search and screening of medicinal plants with aphrodisiac potential in male. *Vitex negundo* L. is a hardy plant belonging to the family Verbenaceae. *Vitex negundo* Linn., commonly known as Five-leaved Chaste tree or Monk's Pepper is used as medicine fairly throughout the

greater part of India and found mostly at warmer zones and ascending to an altitude of 1500m in outer Western Himalayas [4]. Plant is bitter, acrid, astringent, cephalic, stomachic, antiseptic, alterant, thermogenic, depurative, rejuvenating, ophthalmic, anti-gonorrhoeic, antiin flammatory, antipyretic and useful in bronchitis, asthma and enlargement of spleen. Roots are tonic, febrifuge, anti-rheumatic, diuretic, expectorant and are useful as a demulcent in dysentery, in cephalalgia, otalgia, colic, uropathy, wound and ulcers. Bark is useful in odontalgia, verminosis and ophthalmopathy. Leaves are aromatic, bitter, acrid, astringent, anodyne, anti-inflammatory, antipyretic or febrifuge, tranquillizer, bronchial smooth muscle relaxant, anti-arthritic, antihelmintic and vermifuge. Flowers are cool, astringent, carminative, hepatoprotective, digestive, febrifuge, vermifuge and are useful in haemorrhages and cardiac disorders. Fruit is nervine, cephalic, aphrodisiac, emmenagogue and vermifuge [5].

Leaves contain an alkaloid nishindine, flavonoids like flavones, luteolin-7- glucoside, casticin, iridoid glycosides, an essential oil and other constituents like vitamin C, carotene, gluco-nonital, benzoic acid, β – sitosterol and C-glycoside. Seeds contain hydrocarbons, β -sitosterol, benzoic acid and phthalic acid [5], antiinflammatory diterpene, flavonoids, artemetin and triterpenoids [6]. Fatty acids, β -sitosterol, vanillic acid, p -hydroxybenzoic acid and luteolin have been isolated from bark and stem bark yields leucoanthocyanidins [4,5]. The modern sexual stimulant agents which are currently available have adverse effects, so there is growing need to look for herbal aphrodisiacs. The present study is conducted, in order to prove the folklore application of *Vitex negundo* fruit for its aphrodisiac activity in Wistar albino male rats.

MATERIALS & METHODS

The Plant *Vitex negundo* was collected near Perundurai, Erode district in the month of October. The plant was identified and authenticated as *Vitex negundo* by the botanist, Agricultural University, Coimbatore. The Voucher specimen (34/2014) has been deposited in Herbarium for further references.

Preparation of Extract

The fruits of *Vitex negundo*, washed with water and shade dried. With the mechanical blender, dried fruits were coarsely powered. Dried course powders of the fruits were extracted with 70% ethanol by using Soxhlet apparatus. The excess ethanol was removed by evopration, until to get semi solid mass and stored in desiccators.

Animals

Healthy adult albino rats of Wistar strain of both sex, weighing about 150-200 g were obtained from the animal house of Nandha College of Pharmacy, Erode.

The rats of either sex were isolated and housed in separate cages during the course of experimental period and kept them at room temperature (24± 2°C) with a 12 : 12 h light/dark cycle. The animals were fed with standard pellet diet and provided water *ad libitum*. All the procedures and protocols were reviewed and approved by the Institutional Animal Ethics Committee (688/02/C/CPCSEA) of Nandha College of Pharmacy, Erode

Preparation of male rats

The male rats were trained, for sexual behavior, two times a day for a period of minimum of 10 days. The male rat which did not show any sexual interest during the test period was considered as an inactive male. The sexually active male rats were selected for testing aphrodisiac activity of the extracts.

Preparation of female rats

Female rats were housed in separate cages with food and water *ad libitum*. The female rats were brought in oestrous phase by treating them with estradiol valerate (10 µg / kg. s.c.) and hydroxy progesterone 1.5mg/kg. s.c)., for 48 hours and 5 hours prior to experimentation, respectively, to make them sexually acceptable and were selected for the study.

