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Research Article

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In vivo evaluation of analgesic activity of methanolic extract of *Laportea interrupea* (L.) leaves

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ABSTRACT

The study was carried out to evaluate analgesic activity of methanolic extract of Laportea interrupea (L.). For evaluation of analgesic activity acetic acid induced writing test and tail immersion test were performed in Swiss albino mice. The leaves extract were administered orally at different doses and the obtained effects were compared with commercially available analgesic and anti-inflammatory drug paracetamol and acetyl salicylic acid (ASA) respectively. In analgesic bioassay the leaves extract significantly reduced the writhing induced by acetic acid as well as increase pain reaction time (PRT) in tail immersion test. The degree of inhibition of writing of the leaves extract were10.98%, 23.30% and 50.75 % when doses were 25mg/kg, 50mg/kg and 100mk/kg respectively, compared to the effect of standard analgesic drug, paracetamol was 61.17% at a dose 65mg/kg. In tail immersion test, PRT was increased from 2.57±0.05 to 4.52±0.08 with increasing doses from 200mg/kg to 800mg/kg and time from 60 minutes to 120 minutes compared to standard drug, ASA were 4.29±0.18 and 5.68±0.43 at time 60 ,120 minutes respectively at a dose 200mg/kg. In both of the test 0.9 % NaCl was used as control. In conclusion, the experimental data demonstrated that methanolic extract of L. interrupea leaves possess remarkable analgesic activity.

Keywords: *Laportea interrupea*, analgesic activity, Swiss albino mice, NSAIDS.

INTRODUCTION

Inflammation is a complex biological response of living tissue to harmful stimuli such as invasion by an infectious agent, antigen challenge or even just physical, chemical or traumatic damage that leads to local accumulation of plasmatic fluid and blood cells. The classical signs of acute inflammation are pain, heat, redness, swelling, and loss of function. The result of each inflammatory reaction may be beneficial or harmful .The beneficial effect of inflammation is, it acts as a defense mechanism that helps body to protect itself against infection, burns, toxic chemicals, allergens or other noxious stimuli, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases [1]. Damage of surrounding tissues which leads to pain is the harmful effect of inflammation. Pain has been defined by International Association for the Study of Pain (IASP) as an unpleasant sensory and emotional experience associated with actual or potential tissue damage [2].The drugs which are used presently for the management of pain and inflammatory conditions are either narcotics or non narcotics (NSAIDS), and have known toxic and lethal effects.[3] One study suggests that risk of gastrointestinal bleeding was significantly associated with acute use of non-steroidal anti-inflammatory drugs (NSAIDs) like regular-dose aspirin, diclofenac, ketorolac, naproxen or nimesulide. On the contrary, herbal medicines with good absorption, less toxicity, and easy availability have been used since ancient times.[4] It is therefore, essential that efforts be made to introduce new medicinal plants, to develop cheaper and effective drugs.[5] Plants represent a large natural source of useful compounds that might serve as lead for the development of novel drugs.

Laportea interrupea is annual herb which is belongs to the family Urticaceae. The plant is somewhat branched, about 0.5 to 1.5 meters high. Stems are green and succulent; the vegetative parts with scattered, stinging, and spreading hairs, leaves are ovate, 5-15 cm long. From the literature review it was shown that *L. interrupea* use as medicine to cure fever, [6] and also used as diuretic and anthelmintic [7].There is some local area in Bangladesh where *L. interrupea* uses to remove muscle pain. We know that inflammatory reaction in tissues and vessels one the prominent cause of fever. These clues inspire to perform the research activities.

EXPERIMENTAL SCETION

Collection and identification

The leaves of *L. interrupea* was collected from Hathazari, Chittagong, Bangladesh and authenticated by Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh. These are sun dried and finally dried in oven. The leaves are grinded into coarse powder and stored in an air tight container for further use.

Preparation of plant extract

250grams powder of plant material was taken and soaked in 750 ml of methanol for 6 days. It was shaking periodically and filtered by cotton and filter paper respectively. The filtrate was concentrated to small volume removing entire methanol by using rotary evaporator. Then the thick, gummy extract was stored in a refrigerator for studies.

Procurement of animals and its care

Swiss albino mice of 5-7 weeks old, weighing 25-30 gm were collected from International Centre for Diarrheal Disease Research, Bangladesh (ICDDR'B) Mohakhali, Dhaka. Mice were kept in iron cages with saw dust and straw bedding which was changed once a week regularly.

