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Analgesic effects of ethanolic leaf extract of *Ludwigia adscendens* (L.) H. Hara over selective drugs in *in vivo*

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ABSTRACT

Ludwigia adscendens (L.) H. Hara is commonly referred as weed which mainly grow in paddy field in the Asia, Australia and Africa. A number of antioxidants present in this species and have a large therapeutic background. To evaluate the analgesic activity of the ethanolic extract of Ludwigia adscendens (L.) H.Hara leaf on Swiss-albino mice (as experimental model) through Acetic Acid Induced Writhing Method. Acute toxicity experiment of the ethanolic extract of Ludwigia adscendens (L.) H.Hara leaf were performed whereby dose of 250 mg/ kg and 500 mg/kg was selected in this present study. Analgesic activity was observed using acetic acid induced writhing pain in mice. Ludwigia adscendens (L.) H. Hara showed Significant (P<0.05) in Extract (250 mg/kg) and (P<0.001) in Extract (500 mg/kg) in this experimental model. These studies conclude that Ludwigia adscendens (L.) H.Hara possesses positive analgesic properties which might be due to presence of antioxidants like flavonoids, phenolics and polysaccharides. The results provide a scientific basis for the centuries-old usages of aqueous extracts of this medicinal plant. HPLC and NMR might help us to specify different biochemical components, responsible for the therapeutic properties precisely.

Key Words: *Analgesic activity, Ethanolic leaf extract, Diclofenac Na, Phyto-therapeutics and Ludwigia adscendens (L.) H.Hara*

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I. Introduction

Ludwigia species is one of the most aggressive invaders among world plants (Cronk and Fuller, 1995) *Ludwigia species* consider as a natural medicine source historically to treat heal dermatitis, ulcer, impetigo, boil, pimple and gastrointestinal disorders like diarrhea and dysentery (Dutartre *et al.*, 2004). These herbal medicines are investigated with strict rules and must be approved by health organization of developed counties around the world (WHO, 1991). Pain is usually called as stressful

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sensation of a part of body (WHO, 2005). International Organization for the Study of Pain describes it as "an unpleasant sensory and emotional experience related with real or probable tissue injury" (Merskey and Bogduk, 1994) Analgesia or pain is an unpleasant sensation, usually evoked by external or internal noxious stimulus. Excessive pain may be unbearable and causes other effects – sinking sensation, apprehension, sweating, nausea, palpitation, rise or fall in BP (Amrita *et al.*, 2013). Scientists believe that pain evolved in animal kingdom as a valuable 3-part warning system -It warns of injury, Pain protects against further injury by causing a reflexive withdrawal from the source of injury and pain leads to a period of reduced activity, enabling injuries to heal more efficiently (Tripathi, 2001). Analgesic is an agent that reduces or eliminates pain by acting on the sensory nervous system, either centrally or peripherally without significantly altering consciousness. Analgesics are divided into two groups as Opioid analgesics: these are strong analgesics & depress CNS (e.g., Morphine, heroine, codeine) and Non-opioid analgesics: these are weaker analgesics (e.g., Aspirin, Paracetamol) (Brennan, 1984). Analgesic activity of drug or any test sample at different steps of pharmacological investigation can be assessed by different methods. The methods are acetic acid induced writhing method, radiant heat method, formaldehyde induced writhing method, hot plate method, paw-withdrawal test in rats, hot-water tail immersion in mice and tail-flick test in rats (Ganeshpurkar and Rai, 2013).

Observing all the aforementioned facts, the research work actually dealt with the objectives such as the experimental analysis of the analgesic effects of ethanolic leaf extract of *Ludwigia adscendens* (L.) H. Hara through adopted acetic acid induced writhing method in mice and the comparative analgesic effects study of the leaf extract with the prevalent drug Diclofenac Na as standard.

II. Materials and Methods

The whole plant of *Ludwigia adscendens* (L.) H. Hara was collected from Jhenidha area, Bangladesh on January, 2016 and was identified by experts of Bangladesh National Herbarium (BNH), Mirpur, Dhaka as indicated by voucher specimen no.: DACB 38276.

