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Phytochemical Screening and Aphrodisiac Property of *Tinospora cordifolia*

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ABSTRACT
Tinospora cordifolia is an herbaceous vine of the family *Menispermaceae*. This plant is indigenous to the tropical areas and distributed throughout India, Myanmar and Sri Lanka. Traditionally, the plant is being used for the treatment of various diseases but a systematic study is lacking. Further, there are some preliminary reports about using the stems of this plant for treating sexual disorders. To pursue this further, in this study, the total extracts were tested for their constituents and tested for aphrodisiac activity in experimental rats. The preliminary phytochemical screening of hydroalcoholic and aqueous extracts of the stems of *Tinospora cordifolia* showed the presence of alkaloids, carbohydrates, glycosides, steroids, proteins, saponins, gums and mucilages. The hydroalcoholic extract of *Tinospora cordifolia* stem at higher concentration (400 mg/kg body weight) showed significant aphrodisiac activity on male wistar albino rats as evidenced by an increase in number of mounts and mating performance. On the other hand, hydroalcoholic extract at lower dose (200 mg/kg body weight) and aqueous extract (400 mg/kg body weight) showed moderate aphrodisiac property. Thus, in experimental rats, the results of the present study suggest that the extracts of *Tinospora cordifolia* exert significant aphrodisiac activity. Further, detailed studies are needed to know whether *in vivo* administration of the extracts is beneficial for patients suffering from sexual disorders.

Keywords: *Tinospora cordifolia*, Aphrodisiac, Mating, Sex stimulant, Rat.

INTRODUCTION

Tinospora cordifolia which is also known as Giloe, belongs to the family *Menispermaceae*. It is an important medicinal plant used in ayurvedic system of medicine. The stem of the plant is greyish brown-black in colour and bitter in taste. The stems of *Tinospora cordifolia* are rather succulent with long filiform fleshy aerial roots from the branches. The stem is soft wooded, dry, cylindrical and 5mm to 25mm in diameter. [1] Traditionally, the plant has been in use as an anti-spasmodic, anti-inflammatory, jaundice, diabetes, seminal weakness, urinary tract infections, fever, general debility, skin diseases, expectorant, carminative, digestive, anti-stress and aphrodisiac. Piles problem can be controlled by eating this plant mixed with milk or water and thus, preventing the bleeding and constipation. [2] A variety of chemical constituents have been isolated from this plant and their structures have been established. The active ingredients include alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides.

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Male infertility is a world-wide medical and social problem. The fact that more than 15 percent couples worldwide are affected by infertility speaks volume about the worsening reproductive health globally. In India alone, the figure stands at 30 million and in half of such cases, men are responsible for the situation. Excessive alcoholism, smoking, late marriage and stress are all to be blamed. Mushrooming sperm banks and assisted reproduction centres are evident of how this is silently turning into a serious health concern. Male infertility is definitely on the rise and the average sperm count has been decreasing. The World Health Organization (WHO) recently revised its sperm count norm from 20 million to 15 million per milliliter. Virility is sexual desire in the mind whereas fertility is the physical ability of reproducing, which can be judged on two parameters: sperm type (sperm count and motility) and the ability to deposit the sperm into female reproductive tract during a sexual act. [5-6] Aphrodisiacs are defined as foods, herbs or drugs which are believed to increase sexual desire and improve sexual performance. [3] Aphrodisiacs are named after the Greek goddess of love Aphrodite. The belief in sex tonics to stimulate one's sexuality is as old as the human race. Different aphrodisiacs have been in vogue at different times. In the 1960s, an Oriental herb called *ginseng* was very popular. The root of this plant became the largest selling

