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Anti-inflammatory, analgesic and diuretic activity of *Ludwigia hyssopifolia* Linn

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To cite: Das B, Kundu J, Bachar SC, Kundu JK. Anti-inflammatory, analgesic and diuretic activity of Ludwigia hyssopifolia Linn. *Arch Med Biomed Res.* 2014;1(4):139-146. doi:10.4314/ambr.v1i4.3

Publication history
Received: July 07, 2014
Revised: October 10, 2014
Accepted: October 11, 2014

CrossRef Link
http://dx.doi.org/10.4314/ambr.v1i4.3

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ABSTRACT

The effects of hexane, ethylacetate and methanol extracts of whole plant parts of *Ludwigia hyssopifolia* Linn on carrageenan-induced rat paw edema, acetic acid-induced writhing, and diuresis in mice were studied. The hexane extract (LH) and ethylacetate extract (LE) showed maximal inhibition of carrageenan-induced paw edema by 33.96% and 27.39% after 2 h and 3 h of study, respectively, while the methanolic extract (LM) showed no remarkable effects. The study of anti-nociceptive activity of the extracts showed that all three fractions exhibited significant inhibition of writhing reflex in an order of LH > LE > LM. In comparison to furosemide, good diuretic activity was exhibited by all three extracts. The onset of diuretic action of LE and LM was rapid, while that of LH was relatively slow. Results of this preliminary pharmacological screening indicate that the plant *Ludwigia hyssopifolia* Linn holds the promise of being utilized in developing herbal medicines.

KEY WORDS: ***Ludwigia hyssopifolia Linn; Anti-inflammatory activity; Carrageenan; Diuretic activity***

INTRODUCTION

Numerous clinically used medicines are derived directly or indirectly from plant sources[1]. While a good number of purified plant constituents have been developed as modern medicines, a vast majority of world population still uses herbal medicines for primary health care purpose[2]. Herbs, a rich source of structurally diverse classes of secondary metabolites, are effective in the treatment and/or prevention of various chronic diseases, such as diabetes[3], infection[4], cardiovascular disorders[5], cancer[6], etc. The therapeutic effects of herbs and spices in traditional medicines have been documented in early literature, for example, the Ayurveda, mainly based on their folkloric use. However, many of the medicinal herbs are still used in traditional therapy without being examined for their claimed therapeutic benefits. Thus, the systematic evaluation of the biological activities and chemical properties of medicinally important herbs and spices is, therefore, an utmost necessity.

Ludwigia hyssopifolia Linn (synonym *Jussiaea linifolia* Vahl or *Jussiaea hyssopifolia* Linn, Family- *Onagraceae*; Bengali Name- Lalbunlunga) is extensively grown in Bangladesh, in all parts of India and Ceylon. The plant is considered as astringent, anthelmintic, carminative and diuretic. A decoction is used in diarrhea and dysentery, flatulence, leucorrhoea, and spitting of blood[7]. The leaves are used for poulticing in orchitis and glands in the neck. A decoction is also used as a vermifuge and purgative[7]. Previous phytochemical investigation of the plant revealed the presence of chemical constituents namely vitexin, isovitexin, orientin and isoorientin[8]. We have previously reported that different organic extracts of the plant possess antidiarrheal activity[9] and inhibit *Agrobacterium tumefaciens*-induced formation of crown-gall tumor on potato disk[10]. In continuation of our work on biological characterization of different medicinal plants of Bangladesh, the present study has been designed to investigate the anti-inflammatory, analgesic and diuretic activity of different extractives of *Ludwigia hyssopifolia* Linn.

METHODOLOGY

Preparation of plant materials

The whole plant *Ludwigia hyssopifolia* Linn was collected at flowering stage from Dhaka during November 2006 and was identified (voucher specimen No. DUH-163) by the Department of Botany, University of Dhaka, Bangladesh. After collection, the whole plant parts were sun-dried for eight days, made into a coarse powder by grinding and kept in airtight container. The coarse powder of the whole plant (1 kg) was extracted with n-hexane, ehtylacetate and methanol by successive cold extraction. All the extracts obtained were filtered and evaporated to dryness in *vacuo* at low temperature and reduced pressure by rotary evaporator. The n-hexane, ethylacetate and methanol extracts were designated as LH, LE and LM, respectively, and were subjected to preliminary qualitative analysis for the presence of various constituents following standard methods of phytochemical analysis[11]. The suspensions of LH, LE and LM in saline solution were prepared separately by using tween-80 as the suspending agent in such a way that each milliliter of the suspension contained 50 or 250 mg of the respective extract.

