

Analgesic activity of *Leea indica* (Burm. f.) Merr.

Talha Bin Emran^{1*}, Md. Atiar Rahman², S.M. Zahid Hosen¹, Md. Mominur Rahman³, Abu Mohammad Toufiqul Islam³, Md. Ashraf Uddin Chowdhury³, Muhammad Erfan Uddin³

¹Department of Pharmacy, BGC Trust University Bangladesh.

²Department of Biochemistry and Molecular Biology, University of Chittagong, Chittagong-4331, Bangladesh

³Department of Pharmacy, International Islamic University Chittagong (IIUC), Bangladesh

*Corresponding Author: talhabmb@gmail.com

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Abstract

The analgesic potential of *Leea indica* (Burm. f.) Merr., a Bangladeshi tribal medicinal plant was studied for the first time. Despite the progress that has occurred in recent years in the development of therapy, there is still a need for effective and potent analgesics, especially for the treatment of chronic pain. One of the most important analgesic drugs employed in clinical practice today continues to be the alkaloid morphine. Analgesic potential of *L. indica* was evaluated for centrally acting analgesic property using formalin induced licking response model and peripheral pharmacological actions using acetic acid-induced writhing test. In acetic acid-induced writhing test, ethanolic extracts at 200 mg/kg dose exhibited significant ($p < 0.05$) reduction of writhing response in a dose dependent manner; in formalin induced licking response model a significant ($p < 0.05 - 0.001$) result was comparable to the standard drug diclofenac sodium. From the results it was concluded that both extracts exhibited anti-nociceptive activity by central and peripheral mechanism(s). Plant-derived substances have, and will certainly continue to have, a relevant place in the process of drug discovery, particularly in the development of new analgesic drugs.

Keywords Analgesic; Antinociceptive; *Leea indica* (Burm. f.) Merr.; writhing;

Introduction

Medicinal herbs have been used as a form of therapy for the relief of pain throughout history (Almeida *et al.*, 2001). The treatment of rheumatic disorder is an area in which the practitioners of traditional medicine enjoy patronage and success (Akah and Nwambie, 1994). Natural products in general and medicinal plants in particular, are believed to be an important source of new chemical substances with potential therapeutic efficacy. Taking into account the most important analgesic prototypes (e.g. salicylic acid and morphine) were originally derived from the plant sources, the study of plant species traditionally used as pain

killers should still be seen as a fruitful research strategy in the search of new analgesic and anti-inflammatory drugs.

Leea indica (Burm. f.) Merr. (Family Leeaceae) is a large evergreen shrub or small tree (Yusuf *et al.*, 1994; Ghani, 2003; Rahman, 2010) indigenous to tropical Asia, Australasia, and the Pacific and grown mostly in Bangladesh, India, China, Bhutan, and Malaysia. Plant pacifies vitiated pitta, diarrhea, dysentery, colic, ulcers, skin diseases, vertigo, and headache. Marma of Chittagong Hill Tracts, Bangladesh, prescribes combined root paste of this plant along with the root of *Oreocnide integrifolia* and *Cissus repens* for bubo and boils (Zhang, 1699; Kirtikar *et al.*, 1998).

Acetic acid is a pain stimulatory agent. Intraperitoneal administration of acetic acid (0.7 %) causes the release of free arachidonic acid from tissue phospholipid by the action of phospholipase A₂ and other acyl hydrolases. There are three major pathways in the synthesis of the eicosanoids from arachidonic acid. All the eicosanoids with ring structures that is the prostaglandins, thromboxanes and prostacyclins are synthesized via the cyclooxygenase pathway (Hossain *et al.*, 2009). The leucotrienes, HETE (hydroxyeicosatetraenoic acids) and HPETE (hydroperoxy eicosatetraenoic acids) are hydroxylated derivatives of straight-chain fatty acids and are synthesized via the lipoxygenase pathway (Adedapao *et al.*, 2009). The released prostaglandins, mainly prostacyclin (PGI₂) and prostaglandin-E have been reported to be responsible for pain sensation by exciting the A δ -fibres. Activity in the A δ -fibres cause a sensation of sharp well localized pain, (Yerima *et al.*, 2009).

The acetic acid induced writhing method (Whittle BA-1964). is an analgesic behavioral observation assessment method that demonstrates a noxious stimulation in mice. The test consists of injecting 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) 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 (Kouadio *et al.*, 2000). It has analgesic, antipyretic and anti inflammatory actions.