aphrodisiac in the American market. In the 1970s, "royal jelly" became a popular energizer for the gonads. Later on, vitamin E and Zinc became the most commonly accepted nutritional aphrodisiacs. [3] According to WHO, sexual health is the integration of somatic, emotional, intellectual and social aspects of sexual being, in ways that are positively enriching and that enhances personality, communication and love. Sexual hygiene involves developing and maintaining a healthy sexuality and preventing sexual dysfunctions. Sexual activity is one of the most important and essential phenomenon of animal world. The human race is in search of the medicine which will help one to maintain, strengthen and rejuvenate the body in order to lead a healthy sexual life. [4] Research during the past two decades has an unfolded focus on impotence (erectile failure), premature ejaculation and male infertility. There are a number of prescription drugs which may act as sex stimulant and enhancing the sexual desire and activity in both men and women. Although the use of allopathic medicines have shown significant improvement in treating sexual disorders, but at the same time there are large number of side effects. These include irregularities of the rhythm of the heart, suicidal tendencies, mental disorders and tremors. [7] The use of synthetic aphrodisiacs results in the dilation of blood vessels in other parts of the body causing headache and fainting. Other side effects include facial flushing, stomach upset, blurred vision and sensitivity to light which usually occur at higher doses.

Thus, there is growing need to look for aphrodisiacs more of natural plant or herbal origin as opposed to synthetic compounds which are known to cause severe unwanted side effects. In this regard, we undertook the present studies on *Tinospora cordifolia* which has been in use by the traditional healers. Although there are some preliminary reports about the aphrodisiac property of *Tinospora cordifolia*, there has been no systematic study to substantiate this activity. Taking the male infertility rate and sexual dysfunctions into consideration, the current studies on aphrodisiac activity on *Tinospora cordifolia* is intended to look for safe and powerful aphrodisiac. In the present study, we have tested the extracts of *Tinospora cordifolia* for their *in vivo* aphrodisiac activity on wistar albino rats at various dosages.

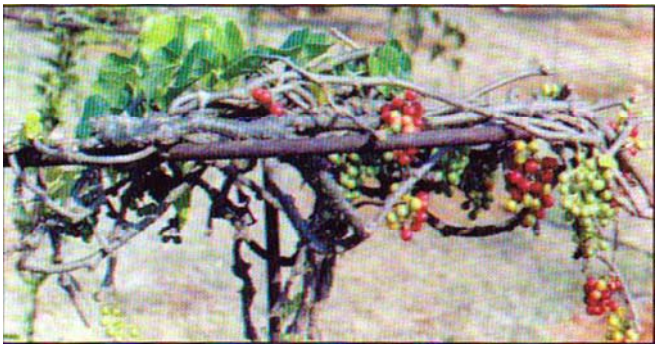

Fig. 1: *Tinospora cordifolia* plant

MATERIALS AND METHODS

Plant material

The fresh plant was collected from University of Agricultural Sciences, Bangalore (Fig. 1). The same were botanically identified, confirmed and authenticated by Regional Research Institute, Bangalore. The fresh stems of *Tinospora cordifolia* were washed with water and cut into small pieces. These were air dried for 10 days and the dried materials were powdered and subjected for different extractions. The extraction was performed by cold maceration method.

Preparation of hydroalcoholic extract

About 1000 gm of *Tinospora cordifolia* stem powder were immersed in hydroalcoholic solution (80% ethanol) in a 5000 ml flat bottom flask and was cold extracted for 7 days with occasional shaking and warming. At the end of the seventh day, the clear filtrate was obtained by filtering through a Buchner funnel. The filtrate was further concentrated by vacuum distillation**,** cooled, transferred into a Petri dish and dried in an oven at 60°C for a period of five minutes. Finally, the hydroalcoholic extract was kept in desiccators for 15 days to remove the excessive moisture and was used for further studies. [8-9]

Preparation of aqueous extract

Tinospora cordifolia stem powder was also subjected to aqueous extraction. About 1000 gm of *Tinospora cordifolia* stem powder was immersed in aqueous solution in a 5000 ml flat bottom flask and was cold extracted for 7 days with occasional shaking and warming. At the end of the seventh day, the clear filtrate was obtained by filtering through a Buchner funnel. The filtrate was further concentrated by vacuum distillation**,** cooled, transferred into a Petri dish and dried in an oven at 60°C for a period of five minutes. Finally, the aqueous extract was kept in desiccators for 15 days to remove the excessive moisture and was used for further studies. [8-9]

