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**ARTICLE**

**Research Article**

**Influence of Chloroform Extract of *Sida acuta* Burm. f. leaves on the sexual behavior of normal rats**

**514**

Alok Semwal, M. Senthil Kumar and Ramandeep Singh

## Full Length Research Paper

# Influence of Chloroform Extract of *Sida acuta* Burm. f. leaves on the sexual behavior of normal rats

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According to the traditional ayurvedic book *Sida acuta* Burm. f. (Malvaceae) claimed to have good aphrodisiac potential, but the actual action is not yet proved by scientific methods. Therefore the present study was conducted to investigate the aphrodisiac potential of *Sida acuta* Burm. f. Acute toxicity test was carried out to determine the nature and extent of untoward reaction which might follow the administration of a single dose (or an overdose) of a drug. The acute toxicity study was carried out on male mice by the administration of PE, CH and ME extracts of *Sida acuta* Burm. f. (Leaves) orally at one dose level (150, 500, 1000, 1500 and 2000 mg/kg) once only. Finally a dose of 200 mg/kg was selected which is 1/10<sup>th</sup> of the toxic dose. Furthermore, male albino rats were distributed into 5 groups consisting of six rats per group. Rats in group I (control) were administered with 1 ml/kg, p.o. of saline. Group II rats were treated with Sildenafil citrate at a dose 5 mg/kg, s.c while those in group III, IV and V were given 200 mg/kg of PEE, CE and ME of *Sida acuta* Burm. f. The Sexual behaviour study was carried out on days 0, 7, 14, 21 and 28<sup>th</sup>. The sexual behaviours were preceded with perceptive and pre-copulatory behaviours in the animals. The increase in Mount Frequency, and Intromission Frequency, and decrease in the Mount and Intromission Latencies, Ejaculation Latency and Post Ejaculatory Phase was observed on 0, 7, 14 and 28<sup>th</sup> consecutive days of treatment period. The present investigation reveals that oral administration of all the extracts of *Sida acuta* Burm. f. leaves showed significant increase in aphrodisiac activity, but CE remarkably enhanced male sexual behavior in male rats.

**Key words:** Sildenafil, Sexual behavior, aphrodisiac, pre-copulatory, toxic, *Sida acuta* Burm. f., chloroform extract (CE), petroleum ether extract (PEE), methanol extract (ME).

## INTRODUCTION

The main essence of marriage in humans is procreation and/or sexual fulfillment of both partners that is initiated by the mating of a male with a female in sexual

intercourse. For there to be a normal sexual intercourse with males, the sexual organs and factors relating to the erection of the copulatory organ must function normally.

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The repeated inability of the male to perform this function, at least effectively or a disorder that interferes with his full sexual response cycle is termed male sexual dysfunction (MSD) (Bose AK., 1981). Male sexual dysfunction is common worldwide among many of all ages, ethnicities and cultural backgrounds. Although, Male sexual dysfunction rarely threatens health, it can take a heavy psychological toll, bringing on depression, anxiety and debilitating feelings of inadequacy (Guay AT et al., 2003).

Male sexual problems include libido, erection, ejaculation and orgasm. Male sexual response cycle is called normal if all the steps are timely and sequentially if any one of the following is not in sequence or delayed than it leads sexual dysfunction in humans. Main causes which are responsible for sexual problems include smoking, obesity, testosterone deficiency, depression, anxiety, alcoholism, and antidepressants and blood pressure medications. Libido refers to sexual need of individuals and it very person to person. Erection is an enlarged condition of male reproductive organ. Ejaculation is ejection of semen during sexual intercourse, and orgasm represents the intense pleasure condition during the climax of sexual response. One of the measure sexual problems arising in the modern time is due to erectile dysfunction which is also associated with depression, endocrine, neurologic, vascular, and systemic disorder (Malviya N et al., 2011). ED is a broad category that includes inability to achieve erection, or the ability to achieve only brief erections (Santosh BT et al. 2011). Besides of ED there are various sexual dysfunctions like Disorders of ejaculations, disorders of orgasm, and failure of detumescence. By the several year's clinical and epidemiological studies, it proves that several risk factors are associated with ED. These risk factors include smoking, age, obesity, and diabetes and also various stress conditions. An important point to notice here is that these risk factors are the same as the risk factors of cardiovascular disease. Synthetic drugs like Sildenafil citrate, Tadalafil citrate, Vardenafil, Tadalafil, Alprostadil, Papaverin are used for ED but these drugs also have fatal side effects like sudden hypotension, hypersensitivity reaction, abnormal vision, infertility, suicidal tendencies, mental disorders and tremors (Cirino et al., 2006; Kasper et al., 2005). 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 (Kulkarni et al., 1998). 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. Some of the most ancient plants- based aphrodisiacs, such as ginseng and yohimbine, are still as popular today as in ancient times.