Anxiety disorders are the most common emotional disorders affecting people in all countries worldwide. It is reported that more than 20% of the adult population suffer from these conditions at some stage during their life (Abid *et al.*, 2006; Wattanathorn *et al.*, 2007). Anxiety is a natural emotion but becomes a problem when it occurs too often. According to the U.S. National Institute of Mental Health (NIMH), anxiety disorders can be related to other mental/emotional disorders, including depression and traumatic events.

Materials and method

Drugs and chemicals

The following drugs and chemicals were used in this study: Diclofenac sodium (Square Pharmaceutical Ltd., Bangladesh), Tween-80 and acetic acid (Merck, Germany), ethanol (Merck, Germany).

Plant materials

Plant material was collected from University of Chittagong campus in 2011. The plant was taxonomically identified by Dr. Shaikh Bokhtear Uddin, Associate professor, Department of Botany, University of Chittagong. The plant leaves were thoroughly washed with water and were dried in a hot air oven at room temperature for 7 days and at 40°C for the next 2 days.

Preparation of plant extract

The collected plant were washed thoroughly water, chopped, air dried for a week at 35-40°C. The powder obtained was successively extracted in ethanol (55-60°C). The extracts were made to dry by using rotary evaporator (Bibby RE200, Sterlin Ltd., England) under reduced pressure.

Animals

White female albino mice (Swiss-webstar strain, 25-35 g body weight) were collected from the animal research branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR). 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.

Acetic acid-induced writhing test

Study design

Experimental animals were randomly selected and divided into three groups denoted as group-I, group-II and group-III consisting of 5 mice in each group. Each group received a particular treatment i.e. control, positive control and a dose of the *L. indica* extract (Test group). Each animal was weighed properly and the dose of the test samples and control materials were adjusted accordingly.

Methodology

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 200 mg/kg orally, vehicle (1% Tween-80 in water, p.o) and diclofenac sodium (40 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, mice were observed and the number of writhings were counted for 15 min. Reduction in the number of writhes compared to the control groups was considered as evidence of analgesic effect (Figure 1). The percent inhibition (% analgesic activity) was calculated as percent inhibition using following equation:

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

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

Formalin induced licking response model

Study design

Experimental animals were randomly selected and divided into three groups denoted as group-I, group-II and group-III consisting of 5 mice in each group. Each group received a particular treatment i.e. control, positive control and a dose of the *L. indica* extract (Test group). Each animal was weighed properly and the dose of the test samples and control materials were adjusted accordingly.

Methodology

Formalin induced pain model was performed according to the method described by Sharma *et al.*, (2010). 20µl of 1.0% v/v 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 (200 mg/kg, orally) and diclofenac sodium (0.5 mg/kg, i.p) were administered 30 min prior to formalin injection. Control animals received 10 ml/kg of distilled water, orally.

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

Acetic acid-induced writhing test

Figure 1 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 induced licking response model

Ethanol extract of *L. indica* (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 8.182% inhibition whereas 66.446 % inhibition was obtained for diclofenac sodium against pain. The results were dose dependent and statistically significant (Figure 2).

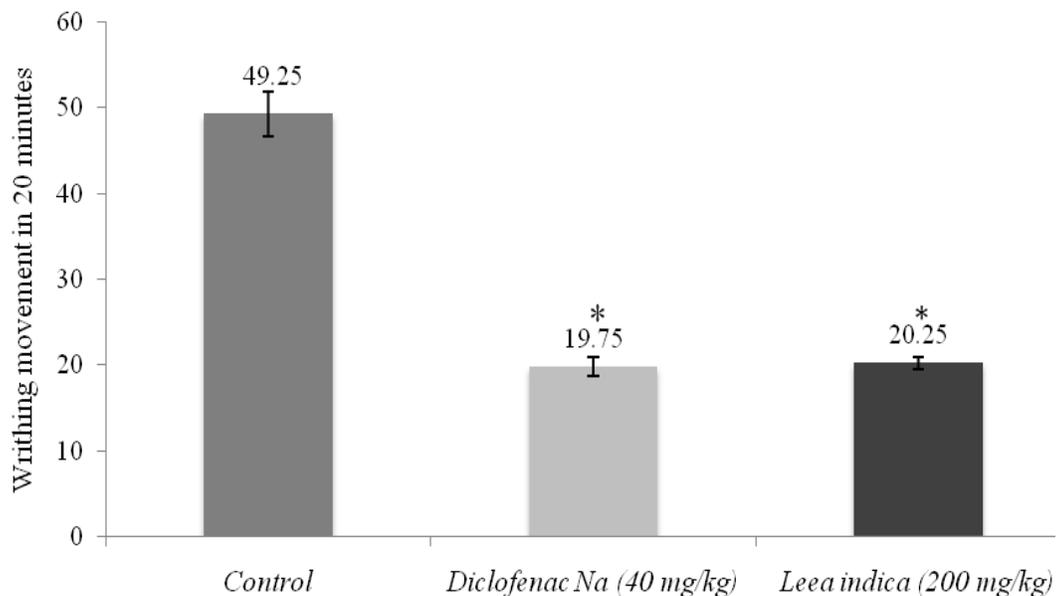


Figure 1. Effect of *Leea indica* extract on acetic acid induced writhing response. *P<0.05 refers to significant compared to control and ** P < 0.01 significant compared to Positive control.

