Phytopharmacology

Inforesights Publishing

Sedative and analgesic activities of *Ludwigia repens*

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Received: 19 November 2011, **Revised**: 28 November 2011 **Accepted**: 29 November 2011

Abstract

The methanolic leaf extract of *Ludwigia repens* was evaluated for possible sedative and analgesic activities in mice. Sedative activity was evaluated by using hole cross, open field, thiopental sodium-induced sleeping time and elevated-plus maze (EPM) tests at 400 mg/kg while the peripheral and central analgesic activity was investigated using acetic acid-induced writhing test, formalin test and tail immersion test at 200 mg/kg body weight orally. The extract decreased the locomotor activity of mice in hole cross, open field and EPM test. Moreover, the extract significantly minimized onset of sleep and maximized the duration of sleeping time when administered with thiopental sodium. The extract also produced a significant ($p < 0.05$) reduction of pain in all three models.

Keywords: *Ludwigia repens*, Sedative, locomotor activity, analgesic

Introduction

Ludwigia repens, belonging to the family Onargaceae, is an evergreen amphibian herbaceous plant which is largely found in Southern parts of North America. Ludwigia sp. is widely distributed in America, Africa, Asia and Australia (Rataj and Horeman, 1977). It is a species of flowering plant in the evening primrose family known by the common name creeping primrose willow and locally known as Kashordum, Tulehan (Rakhaing). It is found in the shallow waters of streams and lakes and freshwater and familiar as an aquatic weed in some regions. Some of the species belonging to Ludwigia genus are used as vegetables, ornamental aquarium plants, pollen source for honey bees, fish feed and medicinal purposes (Brunson, 1988; Brundu *et al*., 2001 Chen *et al*., 1989; Greenway and Wooley, 1999; Kuo *et al*., 1999 Mooi *et al*., 1999). *L. repens* with small yellow flowers (Cirik et al., 2001) and pinkish red to bright green leaves is widely used as an aquarium plant. The leaves are oppositely arranged and up to 4 or 5 centimeters long. The flower has four yellow petals no more than 3 millimeters long nested on a base of four pointed sepals which may be slightly longer. It grows rapidly in slightly acidic waters at 19-28°C.

Materials and Methods

Plant material

The plant *L. repens was* collected from Hajee para village, Chittagong district, Bangladesh in 2011. The plant was previously identified by Dr. Shaikh Bokhtear Uddin, associate professor, Department of Botany, University of Chittagong.

Preparation of Extracts

The collected plant were washed thoroughly water, chopped, air dried for a week at 35-40^{0}C & pulverized in electric grinder. The powder obtained was successively extracted in methanol (55-60^{0}C). The extracts were made to dry by using rotary evaporator under reduced pressure.

Animals

Swiss albino mice having weight 25-30 gm were collected from International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The animals were housed under standard laboratory conditions (relative humidity 55-65%, room temperature 23.0±2.0°C and 12 h light: dark cycle) and acclimatized for 7 days. The animals were fed with standard diet and water. The set of rules followed for animal experiment were approved by the institutional animal ethical committee (Zimmermann, 1983).

Sedative Activity

Hole Cross Test

The test was observed by the method described by Takagi *et al.,* (1971) for screening sedative activity in mice. The animals were divided into three groups- control, positive control and test. The test groups received methanolic extract of *L. repens* at the doses of 400 mg/kg body weight orally whereas the control group received vehicle (1% Tween 80 in water). A steel partition was fixed in the middle of a cage having a size of 30×20×14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. The number of passages of a mouse through the hole from one chamber to other was counted for a period of 3 min on 0, 30, 60, 90 and 120 min after the oral treatment with test drugs. Diazepam was used in the positive control group as reference standard at the dose of 1 mg/kg (i.p).

Open Field Test

The animals were treated as discussed above. The experiment was carried out according to the methods described by Gupta *et al*., (1971). The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a wall of 40 cm height. The number of squares visited by the mice was counted for 3 min, on 0, 30, 60 and 120 min during the study period.

Thiopental sodium induced sleeping time test

The experiment was conducted following the method described by Ferrini *et al* (1974). The animals were randomly divided into three groups consisting of five mice each. The test groups received methanol extract from the leaves of *L. repens* at dose 400 mg/kg (p.o) body weight while the standard group was treated with diazepam (1 mg/kg, p.o) and control group with vehicle (1% Tween 80 in water). Twenty minutes later, thiopental sodium (40 mg/kg, i.p) were administered to each mouse to induce sleep. The animals were observed for the latent period (time between thiopental administrations to loss of righting reflex) and duration of sleep *i.e.* time between the loss and recovery of righting reflex.