Unlike the old-time aphrodisiacs, which were meant only to increase sex drive and/or sexual pleasure, modern stimulants, including Viagra, may rightly be called medications, since their purpose is to correct problems that make sex difficult or impossible. Besides of the fact that several plant sources contain aphrodisiac ingredients (phytochemicals) which can be beneficial as an immune modulator, sex stimulant and also as a medication in erectile dysfunctions, there is a very low range of research work carried out in this field. According to traditional ayurvedic book medicinal plant *Sida acuta* Burm. f. claimed to have good aphrodisiac potential, but the actual action is not yet proved by scientific methods. So our current research work includes collection, identification, extraction, characterization and pharmacological evaluation of aphrodisiac potential of *Sida acuta* Burm. f. is intended to look for safe and powerful aphrodisiac.

## MATERIALS AND METHODS

### Collection, identification and authentication of plant materials

The leaves of *Sida acuta* Burm. f. (Figure 1) was collected from the Srinagar Garhwal (Uttarakhand), India in the months of February to March 2012. The Authentication number for the plant is GUH20726. The plant materials were identified and authenticated by botanist Dr. R. M. Painuli, Department of Botany, H.N.B. Garhwal University, Srinagar Garhwal. Uttarakhand India. The air dried Plant parts were reduced to a coarse powder and stored in air tight containers until the time of use.

### Preparation of plant extracts

The air dried leaves of *Sida acuta* Burm. f. was reduced to a coarse powder. The dry powder of plant material (500 g) was subjected to successive solvent extraction procedure using various solvents such as; petroleum ether, chloroform, acetone and methanol in the increasing order of polarity. The solvents were evaporated under reduced pressure to obtain a semisolid mass and then vacuum dried to yield solid residues (Figure 2) (Kokate, 1994). The dried extracts were stored in airtight container until the time of use.

### Animals

#### Procurements of animals

The experimental protocol was approved by Institution Animal's Ethics Committee (IAEC) of the Himachal Institute of Pharmacy, Paonta Sahib, Himachal Pradesh and from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) vide approval no. HIP/IAEC/03/14/11. Sprague-dawley albino rats (male and female) were used for evaluating aphrodisiac activity.

#### Housing

Animals were housed in polypropylene cages (6 per cage) with dust



**Figure 1.** Plant of *Sida acuta* Burm. f.

free rice husk as a bedding material under laboratory condition with the control environment of temperature (26-28°C), humidity (40-60%) and under reversed light and dark cycle with light from 10:00 P.M to 10:00 A.M. They were provided standard rodent chow/feed and water *ad-libitum*. After a sufficient period of acclimatization, they were used to evaluate aphrodisiac activity.

#### **Acute toxicity study**

##### **OECD Guideline No. 423 (14 Days Study)**

The purpose of an acute toxicity test is to determine the nature and

extent of untoward reaction which might follow the administration of a single dose (or an overdose) of a drug. Male albino mice were used. The acute toxicity study was carried out on male mice by the administration of extracts of *Sida acuta* Burm. f. (Leaves), orally at one dose level (150, 500, 1000, 1500 and 2000 mg/kg) once only. The dose that shows toxicity signs/mortality is the toxic dose and 1/10<sup>th</sup> of this toxic dose is considered for therapeutic explorations. Toxicity study of different extracts of *Sida acuta* Burm. f. (Leaves) was performed as per OECD guideline No. 423. The animals were observed for changes in the skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system, and somato motor activity and behaviour pattern. An attention was given to observation of tremors, con-



**Table 1.** Experimental designs.

To prepare the female rats used in the behavioral study of male rats		Aphrodisiac study in male rats	
48 h before Behavioral Study	6 h before behavioral study	1 h before behavioral study	Male behavioral study
Female rats injected with estradiol benzoate (10 µg/100 g) to bring the animal to estrous phase.	Treated with progesterone (0.5 mg/100 g) to bring rats to estrous phase.	Checked for female responsiveness (vaginal smear test).	Male rats were exposed to estrous female for a period of 2 h and observed for their sexual behavior patterns on days 0, 7, 14, 21 and 28 <sup>th</sup> of drug treatment.