Qualitative Phytochemical analysis

The hydroalcoholic and aqueous extracts of *Tinospora cordifolia* were subjected to different chemical tests for the detection of phytoconstituents such as carbohydrates, glycosides, alkaloids, proteins, amino acids, tannins, phenolics, saponins, flavonoids, triterpenoids, steroids, fixed oils, gums and mucilages.

Tests for carbohydrates

The carbohydrates were tested by using Benedict's test, Fehling's test, Molisch test and Barfoed's test. [10]

Tests for alkaloids

The alkaloids were detected using Dragendroff's test, Wagner's test, Mayer's test and Hager's test. [11]

Tests for proteins and amino acids

The Biuret test, Xanthoprotein test, Lead Acetate test and Ninhydrin test were used for the analysis of proteins and amino acids. [11]

Tests for tannins and phenolics

Test for tannins and phenolics were performed by adding 2-3 drops of ferric chloride to 1ml of extract and the formation of a dark blue or greenish black colour product shows the presence of tannins. [12]

Test for flavonoids

Flavonoids were detected by means of Shinoda Test. [10]

Test for triterpenoids

Test for triterpenoids was done by dissolving two or three granules of tin metal in 2 ml thionyl chloride solution and then, adding 1 ml of the extract into the test tube. The formation of a pink colour indicates the presence of triterpenoids. [13]

Tests for steroids

The steroids were identified by using Libermann Burchard test, Salkowski test and Liebermann's reaction. [11-12]

Test for saponins

The procedure adopted for the identification of saponins was to take 1 ml of extract which is diluted with 20 ml distilled

water and then shaken in a graduated cylinder for 15 minutes. A 1 cm layer of foam indicates the presence of saponins. [11]

Tests for fixed oils

The fixed oils were tested by means of Spot test and Saponification test. [10]

Tests for glycosides

Tests like Legal test, Baijet test, Borntrager's test and Keller Kiliani Test were used for the analysis of glycosides. [10, 13]

Test for gums and mucilages

Test for gums were performed by hydrolyzing the 1 ml of extract using dil. HCl (3ml). Then Fehling's solution was added drop by drop till the appearance of red. [11] Test for mucilages were carried out by treating 1 ml of extract with 2 ml of ruthenium red solution to get red coloured solution. [11]

Animals

Healthy adult albino rats of wistar strain, weighing about 150-200 g were obtained from the J. S. S. Animal house, Ootacamund. 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 in this study were performed in accordance with the NIH guidelines for the care and use of laboratory animals, after getting the approval from the JSS Institutional Animal Ethics Committee (Approval number : JSSCP/IAEC/Ph.D.- 01/84/ 2008-09).

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 microgram/kg body wt. S.C. and hydroxy progesterone 1.5mg/kg b. wt. S.C., for 48 hours and 5 hours prior to experimentation, respectively, to make them sexually acceptable and were selected for the study.

Experimental details

The sexually active male rats were chosen separately and divided into 6 groups; each group consisting of 6 animals. The animals in the divided groups received the treatment orally. Different groups of animals which received the plant extract and the control are as follows:

Group	Treatment	Dose
I	Control (Normal saline)	2ml/kg b.wt.
II	Positive control (Sildenafil citrate)	4.5mg/kg b.wt.
III	Aqueous extract of *Tinospora cordifolia*	200 mg/kg b.wt.
IV	Aqueous extract of *Tinospora cordifolia*	400 mg/kg b.wt.
V	Hydroalcoholic extract of *Tinospora cordifolia*	200 mg/kg b.wt.
VI	Hydroalcoholic extract of *Tinospora cordifolia*	400 mg/kg b.wt.