Elevated plus-maze (EPM) test

The method initially suggested by Handley and Mithani was employed with minor modifications (Lister RG, 1987).The apparatus consist of two open arms (5 × 10 cm) and two closed arms (5 × 10 × l5 cm) radiating from a platform (5 × 5 cm) to give the apparatus a plus sign appearance. The apparatus was situated 40 cm above the floor. The open arms edges were 0.5 cm in height to keep the mice from falling and the closed-arms edges were 15 cm in height. The maze floor and walls were constructed from dark opaque wood. Sixty minutes after administration of the test drugs, each animal was placed at the center of the maze facing one of the enclosed arms. During the 5-min test period, the number of open arms entries was recorded. Entry into an arm was defined as the point when the animal places all four paws onto the arm. The procedure was conducted in a sound attenuated room; observations made from an adjacent corner.

Analgesic Activity

Mouse writhing test

This was based on the method described by koster *et al*., (1959). Swiss albino mice of either sex were selected and divided into three groups of five animals each. The extract 400 mg/kg orally, vehicle (1% tween 80 in water, p.o) and diclofenac sodium (10 mg/kg, i.p) were administered to the respective group 30 min before intraperitoneal injection of 0.7%, 0.1 ml/10 gm acetic acid solution. Immediately after administering acetic acid, mouse were observed and the number of writhing or stretches were counted for 15 min. Reduction in the number of writhes compared to the control groups was considered as evidence of analgesic effect. The percent inhibition (% analgesic activity) was calculated by

$$\% \text{ inhibition} = \{(A-B)/A\} \times 100$$

Where, A= Average number of writhing of the control group; B= Average number of writhing of the test group.

Formalin test

The method was done according to the method described Sharma A *et al*., (2010). 20 µl of 5% formalin was injected subcutaneously into the right hind paw of mice. The time (in sec) spent in licking the paw and the biting responses of the injected paw were taken as an

indicator of pain response. The rats were observed for 30 min after the injection of formalin, and the amount of time spent licking the injected hind paw was recorded. The first 5 min post formalin injection is referred to as the early phase and the period between 15 and 30 min as the late phase. Extract (400 mg/kg, orally) and diclofenac sodium (10 mg/kg, i.p) were administered 30 min prior to formalin injection. Control animals received 10 ml/kg of distilled water orally.

Tail Immersion Test

The experiment was performed according to the methodology depicted by Toma *et al*., (2003). Rats were closely restrained in a wire mesh cage and the tails (1/3rd of the tail) were then dipped in the water bath thermo-statistically maintained at 55±0.5°C. The time in second to withdraw the tail clearly out of the water was taken as the reaction time. All the animals were screened and those that failed to respond within 60 sec were not used for the assay. Measurement of threshold was made just before (0 min) and at 30, 60 and 90 min interval for 1.30 h after administration of the extract (400 mg/kg, orally) or nalbuphine (10 mg/kg i.p). Vehicle (1% tween 80 in water, 10 mg/kg p.o) served as the control.

Statistical analysis

Data are expressed as mean ± STD and were analyzed statistically by one-way ANOVA procedures, followed by using Dunnett's test. A difference was considered significant at $p<0.05$.

Results

Sedative Activity

Hole cross test

The number of hole crossed from one chamber to another by mice of the control group was similar from 0 to 120 min (Table 1). In the hole cross test, the extracts showed a decrease in locomotion in the test animals from the second observation period as evident by the reduction in number of hole crossed by the treated mice compared to the control group. The result was comparable to the reference drug diazepam and was statistically significant ($p < 0.05$).

Table 1. CNS depressant activity of methanolic extract of leaves of *l. repens* on hole cross test in mice

Group	Treatment	Dose, Route	Numbers of movements				
			0 min	30 min	60 min	90 min	120 min
Control	1% tween 80 in water	10 ml/kg, p.o	16.80± 2.387	15.20± 1.923	14.60± 0.837	17.20± 0.742	15.40± 0.570
Standard	Diazepam	1 mg/kg, p.o	18.20± 2.168*	6.60± 2.302*	4.40± 2.966*	4.60± 1.673*	3.40± 1.140*
Test	MELR	400 mg/kg p.o	17.20± 2.775*	4.00± 0.707*	4.20± 0.837*	4.80± 1.923*	3.60± 1.342*

All values are expressed as mean ± STD (n=5); One way Analysis of Variance (ANOVA) followed by Dunnett's test. *P<0.05, significant compared to control.