Source: Yakubu et al. (2005) and Thakur et al. (2009)

vulsions, salivation, diarrhoea, lethargy, sleep and coma (OECD, 1987).

#### Route of administration

Control animal received vehicle (normal saline) while treated animals received extracts of *Sida acuta* Burm. f. leaves. All the extracts were administered orally by gavage and Standard group received (Sildenafil Citrate) by (s.c) route (Table 1).

#### Experimental design

##### Selection of male rats

Sprague-Dawley adult male albino rats weighing 200 to 250 gm were given training for sexual experience. The animals were observed for 2 h for sexual behavior in their transparent arena under dim (25 w) red light. To provide sexual experience, each male rats were allowed 30 min exposure to a stimulus female in behavioral oestrous, several days before testing for copulatory performance. The animals were tested three times over a 10 day 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.

##### Selection of female rats

Adult female rats were brought to oestrus by the sequential administration of estradiol benzoate (10 µg/100 gm) and progesterone (0.5 mg/100 gm) through subcutaneous injections, 48 h and 4 h, respectively prior to pairing. These female rats were divided into five groups, each group consisting of five animals. They were housed in a temperature-controlled room (26 to 28°C) in a reversed dark/light cycle. Food and water were provided *ad libitum*. The female rats, which were in oestrous stage, were used for the study. The highly receptive female was introduced into the male's cage and each male rat is observed for 2 h for copulatory behavior under dim red light. All the rats were tested for copulatory behavior on 0, 7, 14, 21 and 28<sup>th</sup> days, respectively.

#### Animal grouping

Male albino rats were distributed into 5 groups consisting of six rats

per group.

Group I (Control): Normal animals treated with 1 ml/kg, p.o. of saline.

Group II (Standard drug): Animals treated with Sildenafil citrate at a dose of 5 mg/kg, s.c.

Group III: Animals treated with petroleum ether extract (PEE) at a dose of 200 mg/kg (*Sida acuta* Burm f.) p.o.

Group IV: Animals treated with chloroform extract (CE) at a dose of 200 mg/kg (*Sida acuta* Burm f.) p.o.

Group V: Animals treated with methanol extract (ME) at a dose of 200 mg/kg (*Sida acuta* Burm f.) p.o.

#### Experimental procedure

From 2 weeks prior to the screening tests, until the end of the study, the rats were housed individually at 26°C to 28°C under reversed light and dark cycle with light from 10:00 PM to 10:00 A.M. Food and water were given *ad libitum*. The leaf extract was made into suspension with DMSO and administered to 'male rats' p.o in the dose of 200 mg/kg (*Sida acuta* Burm. f.). The control group received only saline (1ml) while standard group received Sildenafil citrate 5 mg/kg s.c.

#### Sexual behaviour study

Sexual behavior studies were monitored in a separate room for 2 h following the administration of standard drug and extracts and were given 20 min adaptation period, after which a primed female was introduced into the study cage. On days 0, 7, 14, 21 and 28<sup>th</sup> sexual behavior study was monitored. An experiment performed in the same environment during the dark phase of the cycle in large cage (e.g. 40 × 40 × 40 cm) with a floor that is similar to the home cages. The following male sexual behavior patterns were recorded, including;

- Mount frequency (MF): number of mounts in series, or number of mounts in a given period of time (30 min)
- Intromission frequency (IF): number of intromissions observed at 30 min.
- Mount latency: the time interval between the introductions of the female to the first mount by the male.
- Intromission latency: the interval from the time of introduction of the female to the first intromission by the male.
- Ejaculatory latency: the time interval between the first intromission and ejaculation.
- Ejaculation frequency: the number of ejaculations in a series.
- Post ejaculatory mounts latency: time from ejaculation to next

mount.

(h) Post ejaculatory interval: times from ejaculation until next intromission.

(i) Copulatory rate: the number of mounts plus the number of intromissions divided by the time from the first mount until ejaculation (Yakubu et al., 2005; Thakur et al., 2009; Suresh et al., 2009).

#### **Guidelines were followed during the experiment**

(a) Males were kept individually, but females were kept in groups.

(b) Training of each male rat for 15 min at a time was done till they elicit sexual behavior. Once the behavior was noticed, males were exposed to receptive females.