Standard mouse diet (recommended and prepared by ICDDR'B) and water were given in adequate.

Study of Analgesic activities

Acetic acid-induced writhing test for analgesic activity

This was based on the method described by koster et al.,(1959) [8]. Swiss albino mice of either sex were selected and divided into five groups of five animals each. The writhes were induced by intraperitoneal injection of 0.6% acetic acid (v/v) (80 mg/kg). Three different doses (25mg/kg, 50mg/kg and 100 mg/kg) of plant extract were administered orally to groups of five animals each, 30 minutes before intraperitoneal injection of acetic acid. Vehicle (0.9 %, 0.2 ml NaCl) was introduced orally and paracetamol (65mg/kg) was introduced by intraperitoneal injection, where vehicle and paracetamol were used as control and standard drug respectively. The numbers of muscular contractions were counted over a period of 20 minutes after acetic acid injection. Reduction in the numbers of writhes was compared to the control group was considered as evidence of analgesic effect. The percent of inhibition (% analgesic activity) was calculated by the following formula.

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

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

Tail immersion test for analgesic activity

The procedure was based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice [9]. Swiss albino mice of either sex were selected and divided into five groups of five animals each. Difference concentration of methanolic extract of *L. interrupea* (200mg/kg, 400mg/kg, 800mg/kg) and vehicle (0.9% NaCl) were administered orally while acetyl salicylic acid was introduced by intraperitoneal injection which acts as standard drug for the experiment. The distal part of the tails of the animals was immersed in hot water maintained at 55.0 ± 1.0 °C. The time taken to withdraw the tail known as the pain reaction time (PRT) was recorded [10]. A cut-off time of 10 s was maintained at 55 °C to prevent tissue damage. The reaction time was measured at 0, 60, 90, and 120 min after treatment, respectively.

Statistical Analysis

All the results obtained by in vitro experiment were expressed as mean ± STD of five measurements.

RESULTS

Acetic acid-induced writhing reflex

The observation demonstrates that the methanolic extract of *L. interrupea* inhibit acetic acid-induced writhing significantly. The results of analgesic effects are presented in table 1. It showed that the plant extract reduced the mean number of writing, in other word percentage of inhibition of writing in a dose dependent manner. The percentage of inhibition increased from 10.98 to 50.75 while dose was increased from 25mg/kg to 100mg/kg. paracetamol was used as standard drug with a dose 65 mg/kg, showed percentage of inhibition is 61.17. There was little difference between the group that was given 100 mg/kg and those treated with the reference drug paracetamol (65mg/kg). Comparison among the doses and standard drug was represented in fig. 1.

Tail immersion response

The result of tail immersion response showed that the mean pain reaction time (PRT) increased with the increase of doses. It also showed that PRT increased significantly at a time 60 minutes and 90 minutes after the administration of extract which are presented in table 2. At time 60 minutes, PRT was 2.57± 0.05 when dose was 200mg/kg. PRT increased with time as well as concentration of plant extract and showed PRT at time 120 minutes with a dose 800mg/kg was 4.52± 0.08. In this experiment as a standard reference drug acetyl salicylic acid (ASA) was used with a dose 200mg/kg and its PRT was increased from 4.29±0.18 to 5.68±0.43 at time 60minutes and 120 minutes respectively. Effect of plant extracts as well as standard (ASA) has been represented in fig. 2.

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Table 1: Effect of the methanolic extract of *L. interrupea* (MELI) on acetic acid induced writhing of test mice

Group	Dose (mg/kg)	Frequency of writhing (Mean±SD)	% of inhibition
Control (.9% NaCl)	-	52.8± 0.5	
Standard (paracetamol)	65	20.5± 0.5	61.17
MELI	25	47± 2.5	10.98
	50	40.5± 1.5	23.30
	100	26± 1.5	50.75

Table 2: Effect of MELI on tail immersion method of analgesic activity test in mice

Group	Dose mg/kg	Mean PRT in second at a time of drug administration				% of increase of PRT with respect of control		
		0	60	90	120	60	90	120
control	-	2.25± 0.21	1.86±0.43	1.60±0.25	1.15±0.24			
Std. Drug	200	2.56±0.12	4.29±0.18	5.53±0.34	5.68±0.43	56.64	71.06	81.18
MELI	200	2.15±0.09	2.57±0.05	2.97±0.31	2.90±0.18	27.62	46.12	60.34
	400	1.95±0.15	3.18±0.12	3.56±0.20	3.86±0.28	41.50	55.05	70.20
	800	1.95±0.31	4.12±0.30	4.88±0.51	4.52±0.08	54.85	67.21	74.55