Preparation of extract: The collected plant parts were separated from undesirable materials or plants or plant parts and were washed with water. They were sun-dried for one week. The plant parts were grinded into coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced. About 450 gm of powered material was taken in a clean, flat-bottomed glass container and soaked in 1600 ml ethanol. The container with its contents was sealed and kept for a period of 8 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton. Then it was filtered through whatman filter paper. The filtrate (Ethanol extract) obtained was evaporated under the air of ceiling fan and in a water bath until dried. After drying the filtrate, dried adhesive powdered mass was obtained. This powdered mass was designated as crude extract. 10.72 gm free flowing crude extract was obtained from 450gm of dried powder material.

Experimental animal: Young Swiss-albino mice aged 4-5 weeks, average weight 28-35 gm were used for the experiment. The mice were purchased from the animal Research Branch of the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B). They were kept in standard environmental condition for one week in the animal house of the Pharmacy Discipline, Khulna University, Bangladesh for adaptation after their purchase. The animals were provided with standard laboratory food and tap water and maintained at natural day-night cycle. All the experiments were conducted on an isolated and noiseless condition.

Study Design: Experimental animals were randomly selected and divided into four groups denoted as Group-I, Group-II, Group-III, Group- IV consisting of 5 mice in each group. Each group received a particular treatment i.e. negative control, positive control and the two doses of the extract. Each mouse was weighed properly and the doses of the test samples and control substances were adjusted accordingly. In the case of negative control group feeding vehicle (1% tween-80) and positive control group up taking Diclofenac Na (25 mg/kg) are administrated orally. *Ludwigia adscendens* (L.) H.Hara plant extract was administrated orally in 250 mg/kg and 500 mg/kg as treatment groups.

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Acetic Acid Induced Writhing Method: The acetic acid induced writhing method is an analgesic behavioral observation assessment method that demonstrates a noxious stimulation in mice. The test consists of injecting the 0.7% acetic acid solution intraperitoneally and then observing the animal for specific contraction of body referred as 'writhing'. A comparison of writhing is made between positive control (Diclofenac Na), negative control and test sample given orally 30 minutes prior to acetic acid injection. If the sample possesses analgesic activity, the animal that received the sample will give lower number of writhing than the control, i.e. the sample having analgesic activity will inhibit writhing. Diclofenac Na is used as reference standard drug. It has analgesic, antipyretic and anti-inflammatory actions at different steps of pharmacological investigation with mild adverse effects. So the drug is used widely.

Analgesic activity: Test samples, positive and negative control solution were given orally by means of a feeding needle. A thirty minutes' interval was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, acetic acid solution (0.7%) was administered intra-peritoneally to each of the animals of each group. After an interval of 5 minutes, which was given for absorption of acetic acid, number of squirms (writhing) was counted for 15 minutes.

III. Results and Discussion

Each mouse of all groups was observed carefully to count the number of writhing that they had made in 15 minutes. The animal does not always perform full writhing, because sometimes the animals begin to produce writhing but they do not complete it. This incomplete writhing was taken as half-writhing, so two half-writhing were taken as one full writhing.

The results of the test showed that ethanolic extract of leaves of *L. adscendens* (L.) H.Hara. at dose of 250 mg/kg and 500 mg/kg exhibit significant inhibition of writhing reflex by 50.77% and 46.92% respectively while the standard drug Diclofenac Na inhibition was found to be 41.53% at a dose of 25 mg/kg body weight**.** The results of statistical analysis are Control Vs Diclofenac Sodium: Significant ($P<0.001$), Control Vs Extract (250 mg/kg): Significant ($P<0.05$) and Control Vs Extract (500 mg/kg): Significant ($P<0.001$).

