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INVESTIGATION OF PRECLINICAL APHRODISIAC ACTIVITY OF *LAGENARIA SICERARIA* (MOLINA) STANDL. FRUIT ETHANOL EXTRACT

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ABSTRACT
Erectile dysfunction is readily increasing worldwide because of busy and changed modern lifestyle. Its effective management becomes difficult due to side effects, cost and easy availability of better treatment. In the present work the effect of ethanolic extract of *Lagenaria siceraria*(Molina) Standl. fruit as an aphrodisiac on albino rats *in vivo* studied. Extract was administered orally at doses of 200 and 400 mg/kg body weight for 45 days. The effect on general mating behavior, potency test, morphological studies, and organ weight and sperm motility were tested. The acute toxicity of the extract was also checked. The results were compared with standard reference sildenafil citrate. On oral administration of ethanolic extract on 15th, 30th and 45th day of treatment in all treated groups significantly increased intromission frequency, mounting frequency, ejaculatory latency and significantly decreased parameters are mounting latency, intromission latency, inter intromission interval and post ejaculatory interval. Test for potency showed significant increase. In all experimental animals morphological study showed significant increase in reproductive organs weight and non significant increase in sperm motility. The prolonged treatments for all treated groups were highly effective as compared to the control group. Results revealed that ethanolic extract of *L. siceraria* fruit showed aphrodisiac activity less than the standard drug. Future study of different fractions of ethanolic extract identifies the potential aphrodisiac chemical constituents.

KEYWORDS: Potency test, Morphological study, Ethanolic extract.

INTRODUCTION
Erectile dysfunction (ED) is inability to achieve or maintain an erection sufficient for normal sexual intercourse. Throughout the world male ED occurrence estimated over 152 million men in 1995 and 2025 projections suggest it is approximately 322 million.[1] For the management of ED modes used are surgery, psychotherapy, mechanical devices, drugs and penile implants.[2] But these options has serious side effects of these treatments include infection complicacy in surgical procedures, acceptability, high cost, mechanical failure of devices, side effects of drugs such as flushing, visual disturbances, headache, dizziness, priapism and nasal congestion.[3,4] From historical time medicinal plants were used to treat ailments because of they are having low cost, good efficacy and better tolerability. The aphrodisiac plants are used to treat erectile dysfunction by increasing sexual pleasure and desire.

The *Lagenaria siceraria* (Molina) Standl. belongs to the family Cucurbitaceae is an important medicinal plant. It is a large softly pubescent climbing or trailing herb. The plant is found throughout in India, the Moluccas and in Abyssinia. The fruit is oleaginous; sweet, cardiotonic, general tonic, aphrodisiac, laxative, cooling, increases "vata", flattening; improves taste; cures leucorrhoea and biliousness; wholesome to the fetus. The fruit is indigestible; liver tonic, vulneraray, antiperiodic; cures blood diseases; cures muscular pain, piles, dry cough. The flesh of the fruit is diuretic, refrigerant and antibilious.[5] The fruit has anti-swelling effect.[6] Significant antioxidant activity shown by fruits in vitro.[7] Fruits shown antihyperlipidemic and hypolipidemic effects in triton-induced and normocholesterolemic hyperlipidemic rats.[8] The hepatoprotective activity of different fractions of the ethanolic fruit extract studied in rats. In that petroleum ether has higher activity and from that two steroids were isolated and identified as campestrol and fucosterol.[9] The fruit extract showed presence of terpenoids, saponins, quinone, sterols, tannins, phenolics, flavonoids, proteins and carbohydrates.[10] So the study is selected to know the potential of the plant as an aphrodisiac.

MATERIALS AND METHODS
Collection of Plant Material
The *Lagenaria siceraria* (Molina) Standl. fruits were collected during flowering of the plant (October to January, 2013) from Latur district Nilanga region and

identification was done by Botanical survey of India, Pune.

Extract and Drug Preparation

The fruits of *L. siceraria* (1Kg) were converted into coarse powder. After complete drying, the powder was extracted with solvent ethanol by cold maceration method for 24 hrs. The extract was concentrated using water bath at 40^0 C. Finally dried extract stored till use in an air tight container.