The sexual behavior of the experimental rats was observed in a dim light in a specially designed cage that has 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 extract or the drug was introduced and the activity of male rat in each group was recorded individually for 60 minutes, after 30 minutes of drug administration. [14–17]

To determine the aphrodisiac activity of the extracts, several parameters were observed. These include measuring and observing the mount frequency, mount latency, intromission frequency, intromission latency, genital grooming and ano-genital sniffing.

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. [18]

RESULTS AND DISCUSSION

Phytochemical screening

The hydroalcoholic and aqueous extracts of *Tinospora cordifolia* were subjected to qualitative photochemical screening for the detection of phytoconstituents like carbohydrates, alkaloids, proteins, amino acids, tannins, phenolics, saponins, flavonoids, triterpenoids, steroids, glycosides, fixed oils, gums and mucilages. As shown in Table 1, the results revealed the presence of alkaloids, steroids, carbohydrates, glycosides, proteins, saponins, gums and mucilages.

Aphrodisiac activity

The aphrodisiac activity of aqueous and hydroalcoholic extracts of *Tinospora cordifolia* were studied on male Wistar albino rats at various dosages. The parameters observed during the study were mount frequency, mount latency, intromission frequency, intromission latency, ano-genital sniffing and genital grooming (Table 2).

Mount frequency

The results revealed that a significant increase in mount frequency was observed in animals treated with aqueous extract at a higher concentration of 400mg/kg body weight. On the other hand, hydroalcoholic extract at higher concentration 400 mg/kg body weight possesses moderate aphrodisiac activity. However, hydroalcoholic and aqueous extracts at lower concentrations of 200mg/kg body weight were found to be inactive (Table 2; Fig. 2).

Mount latency

The experimental data revealed that a significant decrease in mount latency in animals treated with hydroalcoholic extract of 400mg/kg body weight or hydroalcoholic extract 200mg/kg body weight or aqueous extract at the dose of 400mg/kg body weight. On the other hand, the aqueous extract of 200mg/kg body weight did not show any significant activity (Table 2; Fig. 3).

Intromission frequency

The intromission frequency is expected to increase if the test drug is effective. The results revealed that hydroalcoholic extract at higher and lower concentration possesses moderate activity. On the other hand, aqueous extract at higher and lower concentration was found to be inactive (Table 2; Fig. 4).

Intromission latency

The results revealed that the hydroalcoholic and aqueous extracts at higher concentration of 400mg/kg body weight are highly active and possess potent aphrodisiac activity as compared to control animals. On the other hand, aqueous extract at lower concentration of 200mg/kg body weight possesses moderate aphrodisiac activity in comparison to control animals. The hydroalcoholic extract at lower concentration of 200 mg/kg body weight was least active (Table 2; Fig. 5).

Table 1: Qualitative Phytochemical constituents of *Tinospora cordifolia* stem extracts

S. No.	Phytoconstituents	Hydroalcoholic extract	Aqueous extract
1.	**Alkaloids**		
	Dragendroff's test	+	+
	Wagner's test	+	+
	Mayer's test	+	+
	Hager's test	+	+
2.	**Carbohydrates**		
	Benedict's test	+	+
	Fehling's test	+	+
	Molisch test	+	+
	Barfoed's test	-	-
3.	**Glycosides**		
	Legal test	+	+
	Baljet test	+	+
	Borntrager's test	-	-
	Keller Kiliani test	-	-
4.	**Steroids**		
	Libermann Burchard	+	+
	Test Salkowski test	+	+
	Liebermann's test	+	+
5.	**Triterpenoids**	-	-
6.	**Proteins & Amino acids**		
	Biuret test	+	-
	Xanthoprotein test	+	+
	Lead Acetate test	-	-
	Ninhydrin test	+	+
7.	**Fixed oils and Fats**		
	Spot test	+	-
	Saponification test	-	-
8.	**Tannins & Phenolics**		
	Ferric Chloride test	+	+
	Potassium dichromate test	-	-
9.	**Saponins**		
	Foam test	+	+
10.	**Flavonoids**		
	Shinoda test	-	-
11.	**Gums**	+	+
12.	**Mucilages**		
	Ruthenium Red Test	+	+

