

## EVALUATION OF EROGENIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *LEMANEA FLUVIATILIS* IN RATS

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### ABSTRACT

**Objective:** To evaluate the hydro alcoholic extract of *Lemanea fluviatilis* for its erogenic activity Ancient literature included the use of a number of plants/algae as a sex stimulants. *L.fluviatilis* is one of the important algae which is used as an aphrodisiac. Dopamine and testosterone are known to facilitate male-sexual function. In this study, the hydro-alcoholic extract of whole part of *L.fluviatilis* at higher concentration (500mg/kg body weight) showed significant ( $P<0.01$ ) aphrodisiac activity on male wistar albino rats by increase in number of mounts, mating performance, testosterone and DA level. The effect on sexual behaviour such as mount frequency, Intromission frequency, ano-genital sniffing and genital grooming were also significantly ( $P<0.05$ ) increased in comparison with the normal control group.

**Results:** In the present study the extract of the *L. fluviatilis* exert significant aphrodisiac activity. However, further studies are needed to evaluate whether in-vivo administration of the extract is beneficial for people suffering from psycho-social sexual disorders.

**KEYWORDS:** Lemanea fluviatilis, Aphrodisiac, mounting frequency, testosterone, dopamine, body weight.

### 1. INTRODUCTION

Erectile dysfunction is a serious medical and social symptom that occurs in about 31% of men and 43% of women.<sup>[1]</sup> Male sexual behavior comprises a complex pattern of genital and somatomotor responses, elicited, directed and maintained by external and internal signals.

Management therapies for erectile dysfunction include the use of psychotherapy, vacuum devices, surgery, penile implants and drugs. Pharmacotherapy involves use of locally acting vaso-active drugs such as papaverin and alprostadil<sup>[2]</sup>, phosphodiesterase inhibitors such as sildenafil, vardenafil, and tadalafil and central stimulants like apomorphine.<sup>[3]</sup> The basic fundamental purpose of sex and sexuality is the “continuation of progeny” and the survival of human race. The sex is the most intimate, indispensable and an integral part of every individual and can be a source of pleasure and fulfillment. However, unfortunately, there has been a lot of ignorance, wrong information, fear and negative attitude as far as sex is concerned. Myths and misconceptions are rampant and are passed on from generation to generation. These sexual myths can result in sexual dysfunctions, misery, silent suffering, disturbed interpersonal relationships and even divorce. Sexual ignorance is a social disease and can only be resolved through comprehensive sex education, which can increase awareness and improve the environment.<sup>[4]</sup> Nowadays, infertility is a challenging problem to world-wide medical and social lifestyles. WHO estimates that there are 60-80 million infertile couples world-wide. In many countries, the stigma of infertility often leads to marital disharmony, divorce or ostracism.<sup>[5,6]</sup>

Within the central nervous system, the master organ is the brain, which sends both stimulatory and inhibitory projections on the spinal centers driving the sexual organs.<sup>[7]</sup> Dopamine (DA) is one candidate neurotransmitter among others for the control of sexual functions at the level of the central nervous system.<sup>[8,9]</sup> DA is released in several major integrative areas before and/or during copulation, facilitates sexual motivation, motor performance, and genital reflexes. DA has long been known to facilitate male sexual function. L-Dopa (the precursor of DA) administered to Parkinson patients increases libido and sexual potency, and the nonspecific DA agonist apomorphine has been used to treat sexual dysfunction.<sup>[10]</sup>

Any substances which increases the sexual desire are called Aphrodisiacs and consequently enhances fertility rate. There are number of drugs which are used as a sex stimulant that enhances the sexual desire and activity in male. Aphrodisiac drugs have shown significant improvement in treating sexual disorders, at the same time these drugs are also associated with a large number of side effects which includes irregularities of the rhythm of the heart, mental disturbances and tremor. 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.<sup>[11]</sup>

Many parts of the plant like bark, leaf, flower, and gum are useful as per Ayurvedic literature and have been reported to have aphrodisiac properties.<sup>[12]</sup> Thus, there is increasing rate to look for aphrodisiacs more on natural plant or herbal origin as they possess less side effects compared to synthetic drugs which are known to cause severe unwanted side effects.<sup>[13]</sup> Traditional medicines are gaining importance and nowadays are being studied to find the scientific basis of their therapeutic actions. The use of herbal medicine has become increasingly popular world-wide especially in the Asian and African countries.

