

The Journal of Phytopharmacology

(Pharmacognosy and phytomedicine Research)

Research Article

ISSN 2320-480X

JPHYTO 2018; 7(3): 319-324

May- June

Received: 13-04-2018

Accepted: 24-05-2018

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Preclinical aphrodisiac investigation of ethanol extract of *Flueggea leucopyrus* Willd. leaves

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ABSTRACT

Sexual dysfunction is a widely found disorder worldwide now a day because of busy lifestyle and stress. Management of disease is somewhat difficult because of cost and shortage of effective treatment. In the present paper authors have investigated effect of ethanolic extract of *Flueggea leucopyrus* Willd. leaves as aphrodisiac potential on albino rats. The dried leaves of plant were extracted by cold maceration method using ethanol. It was investigated for in vivo aphrodisiac activity. Plant extract was administered at doses 200 and 400 mg/kg body weight for 45 days. The effect of extract on general mating behavior, potency test, morphological studies, organ weight and sperm motility were tested. The results were compared with standard reference sildenafil citrate. The acute toxicity of the drug extract was also checked. On oral administration of ethanol extract 200 and 400 mg/kg doses on 15th, 30th and 45th day of treatment in all treated groups significantly increased intromission latency, mounting frequency, ejaculatory latency and significantly decreased intromission latency, mounting latency, inter intromission interval and post ejaculatory interval. Test for potency showed significant increase in long flips, quick flips and erections. In all experimental animals morphological study showed significant increase in main and accessory reproductive organs weight and sperm motility. As compared to the control the prolonged treatments for all treated groups were highly effective. This showed that aphrodisiac activity has been shown by ethanol extract but it is less than the standard. The present study showed that ethanol extract of *Flueggea leucopyrus* Willd. leaf increase sexual behavior in rats. Thus it supports the claim for this drug as an aphrodisiac.

Keywords: Aphrodisiac activity, Cold maceration, sperm motility.

INTRODUCTION

Sexual dysfunction is a repeated inability to attain normal sexual intercourse including erectile dysfunction, arousal difficulties, premature ejaculation, retrograded, or retarded ejaculation, etc. Management of this has many options but having serious side effects with high expenses and less availability. Natural drugs from medicinal plant used because of their easy availability, reduced cost and less side effects. An aphrodisiac increases sexual desire. Many plant drugs were used from historical time as an aphrodisiac all over world to increase sexual desire, pleasure and to treat sexual dysfunction.

Flueggea leucopyrus (Willd.) belongs to the family Euphorbiaceae is medicinally useful plant. It is bush weed and when young it is an erect shrub 1.5-4m tall having cylindrical or obtusely angular branches. The plant found in southern India, Sri Lanka and south eastern Queensland. The leaves and stem exudates a white substance [1]. The plant has been used traditionally for treatment of hay asthma, cough, bowel complaints, disinfections, for diarrhea, laxatives, gonorrhea, constipation, kidney stones and mental illness [2, 3]. A paste of leaves and tobacco used to destroy sore worms [4]. It is used as fish poison, for stomachache, anti-cancer treatment [4, 5]. The other uses of plant are aphrodisiac, diuretic, cooling, sweet, tonic, used in vitiated pitta, strangury, burning sensation, seminal weakness, larvicide, general debility, paralysis, insecticide, piscicide and paresis [6]. The bark and leaves extracts were showed presence of glycosides, terpenoids, alkaloids, saponins, unsaturated sterols, tannins, phenolics, flavonoids, proteins and carbohydrates [7].

MATERIALS AND METHODS

Collection of Plant Material

The *Flueggea leucopyrus* (Willd.) leaves collected in flowering of plant October to January from Latur district's Nilanga region, identification and authentication is done by Botanical survey of India, Pune.