(c) Repeated training to overcome the lack of sexual response in the presence of observers.

(d) The experiment was conducted in a dark and silent room.

(e) Any jerking movements of the mating arena were avoided.

(f) Sufficient space for animals in the mating arena was provided to enable them to chase each other.

(g) Cleaning of the mating arena was done after each trial, since the urine trails left by one rat may have marked effects on the behavior of his successor.

#### **Statistical analysis**

All the results were expressed as Mean  $\pm$  Standard Error (SEM). Interpretation of the result was supported by statistical analysis. The 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 5.

## **RESULTS AND DISCUSSION**

### **Acute toxicity study**

OECD Guideline no. 423 (14 Days Study): Test drug is administered at one of the dose level (150, 500, 1000, 1500 and 2000 mg/kg) once only. Toxicity studies of PFF, CE and ME of *Sida acuta* Burm. f. at 150, 500, 1000, 1500 and 2000 mg/kg dose on mice was performed. All parameters were found to be normal. Thus, all extracts were preventing all behavioral attention and toxicity indication in mice, so it was concluded that LD<sub>50</sub> (50% Lethal dose) is more than 2000 mg/kg body weight.

### **Sexual behavior study**

Several female perceptive and male pre-copulatory behavior parameters were observed from the cage side when the extract treated male rats were introduced to the receptive female rats. The perceptive behavior displayed a rapid anteroposterior 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 pre-copulatory behavior such as chasing, anogenital sniffing which eventually culminated into mountings. 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 extract produced no sedative effect on the male rats since none of the animals showed evidence of tiredness throughout the observatory period. Aphrodisiac studies in male rats were carried out for 28 days. The following observations were recorded in *Sida acuta* Burm. f. extract treated rats (Tables 2 and 3).

### **Mount frequency**

#### ***Intromission frequency***

CE produced a significant increase ( $p < 0.05$  as compared to control group) in a number of intromission at 0, 7, 14, 21 and 28<sup>th</sup> day a significant increase ( $p < 0.01$  as compared to standard group) in male rats (Table 4).

#### ***Ejaculation frequency***

CE produced a significant increase ( $p < 0.01$  as compared to control group) in number of mounts were observed from day 0 till day 28<sup>th</sup> in male rats (Table 5).

#### ***Mount latency***

CE treated rats showed a significant decrease ( $p < 0.001$  as compared to control group) in mount latency from 0 days till the 28<sup>th</sup> day in male rats (Table 6).

#### ***Intromission latency***

CE produced a significant decrease ( $p < 0.001$  as compared to control group) in intromission latency from 0 days till the 28<sup>th</sup> day in male rats (Table 7).

#### ***Ejaculation latency***

CE treated rats showed a significant decrease ( $p < 0.001$  by the female rats included ear-wiggling characterized by as compared to control group) in ejaculation latency from 0 days till the 28<sup>th</sup> day of treatment (Table 8).

#### **Post ejaculatory mount latency**

CE treated rats showed significant decrease ( $p < 0.001$

**Table 2.** Effect of *Sida acuta* Burm. f. leaves extracts on Mount Frequency in male albino rats.

S/No.	Treatment	Dose (mg/kg)	Number of Mount Frequency in 30 min (Mean $\pm$ SEM)				
			0	7	14	21	28
1.	Control	1 ml	20.72 $\pm$ 1.5	20.72 $\pm$ 1.5	21.92 $\pm$ 1.7	23.52 $\pm$ 0.9	26.72 $\pm$ 2.2
2.	Standard	5 mg/kg	20.15 $\pm$ 1.1	20.15 $\pm$ 1.5	21.43 $\pm$ 1.8**	24.35 $\pm$ 1.2****	32.37 $\pm$ 1.7***
3.	PEE	200	6.36 $\pm$ 1.2	7.96 $\pm$ 2.6	14.30 $\pm$ 1.4	18.30 $\pm$ 1.9	21.50 $\pm$ 2.4
4.	CE	200	12.57 $\pm$ 2.1*	14.96 $\pm$ 2.1*	18.57 $\pm$ 1.6**	26.40 $\pm$ 2.3***	30.76 $\pm$ 1.6***
5.	ME	200	10.24 $\pm$ 0.8	12.50 $\pm$ 1.6	15.80 $\pm$ 2.3	16.50 $\pm$ 2.6	20.40 $\pm$ 1.6

Values are expressed in mean  $\pm$  S.E.M. Where n = 5), \* = p < 0.05, \*\* = p < 0.01, \*\*\* p < 0.001; compared with vehicle treated group. Statistical analysis done by one- way ANOVA followed by Dunnett's test.