*L.fluviatilis* is an important drug of the Meitei communities which is used to treat variety of diseases. *L.fluviatilis* is a fresh water red algae found in Manipur (India). The algae is locally known as “**nungsham**”(nung-stone,sham-hair), due to its hair like growth on stone, it grows profusely in the rocky and shallow river beds of the Chakpi and Imphal river. Peoples of the Manipur used it in the form of dried, fried, roasted and as local delicacy because of the fishy smell, taste and flavor it possesses since times immemorial.<sup>[14]</sup> Traditional uses primarily focus on hepatoprotective, antidiabetic, antimicrobial, antiviral, aphrodisiac, antitumor, anticoagulant many others<sup>[15]</sup> It is chief source of protein, carbohydrate, lipids and many important trace elements and ash. However, there is no scientific research about the effect of hydroalcoholic extract of *L.fluviatilis* on sexual behavior in literature. So, the present study was performed to determine the aphrodisiac activity of *L.fluviatilis* on the sexual behavior in male rats.

## 2. MATERIALS AND METHODS

### 2.1 Procurement of *L.fluviatilis*

The fresh water algae “**nungsham**” used for the study 1.5 kg was collected from Imphal river (Manipur, India). The identity of the specimen was confirmed as *L.fluviatilis* by Dr. N. Beenakumari devi (Asst. Professor in Botany, D.M. College of Science, Manipur, India). A voucher specimen was deposited in the Department of Pharmacology and Toxicology, C. L. Baid Metha College of Pharmacy, Chennai for future used.

### 2.2 Preparation of the extract

The collected algae 1.5 kg was shade dried and made into a coarse powder. The powder was then passed through sieve no- 40 to obtain uniform particle size and used for the purpose of

extraction. A weighed quantity of the powder was subjected to Soxhlet extraction using hydroalcohol (water:ethanol,1:1) as solvent. The algae extract was evaporated to dryness using a rotary vacuum evaporator.<sup>[16]</sup>

### 2.3 Animals

Healthy 24 adult wistar rats weighing 200-220 g were procured from the animal house of C.L.Baid Metha College of Pharmacy. The rats of either sex were housed in polypropylene cages and fed with standard rodent pellet obtained from standard pellet diet (Lipton rat feed Ltd, Pune), and water *ad libitum*. Animals were kept in cages under standard environmental conditions i.e. ambient temperature of  $22 \pm 2^{\circ}\text{C}$  and at 45 to 55% relative humidity for 12 hours each of dark and light cycle. All protocols were performed in accordance with the Institutional Animal Ethical Committee (IAEC) as per the directions of the Committee for the purpose of Control and Supervision of Experiments on Animals (Approval number:IAEC/III/02/CLBMCP/2014 dated 18/01/2017).<sup>[17]</sup>

The male rats were trained for sexual behavior, two times a day for a period of minimum of 10 days.<sup>[18]</sup> The male rat which did not show any sexual interest during the test period was rejected and only the sexually active male rats were selected for testing the aphrodisiac activity of the extracts.