Control group received distilled water. The test groups received ethanolic extract of fruits of *L. siceraria* in the doses 200mg/kg and 400mg/kg body weight. Last group is standard group received sildenafil citrate (Vigora tablets, German Remedies) at the dose 5mg/kg body weight. The ethanolic extracts and sildenafil citrate suspended in water for animal administration.

Animals

Wistar strain of albino rats (200-300g) were used for study. Standard laboratory conditions were maintained including relative humidity 50-60%, temperature 20-40^0 C and a 12 h light cycle beginning in the morning. The animals freely allowed access to water *ad libitum* and food (Golden Feed, New Delhi) except during the short time they were removed from their cages for testing. All the animals were acclimatized for a week before starting the study and randomized into different groups. Food, not water was withdrawn 3-4 h before the experiment. The experiment was conducted in the evening. The study protocol approved by the Institutional Animal Ethics Committee (No.IAEC/ABCP/08/2014-15 Date 17-10-2014) and conducted according to CPCSEA Guidelines, Govt. of India.

Extract and Drug Administration

Group 1 control group received distilled water 10ml/kg orally. Group 2 and 3 test groups received suspension of ethanolic extract of *L. siceraria* fruits in water orally with doses 200mg/kg and 400mg/kg daily for 45 days at 18.00h. Group 4 standard group received water suspension of sildenafil citrate (Vigora tablets, German Remedies) orally at the dose of 5mg/kg body weight, 1 h before starting of the experiment.

Acute Toxicity Study

Healthy male rats for acute toxicity study were starved for 3-4 hr as per Organization of Economic Co-operation and Development (OECD) guidelines No: 423.[11] The rats were made into four groups of six animals each and kept singly in separate cages. Group 1 control group received 10 ml/kg of distilled water orally. Groups 2-4 received ethanolic extract suspension of *L. siceraria* orally in doses of 1000, 2000 and 4000 mg/kg daily for seven days respectively. The rats were observed for autonomic, neurological, and behavioral profile continuously for 2 hr interval up to 72 hrs for any mortality.

Mating Behavior Test

For this study healthy male and female rats were selected. After start of the test, sample treatment tests for sexual desire were carried out on 15^{th}, 30^{th} and 45^{th} day. Experiment was conducted in the evening at uniform laboratory conditions. The male and receptive female rats were introduced at the ratio of 1:1 into the mating cages. The observed mating behaviors were used for further analysis with scoring first four mating series. The phases and frequencies were determined on the following parameters : before ejaculation number of intromission or intromission frequency (IF), number of mounting before ejaculation or mounting frequency (MF), time after introduction of female into male cage up to first mount or mounting latency (ML), time after introduction of the female up to the first intromission by male or intromission latency (IL), number of intromissions (NI), time after first intromission up to ejaculation in series or ejaculatory latency (EL), number of mount (NM), time after first ejaculation up to the next intromission by the male or post-ejaculatory interval (PEI) and time between two successive intromission or inter intromission interval (III).[12,13] Results observed were compared on the basis of statistical interpretation with One Way ANOVA followed by Bonferroni test. $P<0.001$was considered significance level.

Test for Potency

On the day 46^{th} potency effect of the extract was studied. The animal placed on its back in a glass cylinder partial restraint to test the penile reflexes. For a period of 15 min. held the preputial sheath pushed behind the glands using thumb and index finger which gives a cluster of genital reflexes. The following things were recorded: erections (E), quick flips (QF), total reflex (TR) and long flips (LF). Erections (E), is characterized by extension and a swelling of penis with slight reddening. Penile flips were scored when the penis extended ventrally. Quick flips (QF), where flips in an angle acute to perpendicular resulted between glans and ventrum. Long Flips (LF), where the angle exceeded perpendicular of the glans relative the ventrum.[14]

Morphological Studies

Lastly, the animals were sacrificed through anesthesia. After the respiration ceases, immediately the animals were fixed by trans-cardial perfusion using normal saline after flushing the blood with normal saline. Before perfusion, left side of the epididymis was removed and used for morphological study and right side was used for sperm analysis.[15]

Organ Weight- Dissection and weight of Main and accessory reproductive organs were taken.