Table 01. Effects of the crude extract of *Ludwigia adscendensm*(L.) H.Hara. leaves at the doses of 250 and 500 mg/kg-body weight on acetic acid induced writhing of mice

Administered dose to mice Group	Numbering of mice	Weight (gm) of mice	Dose (ml)	Writhing of mice	Total Writhing
Negative control	1	24	0.24	22	130
	2	24	0.24	25	
	3	23	0.23	24	
	4	26	0.26	29	
	5	28	0.28	30	
Positive control Diclofenac Na (25 mg/kg b.w)	1	26	0.26	17	76
	2	29	0.29	16	
	3	29	0.29	14	
	4	28	0.28	13	
	5	29	0.29	16	
Extract (250 mg/kg b.w)	1	26	0.26	8	64
	2	32	0.32	13	
	3	28	0.28	12	
	4	26	0.26	15	
	5	30	0.30	16	
Extract (500 mg/kg b.w)	1	30	0.30	16	69
	2	28	0.28	14	
	3	28	0.28	14	
	4	26	0.26	12	
	5	25	0.25	13	

Legend: b.w- body weight

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So it can be claimed that the analgesic activity of the ethanolic extract of leaves of *L. adscendens* (L.) H.Hara. was significant in comparison with control animals as the extract, at the doses of 250 and 500 mg/kg body weight showed significant decrease in acetic acid induced writhing reflex of mice (Table 01 and Table 02).

The present study was conducted to assess the antinociceptive and anti-inflammatory properties of hydroethanolic extract of *L. adscendens* (L.) H. Hara. The method selected were chemical Diclofenac Na in the test model of acetic acid-induced writhing test (Figure 01 and Figure 02).

Table 02. Comparative study of the % inhibition of writhing between the standard drug and ethanolic leaf extract

Animal group	Mean of Writhing	% Writhing	SD	SEM	% Inhibition of writhing	T-test (value of p)
Negative control	26	100	3.391	1.517	-	-
Positive control Diclofenac Na (25mg/kg)	15.2	58.46	1.643	0.735	41.53	6.4069 P<0.001
Extract (250 mg/kg)	12.8	49.23	3.114	1.392	50.77	6.4113 P<0.05
Extract (500 mg/kg)	13.8	53.06	1.483	0.663	46.92	7.369 P<0.001

Legends: SD = Standard Deviation, SE = Standard Error

The writhing test is a relatively sensitive method for first stage evaluation of analgesic activity and the ED50 values obtained in animals using this test can be compared with the analgesic doses in humans Collier *et al.* (1968), but still indication whether the effects result from central and/or peripheral actions is unpredictable.