**Table 3.** Effect of *Sida acuta* Burm. f. leaves extracts on Intromission Frequency in male albino rats.

S/No.	Treatment	Dose (mg/kg)	Number of Intromission Frequency in 30 min (Mean $\pm$ SEM)				
			0	7	14	21	28
1.	Control	1 ml	7.96 $\pm$ 0.21	7.96 $\pm$ 0.13	8.56 $\pm$ 0.27	9.36 $\pm$ 0.27	10.30 $\pm$ 0.34
2.	Standard	5 mg/kg	9.01 $\pm$ 0.13**	9.54 $\pm$ 0.24	10.34 $\pm$ 0.25*	11.34 $\pm$ 0.23	12.14 $\pm$ 0.36***
3.	PEE	200	6.59 $\pm$ 0.31	6.87 $\pm$ 0.48	6.4 $\pm$ 0.35**	6.3 $\pm$ 0.26**	8.25 $\pm$ 0.68
4.	CE	200	2.58 $\pm$ 0.36***	2.78 $\pm$ 0.52***	3.3 $\pm$ 0.22***	4.16 $\pm$ 0.44***	5.09 $\pm$ 0.22**
5.	ME	200	7.21 $\pm$ 0.19	7.23 $\pm$ 0.24	6.6 $\pm$ 0.20**	7.4 $\pm$ 0.41*	7.98 $\pm$ 0.53

Values are expressed in mean  $\pm$  S.E.M. Where n = 5), \* = p < 0.05, \*\* = p < 0.01, \*\*\* p < 0.001; compared with vehicle treated group. Statistical analysis done by one- way ANOVA followed by Dunnett's test.

**Table 4.** Effect *Sida acuta* Burm. f. leaves extracts on Ejaculation Frequency in male albino rats.

S/No.	Treatment	Dose (mg/kg)	Number of ejaculation frequency in 30 min (Mean $\pm$ SEM)				
			0	7	14	21	28
1.	Control	1 ml	2.26 $\pm$ 0.13	2.66 $\pm$ 0.29	2.80 $\pm$ 0.26	3.31 $\pm$ 0.18	3.34 $\pm$ 0.17
2.	Standard	5 mg/kg	2.20 $\pm$ 0.08	3.05 $\pm$ 0.32	3.22 $\pm$ 0.32	3.45 $\pm$ 0.19	3.27 $\pm$ 0.28
3.	PEE	200	1.80 $\pm$ 0.16	2.01 $\pm$ 0.06	2.40 $\pm$ 0.35	2.5 $\pm$ 0.10	2.92 $\pm$ 0.34
4.	CE	200	1.82 $\pm$ 0.14	2.01 $\pm$ 0.21	2.29 $\pm$ 0.09	2.9 $\pm$ 0.12*	2.68 $\pm$ 0.12**
5.	ME	200	1.62 $\pm$ 0.35	2.23 $\pm$ 0.18	2.6 $\pm$ 0.05	2.36 $\pm$ 0.32	2.39 $\pm$ 0.08

Values are expressed in mean  $\pm$  S.E.M. Where n = 5), \* = p < 0.05, \*\* = p < 0.01, \*\*\* p < 0.001; compared with vehicle treated group. Statistical analysis done by one- way ANOVA followed by Dunnett's test.

**Table 5.** Effect of *Sida acuta* Burm. f. leaves extracts on Mount Latency in male albino rats.

S/No.	Treatment	Dose (mg/kg)	Number of Mount Latency seconds (Mean $\pm$ SEM)				
			0	7	14	21	28
1.	Control	1 ml	64.76 $\pm$ 4.4	69.83 $\pm$ 1.3	71.30 $\pm$ 0.7	68.21 $\pm$ 0.5	53.16 $\pm$ 2.1
2.	Standard	5 mg/kg	69.89 $\pm$ 4.3	64.34 $\pm$ 2.4	48.33 $\pm$ 1.2***	38.75 $\pm$ 0.7	35.76 $\pm$ 1.6
3.	PEE	200	62.10 $\pm$ 6.8	49.50 $\pm$ 1.8**	43.62 $\pm$ 1.8*	36.66 $\pm$ 0.5**	30.83 $\pm$ 1.8**
4.	CE	200	68.18 $\pm$ 2.6	53.66 $\pm$ 0.7	50.57 $\pm$ 1.9	39.66 $\pm$ 0.4	35.11 $\pm$ 1.9
5.	ME	200	62.08 $\pm$ 2.7	60.85 $\pm$ 1.9*	55.50 $\pm$ 0.9**	30.15 $\pm$ 0.8**	15.40 $\pm$ 0.6***