Sperm Analysis- The study samples were obtained by taking small cuts in the vas deferens and epididymis, and placed in 1 ml of Krebs Ringer bicarbonate buffer (pH7.4). Sperm motility was done with haemocytometer. It was graded semi quantitatively on a scale of 0-5 and

the spermatozoa were evaluated for the rate of forward movement and graded accordingly: 0 = no movement, 1 = sluggish or tail movement alone, 2 = intermittent sluggish movement, 3-4 = fair and good movement and 5 = maximum movement in forward direction.[16]

RESULTS

Acute Toxicity Study

Symptoms of respiratory distress, weight loss, salivation, maternal mortality and change in hair appearance were not observed during experiment. In the autonomic, behavioral and neurological profile and mortality no changes were observed in treated groups of the rats with highest dose up to 4000 mg/kg body weight. That's why the one tenth and one twentieth doses were selected for present investigation.

Effect of the Extract on Mating Behavior

The animals were treated with ethanolic extract of *L. siceraria* fruits showed marked increase with different parameters in the sexual action,. The fruits ethanolic extract at the doses of 200 and 400 mg/kg body weight showed the results on mating behavior test which significantly increased the Ejaculatory Latency (EL) (P<0.001), Intromission Frequency (IF) (P<0.001), and Mounting Frequency (MF) (P<0.001). It significantly reduced the Mounting Latency (ML) (P<0.001), Intromission Latency (IL) (P<0.001), Post Ejaculatory Interval (PEI) (P<0.001) and Inter Intromission Interval (III) (P<0.001) on 15th, 30th and 45th day in experimental animal as compared to control group. The standard drug increased the NI, MF, EL, IF and NM and also decreased the IL, ML, PEI and III in a highly significant manner on 15th, 30th and 45th day greater than ethanolic extract as compared to control group (**Table 1(A-I)**).

Table 1: Ethanolic Extract of *L. Siceraria* for Its Different Mating Behaviors on The Experimantal Rats.