Experimental animals

Swiss albino mice (20-25 g) and *Long Evans* rats (140-160 g) of either sex were obtained from the animal house of International Center for Diarrheal Disease and Research, Bangladesh (ICDDRB). The mice were divided into six groups and the rats into eight groups containing five animals in each group. The animals were given standard mouse/rat feed developed by ICDDRB and water *ad libitum* and kept in the laboratory environment for seven days. They were fasted overnight and weighed before the experiment. All animal experiment protocols were reviewed and approved by Dhaka University Research Ethics Committee.

Anti-inflammatory activity assay

The effect of LH, LE and LM on carrageenan (1%)-induced inflammation in rat paw was investigated by following the method of Winter *et al*[12] with minor modifications[13]. Rats were randomly divided into eight groups, each consisting of five animals. One hour prior to challenge with sub-planter administration of carrageenan, LH, LE or LM were given by gavage to animals of group I, III and V, respectively, at a dose of 50mg/kg body weight. Rats belonging to group II, IV and VI were treated with LH, LE and LM, respectively, at a dose of 250 mg/kg body weight. The dose of the test samples was selected on the basis of the folkloric use of

the plant as well as our previous studies with these plant extractives[9,10]. Group VII was given the standard drug phenylbutazone at a dose of 100 mg/kg body weight, while group VIII was kept as control giving only saline water containing 1% tween-80. One hour after the oral administration of the test materials, standard drug and saline solution, 1% carrageenan solution was injected to the right hind paw of each animal. The volume of paw edema was measured at 1, 2, 3, 4 and 24hrs after carrageenan administration. For the measurement of paw volume, the inflamed paw was immersed into the mercury contained in a U-tube, which consists of a right cylindrical glass tube (8.0 cm x 2.2 cm) connected to a narrow side arm (10.0 cm x 0.72 cm) having a wall of uniform cross section and open upper end. The volume of mercury displaced was recorded by using traveling microscope (ELFO Scientific Apparatus, India). Prior to immersion into mercury, each of the inflamed right hind paw was labeled with permanent ink so that the immersion would be uniform in each episode.

The average percent increase in paw volume with time was calculated and compared against the control group. Percent inhibition was calculated using the formula:

% inhibition of paw edema = [(Vc- Vt)/Vc] x 100

Where Vc and Vt represent average paw volume of control and treated animals, respectively.

Acetic acid-induced writhing reflex

Swiss albino mice (6-8 weeks) weighing between 20 to 25 g were used to study the analgesic activity by recording acetic acid-induced writhing reflex as described by Saha *et al*[13]. Animals of various groups were treated with either test material LH, LE and LM respectively at a dose of 250 mg/kg body weight or standard drug aminopyrine at a dose of 50 mg/kg body weight 40min prior to the intraperitoneal administration of acetic acid solution (0.7%, 0.1 ml/10 gm body weight). After an interval of 10 min, numbers of writhing were counted for 10 min. The percent inhibition of writhing was measured using the formula:

Percent inhibition of writhing = (1-W_t/W_c) x 100

Where, W_C and W_t represent the average number of writhing produced by the control and test group, respectively.