Acute Toxicity Study

According to Organization of Economic Co-operation and Development (OECD) guidelines No: 423 healthy male albino rats were starved for 3-4 hrs and subjected to acute toxicity studies^[8]. The rats were divided 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. Group 2-4 received ethanol extract suspension of *Flueggea leucopyrus* (Willd.) leaf orally in doses of 1000, 2000 and 4000 mg/kg daily for seven days respectively. The rats were observed for neurological, behavioral and autonomic profile continuously for 2 hrs and up to 24 and 72 hrs for any lethality.

Mating Behavior Test

For the study healthy male and female rats were selected. After start of the test sample treatment the tests for sexual desire were carried out on 15th, 30th and 45th day. With uniform laboratory conditions this experiment was conducted in the evening. With one female to one male ratio, the male and receptive female rats were introduced in mating cages. The observed mating behaviors were used for further analysis with scoring first four mating series. The phases and frequencies were determined on 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)^[9, 10]. 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. 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 46th day the effect of the extract on potency 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), were 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^[11].

Morphological Studies

Lastly, by using extra dose of anesthesia the animals were killed. 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-hand side of the epididymis was removed and used for morphological study and right-hand side was used for sperm analysis^[12].

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^[13].

RESULTS

Acute Toxicity Study

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

Effect of the Extract on Mating Behavior

The male rats given with ethanol extract of *Flueggea leucopyrus* (Willd.) leaves showed marked increase in the sexual action, with different parameters. The ethanol extract of plant leaves at the doses of 200 and 400 mg/kg body weight show the results of mating behavior test significantly increased the Mounting Frequency (MF) ($P < 0.001$), No. of Intromission (NI) ($P < 0.001$), Intromission Frequency (IF) ($P < 0.001$), Ejaculatory Latency (EL) ($P < 0.001$) and No. of Mount (NM) ($P < 0.001$). It shows significant reduction in Intromission Latency (IL) ($P < 0.001$), Mounting Latency (ML) ($P < 0.001$), Inter Intromission Interval (III) ($P < 0.001$) and Post Ejaculatory Interval (PEI) ($P < 0.001$) on 15th, 30th and 45th day in experimental animal as compared to control group. Likewise, the standard drug increased the MF, NI, IF, EL and NM as well as decreased the IL, ML, III and PEI in a highly significant manner on 15th, 30th and 45th day as compared to control group but greater than ethanol extract (Table 1A-I).

Table 1: Mating Behaviour Test

Table 1A: Mounting Frequency (MF)

S.NO.	Treatment	Dose	15th Day	30th Day	45th Day
1	Control	-	56.93±5.700	57.31±5.506	57.61±5.307
2	FLL Ethanolic Extract	200MG/KG	96.56±6.194*	117.03±9.485*	133.53±10.302*
3	FLL Ethanolic Extract	400MG/KG	108.46±9.883*	137.23±6.933*	170.55±8.717*
4	Sildenafil citrate	5 MG/KG	120.63±9.427*	151.51±9.725*	182.9±10.076*

Table 1B: Intromission Frequency (IF)

1	Control	-	63.90±5.470	64.06±5.550	64.36±5.517
2	FLL Ethanolic Extract	200MG/KG	102.06±3.403*	118.70±7.137*	148.28±5.986*
3	FLL Ethanolic Extract	400MG/KG	121.80±5.968*	143.80±7.550*	180.68±9.097*
4	Sildenafil citrate	5 MG/KG	132.10±11.134*	158.38±11.308*	192.48±9.511*

Table 1C: Mounting Latency (ML)(in S)

1	Control	-	15.83±2.926	14.00±3.162	14.16±2.483
2	FLL Ethanolic Extract	200MG/KG	6.33±2.250*	5.50±2.428*	4.83±2.228*
3	FLL Ethanolic Extract	400MG/KG	4.66±1.632*	3.83±1.471*	3.16±1.471*
4	Sildenafil citrate	5 MG/KG	3.50±1.048*	3.00±0.894*	2.33±0.816*

Table 1D: Intromission Latency (IL)(in S)