S.NO.	Treatment	Dose	15th Day	30th Day	45th Day
A. Mounting Frequency (MF)					
1	Control	-	56.06±2.817	57.36±4.940	57.53±1.837
2	LSFR Ethanolic Extract	200MG/KG	73.30±2.254*	87.45±4.912854*	92.17±4.468*
3	LSFR Ethanolic Extract	400MG/KG	70.96±8.429*	83.91±2.620744*	88.12±2.684*
4	Sildenafil citrate	5 MG/KG	120.87±3.884*	151.95±2.63*6	182.56±3.487*
B. Intromission Frequency (IF)					
1	Control	-	63.98±3.789	64.08±3.789	64.96±2.935
2	LSFR Ethanolic Extract	200MG/KG	78.10±7.511*	87.048±3.836*	106.97±3.371*
3	LSFR Ethanolic Extract	400MG/KG	74.55±3.377*	82.99±4.958*	98.73±7.619*
4	Sildenafil citrate	5 MG/KG	133.33±3.783*	158.60±5.786*	192.32±5.754*
C. Mounting Latency (ML)(in S)					
1	Control	-	14.83±1.471	14.66±1.966	14.16±0.983
2	LSFR Ethanolic Extract	200MG/KG	9.00±0.894*	8.33±0.816*	8.00±0.632*
3	LSFR Ethanolic Extract	400MG/KG	9.66±0.816*	8.83±0.752*	8.50±1.378*
4	Sildenafil citrate	5 MG/KG	3.50±1.048*	3.00±0.894*	2.33±1.211*
D. Intromission Latency (IL)(in S)					
1	Control	-	12.33±0.816	12.50±1.048	12.00±1.095
2	LSFR Ethanolic Extract	200MG/KG	8.83±147*	8.66±0.516*	9.33±0.516*
3	LSFR Ethanolic Extract	400MG/KG	9.66±0.816*	9.16±0.752*	9.83±0.983 NS
4	Sildenafil citrate	5 MG/KG	3.33±1.211*	4.16±0.752*	3.66±0.816*
E. Ejaculatory Latency (EL)(in S)					
1	Control	-	214.33±1.861	217.33±3.141	216.66±2.065
2	LSFR Ethanolic Extract	200MG/KG	265.33±4.501*	551.16±7.859*	686.50±4.806*
3	LSFR Ethanolic Extract	400MG/KG	259.66±2.503*	492.50±3.507*	597.83±4.400*
4	Sildenafil citrate	5 MG/KG	373.50±6.685*	1068.83±6.306*	1105.83±2.22*
F. Number Of Intromission (NI)					
1	Control	-	4.00±1.414	4.5.0±1.048	4.66±1.211
2	LSFR Ethanolic Extract	200MG/KG	5.83±1.169 NS	6.16±0.408 NS	6.33±1.032 NS
3	LSFR Ethanolic Extract	400MG/KG	5.66±0.816 NS	5.83±1.169 NS	6.16±1.169 NS
4	Sildenafil citrate	5 MG/KG	9.33±1.032 NS	10.00±1.414 NS	10.50±1.37 NS
G. Number Of Mount (NM)					
1	Control	-	3.33±1.032	4.00±0.894	4.16±0.752
2	LSFR Ethanolic Extract	200MG/KG	5.16±1.169 NS	5.33±1.632 NS	5.83±1.471 NS
3	LSFR Ethanolic Extract	400MG/KG	4.83±1.471 NS	5.16±1.329 NS	5.33±1.032 NS
4	Sildenafil citrate	5 MG/KG	8.66±0.816 NS	8.83±1.329 NS	9.00±1.414 NS
H. Post Ejaculatory Interval (PEI)(in S)					
1	Control	-	386.33±7.339	389.33±6.186	391.67±5.428
2	LSFR Ethanolic Extract	200MG/KG	165.33±3.724*	146.00±3.688*	124.17±3.251*
3	LSFR Ethanolic Extract	400MG/KG	193.50±4.037*	175.50±3.507*	155.50±2.881*
4	Sildenafil citrate	5 MG/KG	16.17±1.169*	11.83±0.753*	8.50±1.049*
I. Interintromission Interval (III)(in S)					
1	Control	-	28.00±2.366	27.67±1.033	27.50±1.049
2	LSFR Ethanolic Extract	200MG/KG	17.83±1.169*	17.50±1.975*	15.50±1.049*
3	LSFR Ethanolic Extract	400MG/KG	18.33±1.366*	17.83±1.169*	16.17±1.472*
4	Sildenafil citrate	5 MG/KG	8.17±0.753*	5.50±0.548*	5.00±0.632*

*Values are expressed as Mean±SD at n=6, One way ANNOVA followed by Bonferroni test, *P<0.001 compared to the control group & values are Significant, NS – Non Significant. LSFR – Lagenaria siceraria fruit.*

Effect of the Extract on Potency

The ethanolic extract of fruits of *L. siceraria* showed significantly increased test for potency at doses of 200 and 400 mg/kg body weight, in the frequency of Erection (E) ($P < 0.001$), Long Flips (LF) ($P < 0.001$) and Quick Flips (QF) ($P < 0.001$) (**Table 2**) and also in the total penile reflexes (TPR) ($P < 0.001$) as compared to control group on 15th, 30th and 45th day. The standard significantly increased the E ($P < 0.001$), LF ($P < 0.001$), QF ($P < 0.001$) and TPR ($P < 0.001$) on 15th, 30th and 45th day greater than ethanolic extract with respect to control group (**Table 3**).

Table 2: Ethanolic Extract of *L. Siceraria* for Its Potency (Erection, Quick Flip, Long Flip) On The Experimental Rats.