### 2.4 Drugs and Chemicals

Sildenafil citrate (PZ0003 SIGMA), estradiol benzoate (46552 FLUKA), and progesterone (P0130 SIGMA) were purchased from Sigma-Aldrich

### 2.5 Oestrous Females

Twelve (12) female rats were used as mating stimulus. Prior to pairing the rats, estrus was induced in 12 female rats by sequential administration of estradiol benzoate (10  $\mu\text{g}/100\text{gm}/\text{bw}$ ) and progesterone (0.5  $\text{mg}/100\text{gm}/\text{bw}$ ) subcutaneously, 48 hours and 4 hours, respectively, before mating the stage of estrus cycle was confirmed as said by Ecksterin.<sup>[19]</sup>

### 2.6 Mounting behavior

A mount is defined as the male assuming the copulatory position but failing to achieve intromission. To observe the libido-oriented mounting behaviour, non-oestrous female rat were paired with treated male rat. Animals were observed for 3 hours and their behaviors were scored. Males were placed individually in a glass cage. After 15 minutes of

acclimatisation, a non-oestrous female was introduced into the arena. The numbers of mounts were recorded during a 15-min observation period at the start of the 1<sup>st</sup> h. Then the female was separated for 105 min. Again the female was introduced and the number of mounts was observed for 15 min as before at the 3rd h. All the experiments were performed at room temperature, 26-27 °C.<sup>[21]</sup>

### 2.7 Experimental details

Healthy and sexually active male rats were chosen separately for the experiment and divided into 4 groups; each group consisting of 6 animals. The animals in the divided groups received the treatment orally once a day for 28 days. Different groups of animals which received the plant extract and the control are as follows:

**Table. 1: Grouping of Experimental animals.**

Group	Treatment	Dose
1	Normal Control (saline)	1 ml
2	HAELF	200(mg/kg)
3	HAELF	500(mg/kg)
4	Sildenafil citrate (+ve control)	1 (mg/kg)

The sexual behavior of the experimental rats was observed by pairing with a oestrous phase female rat and was observed in a dim light at 10 a.m. in a specially designed cages that have glasses on all the sides and measuring 58×40×20cm. 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 was introduced and the activity of male rat in each group was recorded individually for 60 minutes, after 30 minutes of drug administration.<sup>[22,23]</sup>

### 2.8 Male Rat Sexual Behaviors

The aphrodisiac activity of hydro-alcoholic extracts of *L. fluviatilis* 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.<sup>[24]</sup>

#### 2.8.1. Mount frequency

The results showed that a significant increase in mount frequency was observed in animals treated with hydroalcoholic extract at higher conc. 500 mg/kg body weight. On the other hand, hydro-alcoholic extract at lower concentration viz (200 mg/kg body weight) showed no

significant aphrodisiac activity. Therefore, present investigations clearly indicate an enhanced sexual activity in animals treated with plant extracts (Table-4).

### **2.8.2. Mount latency**

The results revealed that a significant decrease in mount latency was observed in animals treated with hydro-alcoholic extracts at the dose of 500 mg/kg body weight whereas other groups has no significant activities (Table-4).

### **2.8.3. Intromission frequency**

The present study revealed that the hydro-alcoholic extract 500 mg/kg body weight was found to be significant whereas remaining groups were showing non significant activity (Table-4).

### **2.8.4. Intromission latency**

The present study revealed that the hydro-alcoholic extract at higher conc. of 500 mg/kg body weight is highly active and possesses potent aphrodisiac activity as compared to control animals. On the other hand, hydro-alcoholic extract at lower doses showed non-significant activity (Table-4).

### **2.8.5. Ano-genital sniffing**

A significant increase in number of ano-genital sniffing was observed in animals treated with hydro-alcoholic extract at 500 mg/kg body weight whereas only moderate increase in number of ano-genital sniffing was observed in animals treated with hydro-alcoholic extract 200 mg/kg body weight<sup>[25]</sup> (Table-4).

### **2.8.6. Genital grooming**

Significant increase in number of genital grooming was observed in animals recieved hydro-alcoholic extract of *Lemanea fluviatilis* at the dose of 500mg/kg body weight. Whereas other groups show nonsignificant activity (Table-4).