1	Control	-	12.33±1.966	12.50±1.870	12.00±1.414
2	FLL Ethanolic Extract	200MG/KG	5.66±2.338*	5.83±1.722*	5.83±1.940*
3	FLL Ethanolic Extract	400MG/KG	4.33±1.861*	4.50±1.870*	4.33±2.250*
4	Sildenafil citrate	5 MG/KG	3.33±1.505*	4.16±1.169*	3.66±1.966*

Table 1E: Ejaculatory Latency (EL)(in S)

1	Control	-	214.33±17.477	217.33±17.339	216.66±17.918
2	FLL Ethanolic Extract	200MG/KG	307.50±10.747*	886.66±15.461*	921.83±21.292*
3	FLL Ethanolic Extract	400MG/KG	343.33±15.870*	993.50±13.501*	1030.33±22.232*
4	Sildenafil citrate	5 MG/KG	373.00±10.526*	1068.66±26.680*	1105.83±21.949*

Table 1F: Number Of Intromission (NI)

1	Control	-	4.00±1.095	4.50±0.836	4.16±0.752
2	FLL Ethanolic Extract	200MG/KG	7.50±1.378*	8.00±0.894*	8.50±1.378*
3	FLL Ethanolic Extract	400MG/KG	8.66±2.160*	9.33±1.861*	9.66±2.160*
4	Sildenafil citrate	5 MG/KG	9.33±1.966*	10.00±2.000*	10.50±2.073*

Table 1G: Number Of Mount (NM)

1	Control	-	3.33±1.211	4.00±0.894	4.16±0.983
2	FLL Ethanolic Extract	200MG/KG	6.66±2.065NS	7.00±2.097*	7.66±2.065NS
3	FLL Ethanolic Extract	400MG/KG	7.83±2.136*	8.16±2.483*	8.33±2.065*
4	Sildenafil citrate	5 MG/KG	8.66±1.632*	8.83±1.940*	9.00±1.788*

Table 1H: Post Ejaculatory Interval (PEI)(in S)

1	Control	-	387.00±16.911	389.83±17.588	390.50±17.306
2	FLL Ethanolic Extract	200MG/KG	64.33±6.055*	51.50±5.431*	42.83±4.875*
3	FLL Ethanolic Extract	400MG/KG	34.83±4.578*	25.83±4.490*	16.83±3.311*
4	Sildenafil citrate	5 MG/KG	16.16±4.070*	11.16±3.488*	8.00±3.224*

Table II: Interintromission Interval (III)(in S)

1	Control	-	26.83±5.269	28.16±5.115	28.00±5.403
2	FLL Ethanolic Extract	200MG/KG	12.33±4.033*	10.33±3.076*	9.33±3.076*
3	FLL Ethanolic Extract	400MG/KG	9.166±1.471*	6.66±1.861*	5.83±2.041*
4	Sildenafil citrate	5 MG/KG	8.00±2.000*	5.50±1.378*	5.00±1.095*

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. FLL - *Flueggea leucopyrus* (Willd.) leaf.

Effect of the Extract on Potency

The ethanol extract of *Flueggea leucopyrus* (Willd.) leaves showed the test for potency at doses of 200 and 400 mg/kg body weight, significant increase in the frequency of Erection (E) (P < 0.001), Long Flips (LF) (P < 0.001) and Quick Flips (QF) (P < 0.001) also the total

penile reflexes (TPR) (P < 0.001) on 15th, 30th and 45th day as compared to control group. 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 with respect to control group but greater than ethanol extract (Table 2A-D).