Table 2. CNS depressant activity of methanolic extract of leaves of *l. repens* on open field test in mice

Group	Treatment	Dose, Route	Numbers of movements				
			0 min	30 min	60 min	90 min	120 min
Control	1% tween 80 in water	10 ml/kg, p.o	56.20± 7.918	60.80± 4.97	48.00± 5.3852	47.20± 2.588	49.60± 4.037
Standard	Diazepam	1 mg/kg, p.o	68.40± 2.302	54.00± 4.303	27.00± 2.121*	21.20± 3.962*	17.60± 1.673*
Test	MELR	400 mg/kg p.o	48.20 ± 23.285	34.40 ± 16.517*	12.80 ± 6.058*	16.40 ± 7.700*	8.20 ± 8.70*

All values are expressed as mean ± STD (n=5); One way Analysis of Variance (ANOVA) followed by Dunnet's test. *P<0.05, significant compared to control.

Open field test

In the open field test, the number of squares traveled by the mice was suppressed significantly in the test group throughout the study period. The CNS depressant activity obtained for extract was more than that of standard drug and the result was statistically significant.

Thiopental sodium induced sleeping time test

In the thiopental sodium induced sleeping time test, the test group treated with the extract at 400 mg/kg showed significant ($p<0.05$) decrease in onset of action and increased the duration of sleep. The extract showed better sedative activity than the standard drug diazepam regarding both onset of sleep and duration of sleep (Table 3).

Elevated plus-maze (EPM) test

Result of EPM test is presented in Table 4. The methanol extract of *L. repens* at the dose of 400 mg/kg body weight, significantly decreased the percentage of entries of mice into the open arms and the percentage of time spent in the open arms of the EPM

Table 3. CNS depressant activity of methanolic extract of leaves of *l. repens* on thiopental sodium induced sleeping time test in mice

Group	Treatment	Dose, Route	Onset of sleep (min)	Duration of sleep (min)
Control	1% tween 80 in water	10 ml/kg, p.o	40.20±3.701	47.00±2.121
Standard	Diazepam	1 mg/kg, p.o	14.80±1.9235*	149.80±7.694*
Test	MELR	400 mg/kg p.o	11.4±4.336*	305.80±5.805*

All values are expressed as mean ± STD (n=5); One way Analysis of Variance (ANOVA) followed by Dunnet's test. *P<0.05, significant compared to control.

Table 4. CNS depressant activity of methanolic extract of leaves of *l. repens* on elevated plus maze test in mice.

Group	Treatment	Dose, Route	% Entry into open arm
Control	1% tween 80 in water	10 ml/kg, p.o	55.88±4.266
Standard	Diazepam	1 mg/kg, p.o	29.40±3.286*
Test	MELR	400 mg/kg p.o	25.26±11.109*

All values are expressed as mean ± STD (n=5); One way Analysis of Variance (ANOVA) followed by Dunnet's test. *P<0.05, significant compared to control.

Analgesic Activity

Mouse writhing test

Table 5 shows the effects of the extract on acetic acid induced writhing in mice. Oral administration of the extract significantly ($p < 0.05$) inhibited writhing response induced by acetic acid which was comparable to the reference drug.

Formalin test

The methanol extract of *L. repens* (200 mg/kg, p.o.) significantly suppressed formalin-induced pain response in mice, with a more potent effect on the second than the first phase. In the late phase (15-30 min) of this test, the extract exerted 64.628% inhibition whereas 66.446% inhibition was obtained for diclofenac sodium against pain.

Tail Immersion Test

There was a significant increased of the tail withdrawal reflex time following administration of the extract at dose of 200 mg/kg. The result was statistically significant ($p<0.05$) and was comparable to the reference drug nalbuphine (Table 7).

Table 5. Analgesic activity of methanolic extract of leaves of *L. repens* by acetic acid induced writhing method in mice.

Group	Treatment	Dose, Route	No. of writhing	Percent inhibition
Control	1% tween 80 in water	10 ml/kg, p.o	49.25±5.188*	-
Standard	Diclofenec Sodium	10 mg/kgi, p	19.75±2.217	59.9
Test	MELR	200 mg/kg p.o	23±3.366*	53.3

All values are expressed as mean ± STD (n=5); One way Analysis of Variance (ANOVA) followed by Dunnet's test. *P<0.05, significant compared to control.

Table 6. Effect of the methanolic extract of barks of *l. repens* on hindpaw licking in the formalin test in mice.