## **2.9 Determination of testosterone level**

On the day 28, after the last administration of the plant extract, blood was collected to determine the level of serum testosterone. The rats were anesthetized using ether in a rectangular glass and a cardiac puncture was performed. Two milliliters of blood were collected in clean sterile non-EDTA coated vacutainer for testosterone assay. The whole blood composition was separated by centrifugation at 33.5 revolutions per second for 15

minutes using a RM12C (Remi motors) micro centrifuge.<sup>[26]</sup> The serum was collected using a Pasteur pipettes into a clean, dry, vials and was stored overnight before being used for the testosterone assay. The Cobas E911 (Hitachi 911E-C501) immune-analyzer machine was used to determine the testosterone level by the use of indirect Elisa immune assay (ELISA) technique.

**Table. 2: Effect of *L.fluviatilis* on testosterone level.**

Groups	Treatment (mg/kg)	Mean±SEM (ng/ml)	95%CI
I	1 ml (dist. Water)	0.6500±0.1346	0.25-1.04
II	HAELF 200	1.245±0.07986 <sup>L</sup>	1.03-1.46
III	HAELF 500	2.578±0.1160 <sup>L</sup>	2.44-2.88
IV	Sildenafil citrate 1 mg/kg	2.080±0.08354 <sup>S</sup>	2.02-2.36

Comparison with normal control: I p<0.05, L p<0.01, S p<0.001

**2.10 Determination of DA level** - After finding the effective dose (500 mg/kg), we measured the DA concentration of nucleus accumbens in male rats by fluorimetric micro method.<sup>[27]</sup>

**Table. 3: Effect of *L. fluviatilis* on Dopamine level.**

Groups	Treatment (mg/kg)	Mean±SEM
I	1 ml (dist. Water)	302.41±4.62
II	HAELF 200	451.50±2.18
III	HAELF 500	861.32±2.26 <sup>S</sup>
IV	Sildenafil citrate (1 mg/kg)	649.79±5.78 <sup>L</sup>

Values are expressed as mean ± SEM of six observations. Comparison between: a) Group I Vs Group II, b) Group I Vs Group III and c) Group I Vs group IV. L p<0.05; S p<0.01; Units = pg/mg of wet tissue.

**2.11 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 test using Graph pad prism v7 software<sup>[28]</sup> and p<0.05 considered statistically significant.

### 3. RESULTS

The effects of various doses of hydro-alcoholic extract of *L.fluviatilis* (HAELF) on sexual behavior are summarized in Tables 4. Administration of various concentration of plant extracts modified all parameters. Maximum effect was observed in rats treated with dose of 500 mg/kg body weight. HAELF produced a significant increase in mounting frequency (the number of mounts in a series), intromission frequency (the number of intromissions in a series), ejaculation frequency (the number of times there was expulsion of semen by males

after vaginal penetration-characterized by rhythmic contraction of the posterior abdomen), genital grooming and decrease in mounting latency (time from introduction of the female until the first mount) and intromission latency (time from introduction of the female until the first intromission) ( $p < 0.05$ ). Treated male rats showed vigorous ano-genital sniffing and mounting on females and restricted them to one side of cage (Table 4).

A significant increase in the Penile erection was produced by HAELF as compared to normal control group. Present study showed that the orientation of male (received plant extract) towards the female rats was frequent as compared to controlled groups.

There was an increase in the blood level of Testosterone (Table-2) and the effective dose for testosterone was 500 mg/kg. Dopamine level was significantly increased in the nucleus accumbens when a receptive female was introduced behind a screen. Sexual behavior and dopamine release was higher in treated rats with extract (500 mg/kg) in comparison with normal control (Table-3).