Table 2: Test for Potency

Table 2A: Erection

S.NO.	Treatment	Dose	15th Day	30th Day	45th Day
1	Control	-	8.50±1.760	9.16±1.722	9.66±1.632
2	FLL Ethanolic Extract	200MG/KG	18.33±3.777*	19.66±3.386*	20.16±3.311*
3	FLL Ethanolic Extract	400MG/KG	21.50±2.738*	24.16±2.483*	25.66±2.160*
4	Sildenafil citrate	5 MG/KG	23.66±3.386*	26.16±2.926*	28.66±3.076*

Table 2B: Quick Flip

1	Control	-	5.66±1.632	6.50±1.516	6.83±1.722
2	FLL Ethanolic Extract	200MG/KG	16.16±2.926*	18.83±2.316*	20.50±2.345*
3	FLL Ethanolic Extract	400MG/KG	19.33±5.046*	24.16±4.792*	26.33±5.006*
4	Sildenafil citrate	5 MG/KG	21.16±3.311*	27.33±3.141*	30.50±3.271*

Table 2C: Long Flip

1	Control	-	2.50±1.048	3.33±0.816	3.83±0.752
2	FLL Ethanolic Extract	200MG/KG	12.66±2.160*	17.16±2.639*	18.66±2.160*
3	FLL Ethanolic Extract	400MG/KG	15.50±1.870*	21.00±2.097*	23.66±2.160*
4	Sildenafil citrate	5 MG/KG	16.66±3.777*	24.50±4.086*	27.33±4.966*

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. FLL - *Flueggea leucopyrus* (Willd.) leaf.

Table 2D: Total Reflex

a. Total Reflex (15th Day)

S.NO.	Treatment	Dose	Erection	QF	LF	TR
1	Control	-	8.50±1.760	5.66±1.632	2.50±1.048	16.66±1.751
2	FLL Ethanolic Extract	200MG/KG	18.33±3.777*	16.16±2.926*	12.66±2.160*	47.16±3.544*
3	FLL Ethanolic Extract	400MG/KG	21.50±2.738*	19.33±5.046*	15.50±1.870*	56.33±5.354*
4	Sildenafil citrate	5 MG/KG	23.66±3.386*	21.16±3.311*	16.66±3.777*	61.5±4.636*

b. Total Reflex (30th Day)

1	Control	-	9.16±1.722	6.50±1.516	3.33±0.816	19.00±2.190
2	FLL Ethanolic Extract	200MG/KG	19.66±3.386*	18.83±2.316*	17.16±2.639*	55.66±3.669*

3	FLL Ethanolic Extract	400MG/KG	24.16±2.483*	24.16±4.792*	21.00±2.097*	69.33±4.676*
4	Sildenafil citrate	5 MG/KG	26.16±2.926*	27.33±3.141*	24.50±4.086*	78.00±4.000*

c. Total Reflex (45th Day)

1	Control	-	9.66±1.632	6.83±1.722	3.83±0.752	20.33±2.581
2	FLL Ethanolic Extract	200MG/KG	20.16±3.311*	20.50±2.345*	18.66±2.160*	59.33±3.386*
3	FLL Ethanolic Extract	400MG/KG	25.66±2.160*	26.33±5.006*	23.66±2.160*	75.66±5.316*
4	Sildenafil citrate	5 MG/KG	28.66±3.076*	30.50±3.271*	27.33±4.966*	86.50±4.460*

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. FLL - *Flueggea leucopyrus* (Willd.) leaf.

Effect of Extract on Morphological Studies

The ethanol extract of *Flueggea leucopyrus* (Willd.) leaves orally given with dose of 200 and 400 mg/kg, significantly increased the

relative weight of reproductive organs like epididymis and testes. As compared to control animal group there is no significant change is observed in ventral prostate. The weight of reproductive and vital organs was significantly increased in standard group on 15th, 30th and 45th day as compared to control (Table 3A).

Table 3: Morphological Studies

Table 3A: Organ Weight

S.NO.	Treatment	Dose	Testis (gm)	Epididymis(gm)	Prostate(gm)
1	Control	-	0.868±0.0079	0.341±0.0073	0.738±0.0050
2	FLL Ethanolic Extract	200MG/KG	1.190±0.0056*	0.475±0.0061*	0.741±0.0040 NS
3	FLL Ethanolic Extract	400MG/KG	1.285±0.0056*	0.559±0.0050*	0.743±0.0050 NS
4	Sildenafil citrate	5 MG/KG	1.319±0.0126*	0.580±0.0068*	0.747±0.0070 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. FLL - *Flueggea leucopyrus* (Willd.) leaf.