(+) : Indicates the presence of chemical constituents;
(-) : Indicates the absence of chemical constituents

Ano-genital sniffing

A moderate increase in number of ano-genital sniffing was observed in animals treated with hydroalcoholic and aqueous extracts at higher concentration (400mg/kg body weight). At lower concentration of hydroalcoholic and aqueous extracts (200mg/kg body weight), the increase in activity was insignificant as compared to the control (Table 2; Fig. 6).

Genital grooming

The studies on the genital grooming revealed that moderate increase in number of genital grooming was observed in animals treated with hydroalcoholic extract of *Tinospora cordifolia* at a concentration of 400mg/kg body weight and 200mg/kg body weight. The aqueous extract at higher as well as lower concentrations was found to be inactive (Table 2; Fig. 7).

CONCLUSION

In the present study, we were interested in establishing whether *Tinospora cordifolia* extracts exhibited prosexual effects and, if this were the case, upon which of the physiological mechanisms the facilitatory actions were exerted. The prosexual effects of so called aphrodisiacs might be exerted at different levels, i.e. sexual arousal or performance. The data obtained in the present study with sexually active animals reveal that the facilitatory actions of the *Tinospora cordifolia* extracts are being exerted on both the mechanisms of sexual arousal and performance.

The data presented here provides evidence about the ability of the crude extracts of *Tinospora cordifolia* root 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 *Tinospora cordifolia* 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. [19]

The plant extracts were subjected for preliminary photochemical studies and aphrodisiac activity. The reports of photochemical studies showed the presence of alkaloids, 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, hydroalcoholic and aqueous extracts of *Tinospora cordifolia* possess potent aphrodisiac activity as evidenced by an increase in number of mounts, ano-genital sniffing attitude, penile erection index, ejaculatory behavior and mating performance.

Finally, based on this preliminary data, it can be concluded that the herb *Tinospora cordifolia* 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.

Table 2: Effect of *Tinospora cordifolia* extracts on sexual behavior of male rats

Group (Dose mg/kg)	No. of animals	Mount frequency	Mount latency (sec.)	Intromission frequency	Intromission latency (sec.)	Ano-genital sniffing	Genital grooming
Control	6	2.16±0.47	307.50±6.80	0.33±0.21	793.33±251.84	3.00±0.57	1.66±0.33
Sildenafil citrate (4.5)	6	10.33±1.40**	106.67±7.49**	1.33±0.21*	191.63±101.67	10.66±1.14**	3.83±0.47**
Aqueous 200	6	3.63±0.49	296.33±2.86	0.16±0.16	516.67±13.76	3.66±0.49	1.66±0.21
Aqueous 400	6	7.00±0.09**	261.00±3.51**	0.50±0.22	465.33±208.23	6.66±0.98*	2.66±1.6
Hydroalcoholic 200	6	4.66±1.14	279.33±2.12**	1.33±0.21*	689.17±10.52	2.66±0.49	3.50±0.42*
Hydroalcoholic 400	6	5.66±0.80*	172.17±4.82**	1.33±0.25*	463.83± 143.68	6.60±1.02*	3.67±0.49*

** = P< 0.01 (Highly Significant), * = P<0.05 (Moderately Significant)

a) Values are expressed as Mean± SEM of six animals in each group.

b) Comparison was done between control group and drug treated groups by using one way ANOVA followed by Dunnett's comparison method.