**Table. 4: Effect of *L.fluviatilis* extracts on sexual behavior of male rats.**

Group (mg/kg)	Mount frequency	Mount latency(sec.)	Intromission Frequency	Intromission latency(sec.)	Anogenital sniffing	Genital grooming
Control	2.46±0.26	297.50±5.80	0.43±0.24	693.33±241.84	3.10±0.58	1.26±0.34
HAELF 200mg/kg	4.733±0.68*	249.35±2.72*	0.86±0.22*	560.00±240.84	5.40±1.04*	2.13±0.20
HAELF 500mg/kg	7.33±0.44**	155.33±2.76**	1.20±0.22**	365.00±239.86	7.50±1.26*	3.10±0.48*
Sildenafil citrate	4.913±0.28*	221.32±3.24*	0.81±0.20*	451.07±247.204	8.01±1.86*	3.04±1.14*

P< 0.01\*\*, P<0.05\*

#### 4. DISCUSSION AND CONCLUSION

Herbal medicine and products derived from plants are still being used in medical practice, though the mechanisms of action of many herbal drugs are unknown, and the active principles in these drugs are seldom satisfied.<sup>[29]</sup> Many plants with medicinal properties are effective as aphrodisiac through mechanisms such as vasodilation, generation of nitric oxide, elevation of androgens and gonadotropins.<sup>[30]</sup> The data presented here provides evidence about the ability of extracts of *L.fluviatilis* to enhance male sexual behavior expression in sexually active rats. The data obtained reveal that an oral administration of different doses of *L. fluviatilis* extracts effectively facilitate several aspects of copulatory behavior.



Administration of the hydroalcoholic extract at two different doses modified rat copulatory behavior as well as orientation activities, the main determinant for measuring male sexual behavior. The results of the present study revealed a significant increase in penile erection and genital grooming. Increased mount frequency and intromission frequency in treated rats, the sexual motivation and efficiency of erection and penile orientation.

Our present results showed that testosterone and DA concentration of nucleus accumbens increased in male rats following their first encounter with a female in various phases of sexual behavior and this increase was higher in extract treated rats in comparison with control. Testosterone enhances sexual desire, index of libido, motivation and sexual performance. In rats, it has been shown that the presence of an estrus female enhances extracellular DA in the medial preoptic area (Hull et al., 1995) as measured by *in vivo* microdialysis followed by HPLC with electrochemical detection (HPLC-EC). Males that exhibited a substantial precopulatory increase in DA in the medial preoptic area (mPOA) copulated with females, but in the absence of this rise in DA they did not (Hull et al., 1995). Enhanced dopamine efflux causes facilitation of sexual behavior and has effects on sexual motivation, copulatory proficiency and genital reflexes. Copulation stimulates mesolimbic DA function in male rats and intromission are associated with increasing of DA. Increasing of DA and testosterone following administration of HAELF indicates that, HAELF can be used as stimulator of sexual behavior and management of male impotency (*i.e.* sexual arousal/erection disorders) and pre-mature ejaculation. Also, dopamine increases IF and reduces IL and ML.<sup>[31]</sup> The present Data suggest potential utility in managing sexual dysfunction. No significant side effects were observed in our study.

As we know that dopamine (DA) plays a vital role in sexual behavior in humans. Moreover, drugs inducing changes in neurotransmitter levels or their action at the cellular level could also change sexual behavior.<sup>[32]</sup> Considering this relation, it should be noted that the action of this drug as an aphrodisiac may be due to its nervine stimulating property. Sexual stimulation is also related to increase in blood circulation in the body. This property may be attributed to the nervous stimulating effect for increased sexual behavior in animals. Therefore, it may be useful to solve the sexual problems such as pre-ejaculation and impotency.

The overall observation of the study suggests a highly significant effect in Group III animals, which were treated with 500 mg/kg of HAELF when compared with other Groups. Effects of HAELF may be due to the presence of steroids, saponins and flavonoids through a multitude

of central and peripheral means.<sup>[33]</sup> Therefore, the present studies showed that *L.fluviatilis* may be useful to solve the sexual problems such as pre-ejaculation and impotency.

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