Sperm analysis of the ethanol extract of *Flueggea leucopyrus* (Willd.) leaves with the dose of 200 and 400 mg/kg showed significant increase in sperm motility. As compared to control group standard group showed significant increase in sperm motility also (Table 3B).

Table 3B: Sperm Analysis

S.NO.	Treatment	Dose	Motility
1	Control	-	1.33±0.816
2	FLL Ethanolic Extract	200MG/KG	3.33±0.516*
3	FLL Ethanolic Extract	400MG/KG	4.00±0.632*
4	Sildenafil citrate	5 MG/KG	4.83±0.408*

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. FLL - *Flueggea leucopyrus* (Willd.) leaf.

DISCUSSION

The *Flueggea leucopyrus* (Willd.) leaves listed for its sexual activity stimulation. So to prove its activity as an aphrodisiac the study is carried out.

The presence of phenolic compounds, flavonoids, xanthenes, alkaloids, other amines and saponins [14] might be leads to improvement of sexual function observed in animals treated with the ethanol extract of *Flueggea leucopyrus* (Willd.) leaves. Further fractionation gives us insight about which active constituents are responsible for aphrodisiac activity.

In this study, symptoms of clinical toxicity were not observed during any time of the experiment. The study showed that, it is apparently safe to use this for short duration of time [15].

Behavioral indications of sexual facilitation and performance in male rats are considered ejaculation and intromission, whereas the sexual indicators of motivation are considered as latency of mount and intromission [16]. The treatment of animals with different doses of ethanol extract of *Flueggea leucopyrus* (Willd.) leaves showed a significant increase in the ejaculation latency and number of ejaculation. There was a significant decrease in the intromission and latency of mount showing an enhancement in sexual motivation. The Mounting frequency (MF) and Intromission frequency (IF) are considered the libido and potency indices increased significantly as compared to control group in the ethanol extracts. Because of ethanol extracts and standard drug the Ejaculation latency (EL) increased significantly suggesting prolonged coitus duration, indicating increased sexual motivation [17]. The drug effect is evaluated for erectile function by using the penile erection index [18]. The ethanol extract of *Flueggea leucopyrus* (Willd.) leaves with doses 200 and 400 mg/kg body weight showed the potency test significantly increased as compared to control group include the frequency of Erection (E), Quick Flips (QF) and Long Flips (LF), but lesser than standard drug treated rats. The experimental and standard animals showed significantly increased aggregate of these penile reflexes (TFR). This shows increased potency of the ethanol extracts of *Flueggea leucopyrus* (Willd.) leaves.

Increase in body weight and sexual organ weight are because of steroids and these are parameters for plant extract effectiveness improving the steroidal hormones production^[17]. Ethanol extract of *Flueggea leucopyrus* (Willd.) leaves at the doses of 200 and 400 mg/kg body weight significantly increased weight of the organs like testes and epididymis but not the prostate.

Some aphrodisiac studies indicated that an increase in level of cytosolic cGMP elevated sperm motility, whereas an increase in level of cytosolic cAMP increased sperm motility and sperm viability^[19].

CONCLUSION

Ethanol extract of *Flueggea leucopyrus* (Willd.) leaves studied for its aphrodisiac activity because of its traditional claim improved sexual function. So we can use it effectively in sexual disorders as safe alternative remedy. Future work includes isolation and characterization of an aphrodisiac active principle in plant extract and investigation of mechanism of action.

This study concluded with dose dependant enhancement of sexual activity by ethanol extract of *Flueggea leucopyrus* (Willd.) leaves and proved existing ancient knowledge.

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HOW TO CITE THIS ARTICLE

Usnale SV, Biyani KR. Preclinical aphrodisiac investigation of ethanol extract of *Flueggea leucopyrus* Willd. leaves. J Phytopharmacol 2018; 7(3):319-324.