Screening of diuretic activity

The diuretic activity of LH, LE and LM was studied in Swiss albino mice following the method of Gujral *et al*[14] with slight modifications. The test animals were divided into six groups, containing six mice in each group. Group I was provided only with saline solution containing 0.1% tween-80 i.e. control group. Group II was given urea at a dose of 500 mg/kg body weight and was considered as positive control group. Group III was provided with standard diuretic drug furosemide at a dose of 3 mg/kg body weight per oral. Group IV, V and VI received the test compounds LH, LE and LM, respectively, at the doses of 250 mg/kg body weight by gavage. The experimental animals were placed into metabolic cages 24 hr prior to the experiment. The urinary output of each group was recorded at different time intervals from the graduated urine chamber of metabolic cages. The volume of urine excreted in 4 hr of study by each group was expressed as percent of the liquid administered giving rise to a measure of urinary excretion (UE):

UE = (Total urinary output/ Total liquid administered) x 100

The ratio of urinary excretion (UE) in test group and control group was denoted as diuretic action, which was used as the measure of degree of diuresis:

Diuretic Action = UE in test group / UE in control group

Diuretic Activity = Diuretic action of drug / Diuretic action of urea

Statistical analysis

Data are expressed as means ± SEM. Statistical significance of changes have been determined by the Student's t-test. A p value < 0.05 has been considered to be statistically significant.

RESULTS AND DISCUSSION

The three extracts namely LH, LE and LM of *Ludwigia hyssopifolia* Linn were obtained as 0.75%, 1.125% and 1% yield, respectively. Phytochemical analysis of the extracts revealed the presence of terpenoids in both LH and LM, while flavonoids and alkaloids were present in LE[9,10]. The effect of test materials on carrageenan induced rat paw edema at different time intervals was compared to that of control for the evaluation of anti-inflammatory activity on the basis of percent inhibition of paw edema volume. LH and LE exhibited statistically significant ($p < 0.001$) inhibition of paw volume by 33.96% and 27.39% at 2 and 3 h of study, respectively (**Figure 1**). However, LM failed to inhibit carrageenan-induced paw inflammation. The inhibitory effects of LH and LE on paw volume were comparable to that of the standard drug phenylbutazone (**Table 1**). The anti-inflammatory response of LH and LE was less than that of phenylbutazone but their duration of action was found to be comparable to that of phenylbutazone till the fourth hour of study. It was also revealed from the experimental results that the order of anti-inflammatory response among the three extracts tested was LH > LE > LM.

The carrageenan-induced rat paw edema model is frequently used to evaluate the effect of non-steroidal anti-inflammatory agents, which primarily inhibit the cyclooxygenase (COX) enzymes involved in the biosynthesis of prostaglandins (PGs)[15,16]. The time kinetics of carrageenan-induced paw edema development in rats is represented by a biphasic curve[12], of which the first phase of inflammation occurring within an hour of carrageenan injection is partly due to the trauma of injection and to the release of histamine and serotonin[17]. The second phase of inflammatory reaction that occurs after 3 h of carrageenan administration is largely contributed by PGs[18]. Therefore, the inhibitory effect of LH and LE on carrageenan-induced inflammation may result from the possible inhibition of the release of histamine or the expression and/or the activity of COX enzymes. Based on these reports, it can be inferred that the inhibitory effect of LH and LE on carrageenan-induced inflammation in rats could be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis. An alkaloid constituent 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl] piperidine, trivial name piperine is isolated from the LE fraction[10]. Previous studies have shown that piperine possesses antioxidative and anti-inflammatory properties[19,20]. Thus, the observed anti-inflammatory effect of LE may be due to the presence of piperine. However, the anti-inflammatory constituent(s) present in LH is/are yet to be examined.

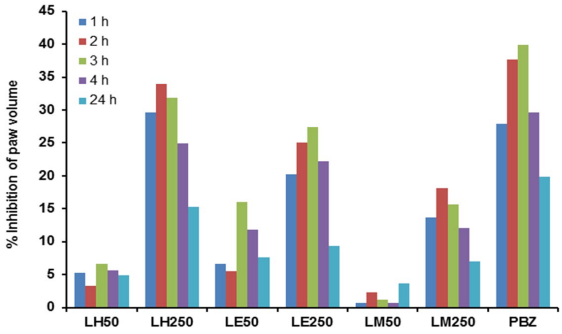

Figure 1: Inhibitory effects of LH, LE and LM on carrageenan-induced rat paw inflammation. LH50 and LH250 indicate hexane extract given at doses of 50 and 250 mg/kg body weight, respectively. LE50 and LE250 indicate extract given at doses of 50 and 250 mg/kg body weight, respectively. LM50 and LM250 indicate methanol extract given at doses of 50 and 250 mg/kg body weight, respectively.