Values are expressed in mean  $\pm$  S.E.M. Where n = 5), \* = p < 0.05, \*\* = p < 0.01, \*\*\* p < 0.001; compared with vehicle treated group. Statistical analysis done by one- way ANOVA followed by Dunnett's test.

**Table 6.** Effect of *Sida acuta* Burm. f. leaves extracts on Intromission Latency in male albino rats.

S/No.	Treatment	Dose (mg/kg)	Number of Intromission Latency seconds (Mean $\pm$ SEM)				
			0	7	14	21	28
1.	Control	1 ml	175 $\pm$ 7.5	160 $\pm$ 7.1	184.4 $\pm$ 9.5	171 $\pm$ 9.3	166.8 $\pm$ 5.5
2.	Standard	5 mg/kg	210.6 $\pm$ 14.1*	156 $\pm$ 5.99	180.2 $\pm$ 5.9	131 $\pm$ 6.8**	109.5 $\pm$ 6.7***
3.	PEE	200	170 $\pm$ 5.4	149 $\pm$ 4.9**	125 $\pm$ 4.6**	128 $\pm$ 7.2**	78.69 $\pm$ 2.6***
4.	CE	200	315 $\pm$ 5.6	296 $\pm$ 9.4	212 $\pm$ 6.4	161 $\pm$ 4.2	158 $\pm$ 2.9
5.	ME	200	162 $\pm$ 4.2**	152 $\pm$ 4.81**	141.97 $\pm$ 3.3**	131 $\pm$ 5.7**	98.7 $\pm$ 6.1***

Values are expressed in mean  $\pm$  S.E.M. Where n = 5), \* = p < 0.05, \*\* = p < 0.01, \*\*\* p < 0.001; compared with vehicle treated group. Statistical analysis done by one- way ANOVA followed by Dunnett's test.

**Table 7.** Effect of *Sida acuta* Burm. f. leaves extracts on Ejaculation Latency in male albino rats.

S/No.	Treatment	Dose (mg/kg)	Number of Ejaculation Latency in min (Mean $\pm$ SEM)				
			0	7	14	21	28
1.	Control	1 ml	7.96 $\pm$ 0.11	6.37 $\pm$ 0.75	8.66 $\pm$ 0.37	8.20 $\pm$ 0.28	8.55 $\pm$ 0.22
2.	Standard	5 mg/kg	8.80 $\pm$ 0.68	9.60 $\pm$ 0.33**	10.23 $\pm$ 0.35	10.44 $\pm$ 0.23*	10.76 $\pm$ 0.19
3.	PEE	200	6.82 $\pm$ 0.35	6.43 $\pm$ 0.37	6.22 $\pm$ 0.56*	6.2 $\pm$ 0.47*	5.77 $\pm$ 0.25**
4.	CE	200	5.119 $\pm$ 0.32**	5.19 $\pm$ 0.37	4.81 $\pm$ 0.19**	4.5 $\pm$ 0.35**	4.32 $\pm$ 0.25**
5.	ME	200	6.17 $\pm$ 0.36*	5.94 $\pm$ 0.50	4.92 $\pm$ 0.37**	3.5 $\pm$ 0.49**	2.49 $\pm$ 0.30**

Values are expressed in mean  $\pm$  S.E.M. Where n = 5), \* = p < 0.05, \*\* = p < 0.01, \*\*\* p < 0.001; compared with vehicle treated group. Statistical analysis done by one- way ANOVA followed by Dunnett's test.