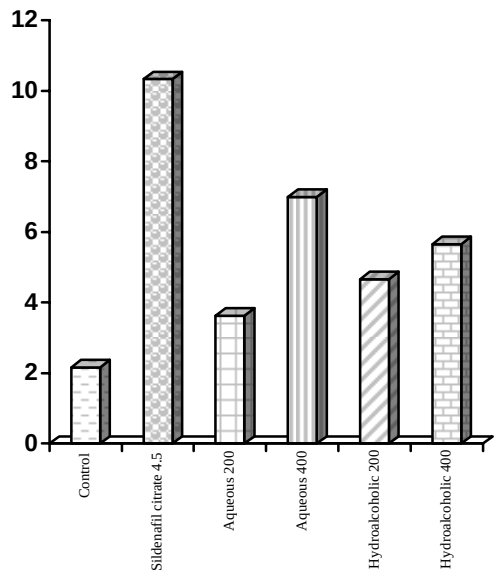

Fig. 2: Specific sexual behavior parameter i.e. mounting behavior of sexually active male rats treated with different doses of *Tinospora cordifolia* extracts. Results are expressed as mean ± SEM, n = 6. The extracts were administered via oral route 30 minutes prior to testing.

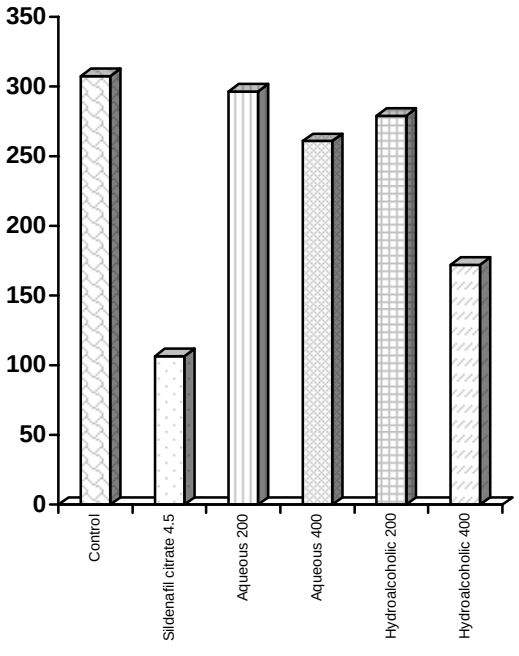

Fig. 3: Effect of administration of different doses of *Tinospora cordifolia* extracts on mount latency behavior in sexually active male rats. Results are expressed as mean ± SEM, n = 6. The extracts were administered via oral route 30 minutes prior to testing.

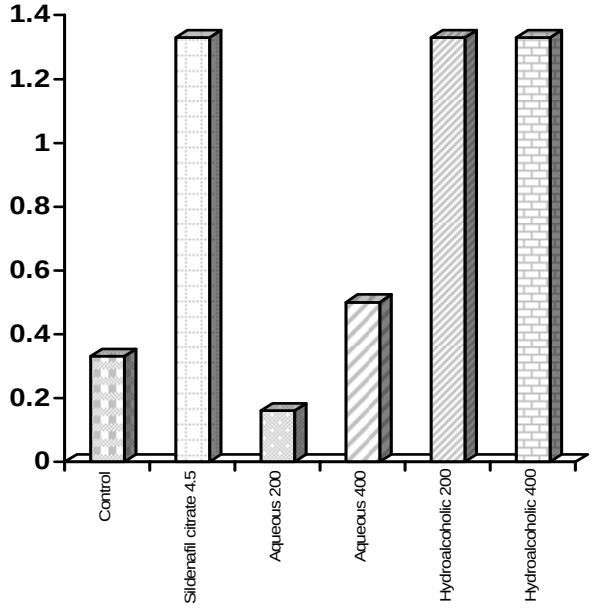

Fig. 4: Specific sexual behavior parameter i.e. intromission behavior of sexually active male rats treated with different doses of *Tinospora cordifolia* extracts. Results are expressed as mean ± SEM, n = 6. The extracts were administered via oral route 30 minutes prior to testing. For Statistical significance, comparison was done between control group and tested groups by means of one way ANOVA followed by Dunnett's comparison method.