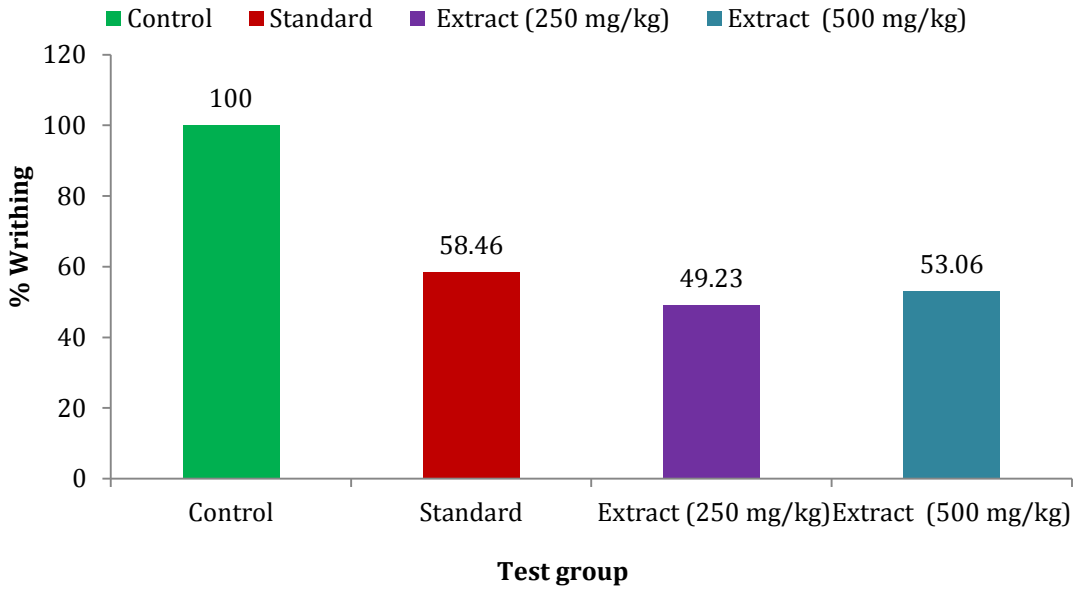

Figure 01. % Writhing of the standard drug (Diclofenac Na) & extract of *L. adscendens* (L.) H. Hara on acetic acid induced writhing in mice.

The potential analgesic effect of the extract was shown by acetic acid test to be significant but that was not specific (Godoy *et al.*, 2004). *Ludwigia* species are known to contain flavonoids, phenolics, polysaccharides and polysaccharopeptides which has been documented to prepare therapeutic properties (Butcher *et al.*, 2009). Ehtanolic leaf extract of *L. adscendens* (L.) H. Hara showed excellent analgesic activity in mice (Figure 01 and Figure 02). Further research should carry on isolation and identification of the active compounds that can provide a suitable source to develop new therapeutic agents.

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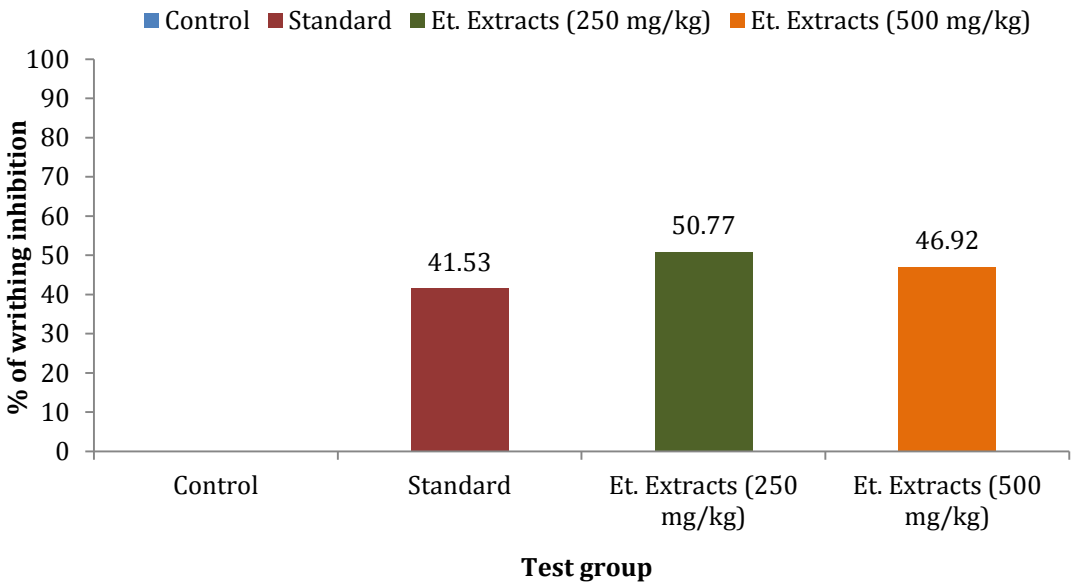

Figure 02. % Writhing inhibition of acetic acid induced writhing in mice by the standard drug (Diclofenac Na) & leaves of *L. adscendens* (L.) H. Hara.

IV. Conclusion

The findings of the research are transparent enough to mention that *L. adscendens* (L.) H. Hara has positive analgesic effects *in vivo*. Finally, HPLC will help us further, providing the information of the specific chemical groups responsible for the activities precisely.

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V. References

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MLA (Modern Language Association)

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