S.NO.	Treatment	Dose	15th Day	30th Day	45th Day
A. Erection (Frequency)					
1	Control	-	9.66±0.816	9.16±0.752	9.67±0.816
2	LSFR Ethanolic Extract	200MG/KG	14.33±1.632*	14.17±0.753*	14.33±1.633*
3	LSFR Ethanolic Extract	400MG/KG	13.16±0.752*	12.67±0.516*	13.17±0.753*
4	Sildenafil citrate	5 MG/KG	28.66±0.816*	26.16±1.471*	28.67±0.816*
B. Quick Flip (Frequency)					
1	Control	-	5.66±1.032	6.5±0.836	6.83±1.169
2	LSFR Ethanolic Extract	200MG/KG	11.83±0.752*	13.66±0.816*	15.83±1.471*
3	LSFR Ethanolic Extract	400MG/KG	9.83±0.752*	12.33±1.032*	14.83±0.752*
4	Sildenafil citrate	5 MG/KG	21.16±1.169*	27.33±1.632*	28.66±1.032*
C. Long Flip (Frequency)					
1	Control	-	2.50±0.547	3.33±0.816	3.83±0.752
2	LSFR Ethanolic Extract	200MG/KG	7.66±1.032*	11.83±1.169*	13.33±0.816*
3	LSFR Ethanolic Extract	400MG/KG	6.50±1.048*	10.50±1.048*	12.83±1.722*
4	Sildenafil citrate	5 MG/KG	16.66±1.211*	24.50±1.048*	27.33±2.065*

*Values are expressed as Mean±SD at n=6, One way ANNOVA followed by Bonferroni test, *P<0.001 compared to the control group & values are Significant, NS – Non Significant. LSFR – Lagenaria siceraria fruit.*

Table 3: Ethanolic Extract of *L. Siceraria* for Its Total Reflexes on The Tested Rats 15th Day, 30th Day And 45th Day.

S.NO.	Treatment	Dose	Erection	QF	LF	TR
Total Reflex (15th Day) (Frequency)						
1	Control	-	9.66±0.816	5.66±1.032	2.50±0.547	2.5±0.547
2	LSFR Ethanolic Extract	200MG/KG	14.33±1.632*	11.83±0.752*	7.66±1.032*	7.66±1.032*
3	LSFR Ethanolic Extract	400MG/KG	13.16±0.752*	9.83±0.752*	6.50±1.048*	6.50±1.048*
4	Sildenafil citrate	5 MG/KG	28.66±0.816*	21.16±1.169*	16.66±1.211*	16.66±1.211*
Total Reflex (30th Day) (Frequency)						
1	Control	-	9.16±0.752	6.5±0.836	3.33±0.816	3.33±0.816
2	LSFR Ethanolic Extract	200MG/KG	14.17±0.753*	13.66±0.816*	11.83±1.169*	11.83±1.169*
3	LSFR Ethanolic Extract	400MG/KG	12.67±0.516*	12.33±1.032*	10.50±1.048*	10.50±1.048*
4	Sildenafil citrate	5 MG/KG	26.16±1.471*	27.33±1.632*	24.50±1.048*	24.50±1.048*
Total Reflex (45th Day) (Frequency)						
1	Control	-	9.67±0.816	6.83±1.169	3.83±0.752	3.83±0.752
2	LSFR Ethanolic Extract	200MG/KG	14.33±1.633*	15.83±1.471*	13.33±0.816*	13.33±0.816*
3	LSFR Ethanolic Extract	400MG/KG	13.17±0.753*	14.83±0.752*	12.83±1.722*	12.83±1.722*
4	Sildenafil citrate	5 MG/KG	28.67±0.816*	28.66±1.032*	27.33±2.065*	27.33±2.065*

*Values are expressed as Mean±SD at n=6, One way ANNOVA followed by Bonferroni test, *P<0.001 compared to the control group & values are Significant, NS – Non Significant. LSFR – Lagenaria siceraria fruit.*

Effect of Extract on Morphological Studies

The ethanolic extract of *L. siceraria* fruits orally given with dose of 200 and 400 mg/kg, significantly increased the relative weight of reproductive organs like epididymis, prostate and testes. In standard group as compared to control the weight of reproductive organs was significantly increased on 15th, 30th and 45th day (**Table 4**).

Sperm analysis of the ethanolic extract of *L. siceraria* fruits with the dose of 200 and 400 mg/kg and standard group showed non-significant increase in sperm motility as compared to control group (**Table 4**).

Table 4: Effect of Ethanolic Extract of *L. Siceraria* Fruit for Its Morphological Changes (Organ Weight and Sperm Analysis) On The Experimental Rats.