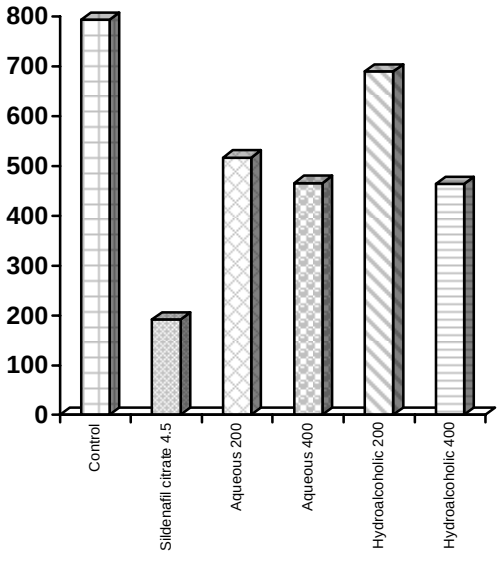

Fig. 5: Effect of administration of different doses of *Tinospora cordifolia* extracts on intromission latency behavior in sexually active male rats. Results are expressed as mean ± SEM, n = 6. The extracts were administered via oral route 30 minutes prior to testing.

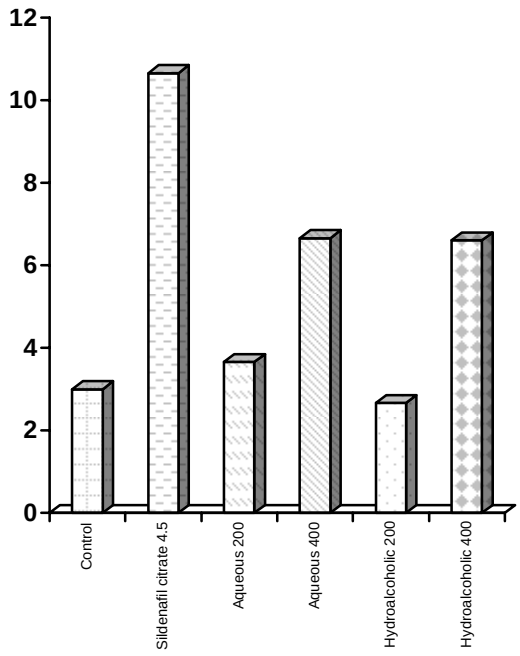

Fig. 6: Effect of administration of different doses of *Tinospora cordifolia* extracts on ano-genital sniffing behavior in sexually active male rats. Results are expressed as mean ± SEM, n = 6. The extracts were administered via oral route 30 minutes prior to testing.

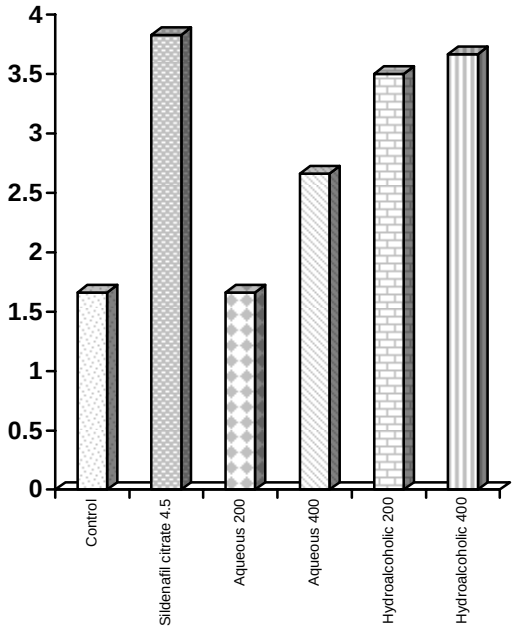

Fig. 7: Effect of administration of different doses of *Tinospora cordifolia* extracts on genital grooming behavior in sexually active male rats. Results are expressed as mean ± SEM, n = 6. The extracts were administered via oral route 30 minutes prior to testing.

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