Group	Treatment	Dose, Route	Early phase (sec)	Inhibition (%)	Late phase (Sec)	Inhibition (%)
Control	1% tween 80 in water	10 ml/kg, p.o	1.44±0.465	-	3.025±0.293	-
Standard	Diclofenec Sodium	10 mg/kg, i.p	1.09±0.379	24.306	1.015±0.85*	66.446
Test	MELR	200 mg/kg p.o	1.2575±0.236	12.673	1.07±0.556*	64.628

All values are expressed as mean ± STD (n=5); One way Analysis of Variance (ANOVA) followed by Dunnet's test. *P<0.05, significant compared to control.

Table 7: Analgesia activity of the methanolic extract of l. *repens* by tail immersion response

Group	Treatment	Dose, Route	Basal Reaction Time (Sec)	Reaction time (Sec)		
				30 min	60 min	90 min
Control	1% tween 80 in water	10 mg/kg, p.o	2.21±0.106	2.50±0187	2.90±0.371	2.39±0.191
Standard	Nalbuphine	10 mg/kg, i.p	2.27±0.147	6.05±0.492	9.23±0.407	11.93±1.05
Test	MELR	400 mg/kg p.o	3.46 ±0.806	8.28±1.8956	6.74±5.76	6.54±2.101

All values are expressed as mean ± STD (n=5); One way Analysis of Variance (ANOVA) followed by Dunnet's test. *P<0.05, significant compared to control.

Discussion

The study has examined some neuropharmacological activities of methanolic extract of *L. repens*. The plant extract possessed central nervous system depressant activity as indicated by the decrease in locomotor activity in mice in hole cross, open field and EPM test. The marked sedative effect of the extract was also found by the reduction in sleeping latency and increase of thiopental sodium induced sleeping time. Gamma-amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. CNS depressant drugs mainly exert their action through $GABA_A$ receptor (Kolawole OT *et al.*, 2007). So, the extract of *L. repens* may acts by hyperpolarization of the CNS via GABA receptor or benzodiazepine receptor located adjacent to the GABA receptor.

The methanol extract was also evaluated in the tail immersion, formalin and acetic acid-induced writhing test for its analgesic activity. The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesic. The response is thought to be mediated by the prostaglandin pathways, peritoneal mast cells and acid sensing ion channels (Hossain *et al.*, 2006; Ronaldo *et al.*, 2000; Voilley, 2004). Therefore, the significant pain reduction of the plant extract may be due to the presence of analgesic

principles acting with the prostaglandin pathways or interfering with other mediators responsible for peripheral pain.

The formalin test is another reliable model of analgesic which is better correlated with clinical pain (Tjolsen A *et al*., 1992; Ghannadi A *et al*., 2005). This method elucidates central and peripheral activities. The response of early phase is supposed to represent a direct chemical stimulation of pain, due to the irritant effect of formalin on sensory C fibers (Heapy *et al*., 1987; Hunskaar *et al*., 1985; Tjolsen *et al*., 1992). The late phase response is most likely secondary to the development of an inflammatory response and the release of allergic mediators (Hunskaar & Hole 1987). Inhibition of licking response of the test drugs in the early phase and late phase signifying the analgesic effect of the extract in the formalin test.

The tail immersion method was used to evaluate the central mechanism of analgesic activity. Here the painful reactions in animals were produced by thermal stimulus that is by dipping the tip of the tail in hot water. Analgesic effect against thermal noxious stimuli may be elicited through opoid receptors or through modulation of several neurotransmitters involved in relevant phenomena. But the extend of activity shown by the crude extracts are less than that of the standard drug nalbuphine but many fold more than that of the control group, which justifies its activity.

Narcotic analgesics inhibit both peripheral and central mechanism of pain, while non steroidal anti-inflammatory drugs inhibit only peripheral pain (Elisabetsky *et al*., 1995; Pal *et al*., 199). The extract inhibited both mechanisms of pain, suggesting that the plant extract may act as a narcotic analgesic.

The pharmacological profiles of the present investigation of the methanol extract of *L. repens* indicate that the extract possess strong CNS depressant and analgesic activities as it significantly reduced locomotion, onset of sleep, increased duration of sleep and inhibition of central and peripheral pain of mice in different experimental model. However, further studies are underway to determine the exact phytoconsituents that are responsible for the neuropharmacological activities of the methanol extract of *L. repens.*

Acknowledgement

Authors wish to thanks Dr. Shaikh Bokhtear Uddin, Associate professor, Department of Botany, University of Chittagong for identification the plant. The authors are also grateful to the authority of International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) for providing the experimental rats and Square Pharmaceuticals Ltd., Bangladesh for providing diclofenac sodium and diazepam.

Conflict of interest

The Authors have declared that there no conflict of competing interest.

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