S.NO.	TREATMENT	DOSE	Testis (gm)	Epididymis (gm)	Prostate (gm)
Organ Weight					
1	Control	-	0.85±0.20	0.33±0.011	0.717±0.007
2	LSFR Ethanolic Extract	200MG/KG	1.12±0.008*	0.429±0.01*	0.74±0.006*
3	LSFR Ethanolic Extract	400MG/KG	1.09±0.008*	0.39±0.005*	0.74±0.007*
4	Sildenafil citrate	5 MG/KG	1.52±0.060*	0.59±0.005*	0.78±0.010*

Sperm Analysis			
S.NO.	**Treatment**	**Dose**	**Motility**
1	Control	-	3.00±0.632
2	LSFR Ethanolic Extract	200MG/KG	4.50±1.049 NS
3	LSFR Ethanolic Extract	400MG/KG	4.83±0.753 NS
4	Sildenafil citrate	5 MG/KG	9.67±0.816 NS

*Values are expressed as Mean±SD at n=6, One way ANNOVA followed by Bonferroni test, *P<0.001 compared to the control group & values are Significant, NS – Non Significant. LSFR – Lagenaria siceraria fruit.*

DISCUSSION

It is coated that the *L. siceraria* fruits stimulated sexual activity. The study is carried out to prove its potential as an aphrodisiac drug.

Due to presence of phenolic compounds, flavonoids, tannins, steroids and saponins[17] there might be improvement in sexual function observed when animals treated with the ethanolic extract of *L. siceraria* fruits. Further fractionation gives us information about active constituents responsible for an aphrodisiac activity.

In this study, symptoms of clinical toxicity were not observed during the experiment. The study showed that, to use this for short duration of time it is apparently safe.[18]

The sexual indicators of motivation in male rats are considered as latency of mount and intromission whereas behavioral indications of sexual facilitation and performance are considered as ejaculation and intromission.[19] The animal treatment with different doses of ethanolic extract of *L. siceraria* fruits showed a significant increase in the ejaculation latency and number of ejaculation. There was a significant decrease in the latency of mount and intromission showing sexual motivation enhancement. The Mounting frequency (MF) and Intromission frequency (IF) are considered the libido and potency indices. These are increased significantly as compared to control group in the ethanolic extracts group. After administration of ethanolic extracts and standard drug, the Ejaculation latency (EL) increased significantly indicating prolonged coitus duration, suggesting increased sexual motivation.[20] The penile erection index is used to evaluate the drug effect on erectile function.[21] The ethanolic extract of *L. siceraria* fruits with the doses of 200 and 400 mg/kg body weight showed significant increase as compared to control group in the potency test. Which include the frequency of Erection (E), Quick Flips (QF) and Long Flips (LF), but the effect of extract is lesser than standard drug treated rats. The ethanolic extract and standard drug treated animals showed significantly increased aggregate of total penile reflexes (TFR). This shows increased potency of the ethanolic extracts.

Increase in body weight and sexual organ weight were because of steroids. These are the parameters improving the steroidal hormones production for plant extract effectiveness.[20] Ethanolic extract of *L. siceraria* fruits at the doses of 200 and 400 mg/kg body weight significantly increased the weight of the organs epididymis, testes and prostate.

The results showed no significant improvement in sperm motility with ethanolic extract and standard as compared to control group. The possible reason might be an increase in level of cytosolic cGMP elevated sperm motility because some of the aphrodisiac studies indicated this fact, whereas an increase in level of cytosolic cAMP increased sperm motility and sperm viability.[22]

CONCLUSION

The purpose of the study is to prove ethanolic extract of *Lagenaria siceraria* (Molina) Standl. fruits as an aphrodisiac drug claimed by literature which will improve sexual function. In the conclusion it is found that sexual activity enhancement by ethanolic extract of *L. siceraria* fruits is dose dependant. All the parameters studied revealed the same result to confirm the activity as an aphrodisiac of ethanolic extract of *L. siceraria* fruit.

Isolation and characterization of an aphrodisiac active principle in ethanolic extract and to investigate the mechanism of action will be done in future.

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