**Table 8.** Effect of *Sida acuta* Burm. f. leaves extracts on Post Ejaculatory Pause in male albino rats.

S/No.	Treatment	Dose (mg/kg)	Number of Post Ejaculatory Pause in min (Mean $\pm$ SEM)				
			0	7	14	21	28
1.	Control	1 ml	8.3 $\pm$ 0.19	8.04 $\pm$ 0.35	8.74 $\pm$ 0.35	8.4 $\pm$ 0.21	7.8 $\pm$ 0.23
2.	Standard	5 mg/kg	5.5 $\pm$ 0.33	5.77 $\pm$ 0.40*	4.75 $\pm$ 0.35	4.3 $\pm$ 0.39***	4.2 $\pm$ 0.36***
3.	PEE	200	7.95 $\pm$ 0.10	7.56 $\pm$ 0.35	7.02 $\pm$ 0.79	6.8 $\pm$ 0.30*	6.26 $\pm$ 0.31*
4.	CE	200	5.79 $\pm$ 0.32***	6.06 $\pm$ 0.27*	6.22 $\pm$ 0.42*	4.9 $\pm$ 0.32***	4.08 $\pm$ 0.41***
5.	ME	200	7.10 $\pm$ 0.21	7.24 $\pm$ 0.47	6.9 $\pm$ 0.99	6.53 $\pm$ 0.21	5.94 $\pm$ 0.32**

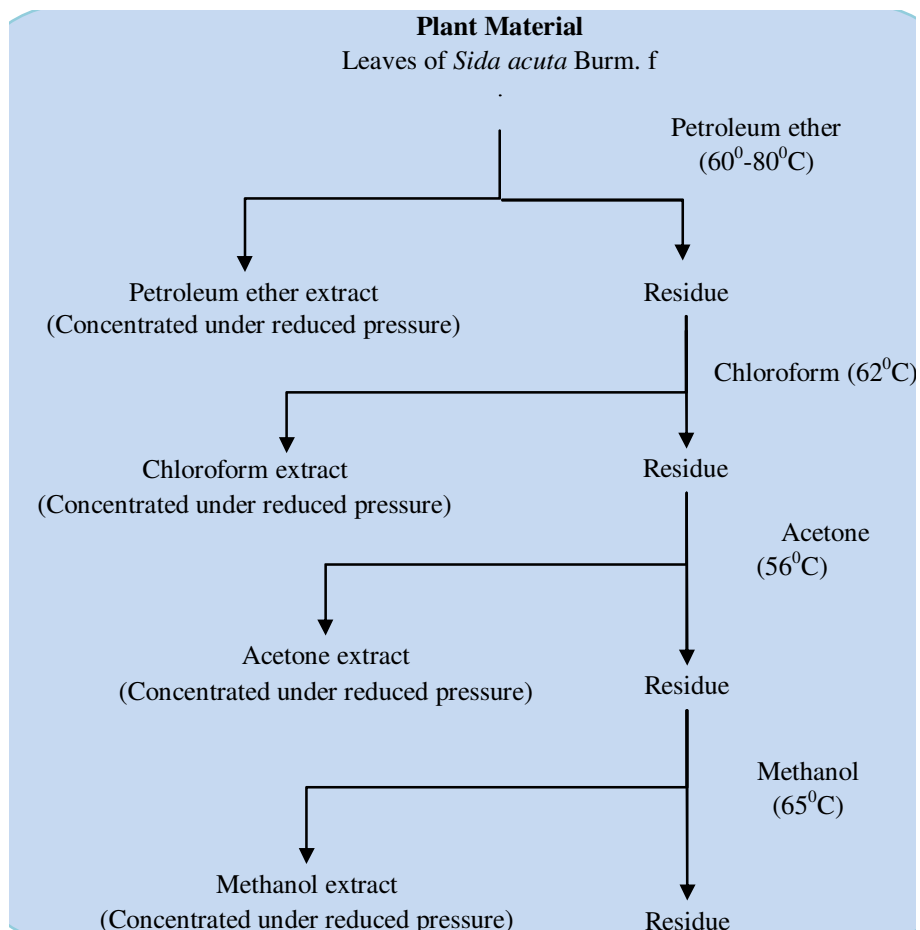
Values are expressed in mean  $\pm$  S.E.M. Where n = 5), \* = p < 0.05, \*\* = p < 0.01, \*\*\* p < 0.001; compared with vehicle treated group. Statistical analysis done by one- way ANOVA followed by Dunnett's test.

as compared to control group) in post ejaculatory mount latency from 0 day till 28<sup>th</sup> day of treatment.