Experimental Design [7]

The sexually active male rats were divided in to 4 groups of 6 animals each. Group I served as normal control received 0.1 % CMC (1ml/kg) through oral route. Group II served as reference control received sildenafil citrate (5mg/kg). Group III & IV received the ethanolic fruit extract of *Vitex negundo* 200 and 400mg/kg respectively. The sexual behavior of the experimental rats was observed in a dim light in specially designed cages that have glasses on all the sides and measuring 50×30×30cm. The male experimental rat was first placed in the cage and then two female rats in estrous phase were introduced. An initial period of 15 minutes was considered as acclimatization period. After 15 minutes, the test drugs were administered and the activity of male rat in each group was recorded individually for 1 hour, after 30 minutes of drug administration.

To determine the aphrodisiac activity of the extracts, several parameters were observed. These include measuring and observing the mount frequency (Mount frequency is the number of mounts made in one hour by the male rats after introducing the female rats), mount latency (Mount latency is the time interval between the introduction of female and first mount by the male), intromission frequency (introduction of one organ to another), intromission latency (Intromission latency is the interval from the time of introduction of the female to the first intromission by the male), genital grooming and ano-genital sniffing.

Statistical Analysis

Results were expressed as mean ± SEM. The data were analyzed by using one way analysis of variance (ANOVA) followed by Dunnett's 't' test using GraphPad version 3. P values < 0.05 were considered as significant.

Table I. Effect of ethanolic fruit extract of *Vitex negundo* on sexual behavior of male rats

Groups	Drug Treatment	Mount Frequency	Mount Latency (sec)	Intromission Frequency	Intromission Latency (sec)	Ano-genital Sniffing	Genital Grooming
I	Normal Control 0.1 % CMC(1ml/kg)	3.22±0.15	297.42±10.62	0.47±0.02	698.64±10.22	3.98±0.23	1.34±0.12
II	Sildenafil Citrate (5mg/kg)	12.36±1.16***	112.21±7.91***	1.96±0.09**	187.36±9.65***	11.87±1.64***	4.69±0.27**
III	*Vitex negundo* (200mg/kg)	7.45±0.34*	195.41±8.66**	0.93±0.06*	256.93±11.66**	6.42±0.45*	2.97±0.16*
IV	*Vitex negundo* (400mg/kg)	10.32±1.62**	148.72±8.39***	1.34±0.06**	202.96±12.67***	8.77±0.71**	3.32±0.26**

Values are in Mean ±SEM; (n=6) *P<0.05, ** P<0.01 and** *P<0.001 Vs Control

RESULT

The ethanolic fruit extract of *Vitex negundo* was studied for its aphrodisiac activity in wistar albino rats. Various sexual behaviors like mount frequency, mount latency, intromission frequency, intromission latency, genital grooming and ano-genital sniffing in male rats were observed. The reference control sildenafil citrate showed significant (P<0.001) increase in mount frequency, intromission frequency, genital grooming and ano – genital sniffing whereas there was significant (P<0.001) decrease in mount latency and intromission latency as compared to control animals. Ethanolic fruit extract of *Vitex negundo* also showed significant increase in mount frequency, intromission frequency, genital grooming and ano – genital sniffing and there was significant decrease in mount latency and intromission latency as compared to control animals. The effect produced by the *Vitex negundo* was dose dependent. 200mg/kg of *Vitex negundo* fruit extract showed moderate aphrodisiac activity, where as 400 mg/kg showed equipotent aphrodisiac activity as that of the reference control sildenafil citrate.

CONCLUSION

From the results of above study, it was concluded that, ethanolic fruit extract of *Vitex negundo* showed dose dependent aphrodisiac activity in Wistar albino rats. Further study is needed in order isolate the active principal responsible for its aphrodisiac activity.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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