The effect of the extracts of *L hyssiopifolia* Linn on acetic acid-induced writhing was compared to that of aminopyrine. Significant analgesic effect of all extracts tested was observed (**Table-2**). The LH showed statistically significant ($p < 0.001$) inhibition (77.62%) of acetic acid-induced writhing, which was comparable to that of standard drug aminopyrine. The extracts LE and LM exhibited a moderate level of inhibition of writhing reflex by 38.57% ($p < 0.001$) and 34.29% ($p < 0.01$), respectively. Acetic acid (0.7%) as a pain stimulus produces localized inflammation by releasing arachidonic acid from membrane phospholipids through the action of phospholipase A2 and other acyl hydrolases[21]. The released arachidonic acid is metabolized by COX enzymes to produce PGs, especially PGE2, which produces pain sensation[22]. Administration of the acetic acid stimulates this peripheral pain perception and induces writhing reflexes. Compounds capable of reducing the number of writhing can thus function as analgesic agents probably by inhibiting the prostaglandin synthesis.

Since the plant *Ludwigia hyssopifolia* Linn has a traditional use as diuretic, the effect of LH, LE and LM on the urine volume was investigated in *Swiss albino* mice. The result of the experiment (**Table 3**) revealed that the diuretic activities of LE and LM at a dose of 250 mg/kg body weight were comparable to that of the standard drug furosemide at a dose of 3 mg/kg body weight. All the three extracts LH, LE and LM at a dose of 250 mg/kg body weight per oral showed maximum diuretic activity at the third hour of study. The diuretic activity[14] of a drug is considered to be good if it is above 1.50, moderate if it is within 1.00 ~1.50, little if it is between 0.72~1.00. A value less than 0.72 indicates lack of diuretic activity. As shown in **Figure 2**, LH showed no diuretic activity until 2 h of its administration, although it gave good diuretic activity (1.91) at the third hour indicating its delayed onset of action. LE and LM were found to cause diuresis at the first hour of the study. Thus, the onset of diuretic activity of LE and LM at an oral dose of 250 mg/kg body weight was about one hour, which was similar to that of the standard drug furosemide given at a dose of 3 mg/kg body weight per oral. All the three extracts tested showed good diuretic activity even after 4th hour of their administration. Though LH, LE and LM appeared to cause marked diuresis, the actual mode of action is unclear. Since the increase in loop permeability, inhibition of antidiuretic hormone secretion, or inhibition of the activity of carbonic anhydrase enzyme are the well-established mechanisms of diuresis[23], it would be worthwhile to examine the effects of LH, LE and LM on these biochemical parameters.

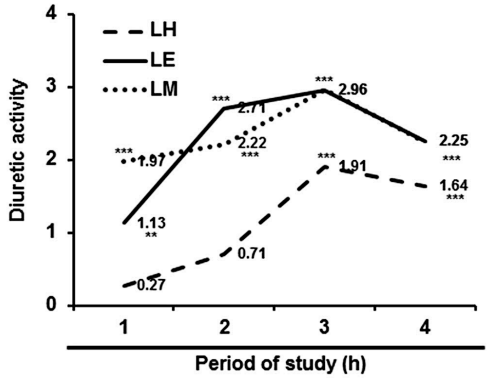

Figure 2: The diuretic activity of different extracts of *L hyssopifolia*. The diuretic activity of LH, LE and LM given at a dose of 250 mg/kg body weight was calculated from the urine volume data as described in Materials and methods. According to method of Gujral *et al* (1955), the diuretic activity is considered as good (***) if the value is greater than 1.50, moderate (**) if the value ranges between 1.00 to 1.50, mild (*) if the value falls between 0.72 to 1.00. A value less than 0.72 indicates no diuretic activity.