Discussion

The ethanol extract was evaluated in the 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). Intraperitoneal administration of acetic acid releases prostaglandins and sympathomimetic mediators like PGE₂ and PGF_{2α} and their levels increase in the peritoneal fluid of the acetic acid induced mice (Deraedt R *et al.*, 1980). The

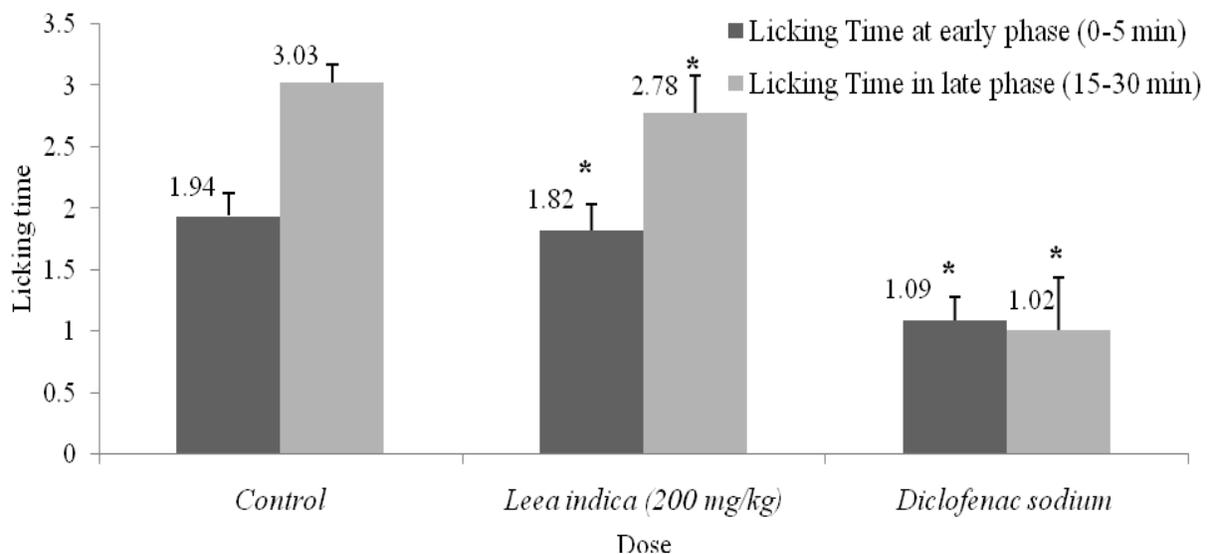


Figure 2. Effect of the ethanolic extract of *L. indica* on hind paw licking in the formalin test in mice. Values are mean ± S.E.M., (n = 5); * p < 0.05, ** p < 0.01, Dunnet test as compared to control.

abdominal constrictions produced after administration of acetic acid is related to sensitization of nociceptive receptors to prostaglandins. It is therefore possible that the extract exerts its analgesic effect by inhibiting the synthesis or action of prostaglandins which may be due to phytochemicals present in the extract. Thermally induced nociception indicates narcotic involvement (Besra SE *et al.*, 1996). The centrally acting analgesics generally elevate the pain threshold of mice towards heat. The extract significantly delayed the response time to thermal pain sensation in tail flick method indicating narcotic involvements. Moreover, since the extract inhibited both peripheral and central mechanisms of pain, it is possible that the extract acted on opioid receptor (Elisabetsky E *et al.*, 1995; Pal S *et al.*, 1999). 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 (Hunnskaar *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 (Hunnskaar & 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.

Results of the study demonstrated that ethanol extract of *L. indica* leaf exerts potential analgesic effect in experimental animal models. which support the claims by traditional medicine practitioners. On the basis of the results, it can be used as a good source of analgesic drugs. However, pharmacodynamic studies should be undertaken to establish the mechanism of action of the plant extracts contributing in nociception. Phytochemical investigation is also proposed in order to isolate the active fraction and eventually the pure compound.

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Conflict of interest

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

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