## DISCUSSION

In this study, an attempt has been made to evaluate aphrodisiac activity of *Sida acuta* Burm. f. in the experimental animals. The acute toxicity was determined by the method of OECD guidelines, which is adequate for most practical purposes. Based on these results, dose of 200 mg/kg of the plant extract was selected for various animal models. To understand the scientific reasons

behind these folk claims, we investigated the effects of PEE, CE and ME of *Sida acuta* Burm. f. During this investigation, treatment of male rats with the PEE, CE and ME of *Sida acuta* Burm. f. enhanced the sexual behavior of the male rats. These sexual behaviours were preceded with perceptive and pre-copulatory behaviours in the animals. For example, the ear-wiggling, darting, hopping and lordosis by the receptive female rats in this study implied intense perceptivity and receptivity whereas the pre-copulatory behavior by the extract treated male rats also suggested that the animals were generally aroused. The pursuit of the female animals (the males running behind the female animals in close contact)



**Figure 2.** The scheme for extraction of leaves of *Sida acuta* Burm. f.

suggested imminent copulation. Mount Frequency and Intromission Frequency are useful indices of vigour, libido and potency. While the number of Mount Frequency (MF) reduces sexual motivation, increase in the number of Intromission Frequency (IF) shows the efficacy of erection, penile orientation and the ease by which ejaculatory reflexes are activated (Agmo, 1997). Therefore, the increase in Mount Frequency, and Intromission Frequency following the administration of CE of *Sida acuta* Burm. f. leaves at 200 mg/kg body weight on day 0 and subsequently at all the other days of observation suggests enhanced libido.

Such enhancement of libido might have arisen from an increase in the number of concentrations of several anterior pituitary hormones and serum testosterone, which in turn stimulated dopamine receptor synthesis and sexual behavior (Mills TM et al. 1996). Furthermore, since intromission is not possible without adequate erection and coordinated activity of penile muscles, the increase in Intromission Frequency by the extract in

this study suggests that the mechanism of penile erection was activated (Agmo, 1997). Therefore, CE of *Sida acuta* Burm. f. leaves, may increase potency by allowing or sustaining erection. The enhanced Intromission Frequency by the extracts of *Sida acuta* Burm. f. leaves, may be associated with the steroidal content of the plant via these mechanisms may await further studies. The significant increase in ejaculation frequency by the extract of *Sida acuta* Burm. f. leaves at dose of 200 mg/kg body weight on day 14<sup>th</sup>, 21<sup>th</sup> and 28<sup>th</sup> successively is an indication of the enhanced aphrodisiacs effect of the plant. The presence of a vaginal plug in the vagina of the female rats indicated that ejaculation occurred which was further complemented by the genital toileting observed in the male rats. Mount Latency and Intromission Latency are indicators of sexual motivation. Mount Latency and Intromission Latency are inversely proportional to sexual motivation. Therefore, the decrease in the Mount and Intromission Latencies observed in CE at 200 mg/kg body weight at 0, 7<sup>th</sup> and 14<sup>th</sup> consecutive days in this

study might imply stimulation of sexual motivation and arousability. It may also be an indication of enhanced sexual appetitive behavior in the male rats. All these further support the sexual function improving effect of the extract at these days. In addition, the higher values of the computed rat sexual behavior parameters following treatment with the CE when compared with the normal saline (control group) are indications of a significant and sustained increase in sexual activity (Yakubu et al., 2005). Ejaculation Latency and Post Ejaculatory phase are indicators of sexual motivation. Therefore, the decrease in the Ejaculation Latency and Post Ejaculatory phase was shortened observed in CE at 200 mg/kg body weight at 0, 7<sup>th</sup> and 14<sup>th</sup> consecutive days. Many plants with medicinal properties are effective as aphrodisiacs through mechanisms such as vasodilation, generation of nitric oxide, elevation of androgens and gonadotropins. It has also been documented that sexual behavior and erection are dependent on androgen which may act through central and peripheral mechanisms (Mills et al., 1996; Majewska, 1995).

## Conclusion

In preliminary phytochemical screening, the chloroform extract (CE) showed positive results for alkaloids, steroids, etc. Therefore, it is possible that the active principle(s) contained in the extract might have crossed the blood brain barrier of the animals to exert its aphrodisiac effect on the hypothalamic-pituitary-testicular axis. From the results of the studies, it is obvious that PE extract of *Sida acuta* Burm. f. has aphrodisiac action. Furthermore a result obtained indicates the possibility of developing cheaper, safer and potent agent for the treatment of sexual dysfunction. These findings scientifically validated the traditional use of these plants for treating sexual dysfunction in the folk medicine.

## Conflict of Interest

The authors have not declared any conflict of interest.

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