Table 1: Anti-inflammatory activity of different extractives of *L hyssopifolia* in rats

Test Material	Paw Volumes (ml ×1000)* (Percent Inhibition)				
	1st h	2nd h	3rd h	4th h	24th h
LH (50 mg/kg)	75.22 ± 1.34 (5.31)	90.31 ± 1.91 (3.33)	100.26 ± 2.01 (6.63)	108.78 ± 1.06 (5.67)	65.33 ± 2.31 (4.96)
LH (250 mg/kg)	55.92 ± 1.44 (29.60) [a]	61.68±1.60 (33.96) [a]	73.18 ±1.88 (31.84) [a]	86.52 ±1.24 (24.97) [a]	58.24 ±1.09 (15.27) [b]
LE (50 mg/kg)	74.13 ± 1.27 (6.68)	88.22 ± 1.84 (5.55)	90.14 ± 2.21 (16.05)	101.66 ± 1.38 (11.85)	63.48 ± 1.91 (7.65)
LE (250 mg/kg)	63.32 ±1.89 (20.29) [a]	70.04 ±1.63 (25.01) [a]	77.96 ±1.94 (27.39) [a]	89.76 ±1.03 (22.16) [a]	62.32 ±1.64 (9.33)
LM (50 mg/kg)	78.88 ± 1.35 (0.70)	91.25 ± 1.66 (2.30)	106.12 ± 1.21 (1.17)	114.54 ± 1.62 (0.67)	66.23 ± 1.89 (3.65)
LM (250 mg/kg)	68.54 ±1.20 (13.72)	76.50 ±1.37 (18.09) [a]	90.60 ±2.20 (15.63)	101.4 ±1.40 (12.07)	63.88 ±1.06 (7.07)
Phenylbutazone (100 mg/kg)	57.28 ±1.67 (27.89) [a]	58.28 ±1.21 (37.60) [a]	64.56 ±1.56 (39.87) [a]	81.12 ±2.04 (29.65) [a]	55.12 ±1.79 (19.81) [b]
Control (saline 10 ml/kg)	79.44 ±2.80	93.40 ±2.05	107.38 ±1.86	115.32 ±3.07	68.74 ±2.57

**Data are presented as Mean ± SE; Figures in parentheses indicate percent inhibition of paw edema; [a]p <0.001 and [b]p <0.01 as compared to control. All values are means ± SEM of data obtained from five rats in each group.*

Table 2: Effects of different extractives of *L hyssopifolia* on acetic acid-induced writhing reflex in Swiss albino mice

Treatment	Dose (mg/kg)	*Number of writhing (Mean ± S.E.M.)	% Inhibition of writhing reflex
LH	250	04.63 ± 0.60[a]	77.72
LE	250	12.88 ± 1.04 [a]	37.96
LM	250	13.50 ± 2.62 [a]	34.94
Aminopyrine	50	03.50 ± 0.47 [a]	83.14
Control	----	20.75 ± 0.73	------

*Six animals per group. LH, LE and LM indicate hexane, ethylacetate and methanol extract of L. hyssopifolia, respectively; 0.7% (v/v) acetic acid (0.1ml/10g body weight) was given intraperitoneally; the number of writhing induced by acetic acid was counted for 10 min; *Values are mean ± SEM; [a]p < 0.001*

Table 3: Effects of *L hyssopifolia* extractives on urine volume in *Swiss albino* mice

Treatment	Dose	Volume (ml) of urine at different time intervals			
	mg/kg bw	Period of study (hours)			
		+1	+2	+3	+4
Control	-	0.00	0.00	1.75	2.00
Urea	500	1.75	2.00	2.00	3.50
Furosemide	3	3.8	6.9	9.2	9.5
LH	250	0.50	1.50	4.00	6.00
LE	250	2.00	5.50	6.00	8.00
LM	250	3.50	4.50	6.00	8.00

In conclusion, the present study demonstrates the preliminary pharmacological activity of the n-hexane-, ethylacetate- and methanol-extract of *L hyssopifolia*. Although this is the first time report that LH, LE and LM inhibit inflammation and pain perception and induce diuresis in animal models, the bioactivity-guided isolation of principal bioactive constituent(s) and the evaluation of their biochemical, toxicological and pharmacokinetic studies merit further investigation.

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