Figure 1: Effect of MELI of difference concentrations on acetic acid induced writing compared to standard drug (paracetamol)

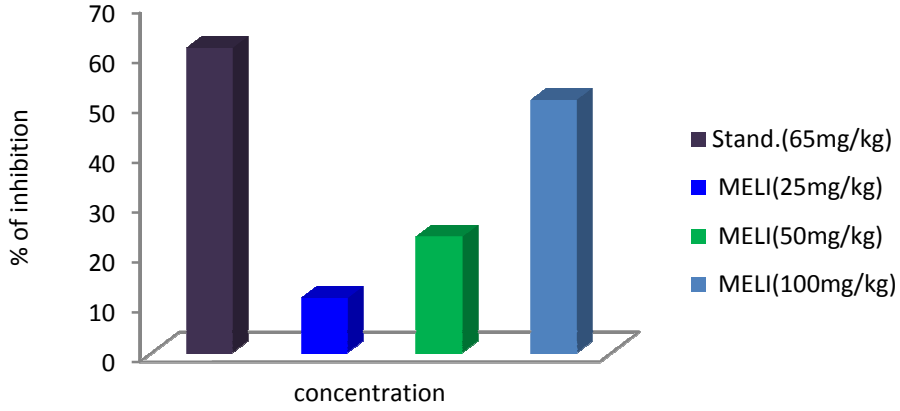
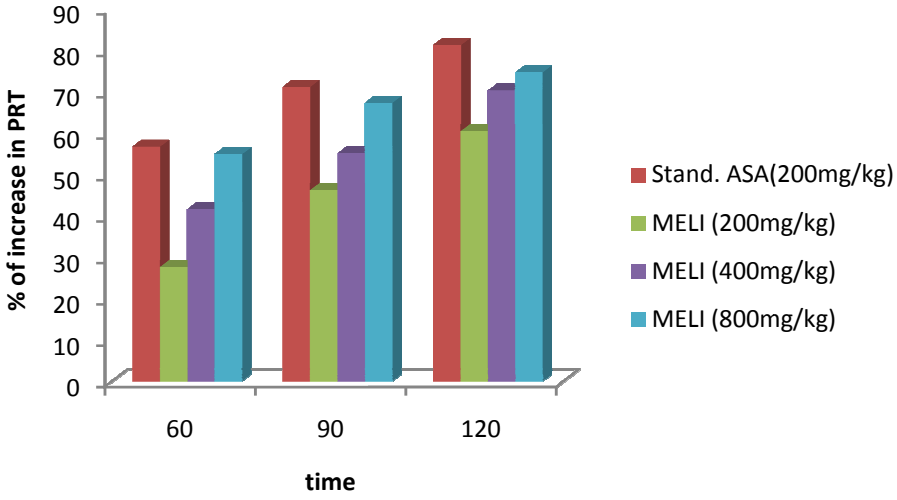


Figure 2: Effect of MELI of difference concentrations on PRT with time compared to standard drug (ASA)



DISCUSSION

It was found that prostaglandins injected into the peritoneal cavity of mice elicited a writhing response and from the response it can be suggested that prostaglandins could be one of the pain mediators released by a noxious stimulus [11] Prostaglandins produced pain in human volunteers when injected subdermally in high doses and the main feature of its action was to produce a long-lasting hyperalgesia i.e. a sensitization to mechanical or to other chemical stimuli. It can be postulated that the analgesic activity of aspirin-like drugs was indeed due to prevention of biosynthesis of prostaglandins inhibiting the cyclooxygenase (COX) enzymes, either COX-1 or COX-2 or both, thus removing this sensitizing effect [12]. On the other hand opioid analgesics relieve pain by acting directly on the central nervous system. Opioids are unique in that they not only block the incoming nociceptive signals to the brain but also act at higher brain centers, controlling the affective components of the pain. Opiod receptors are mainly three types such as mu (μ), delta (δ), kappa (κ) and most clinically used opioid drugs are mu (μ), opioid receptor agonists. A few, however, are kappa agonists [13].

The methanolic extract of *L. interrupea* at the doses of 50-100 mg/kg protected mice against chemical stimulus which was evidenced from the acetic acid-induced writhing test. Acetic acid induces writhing syndromes and causes analgesia by releasing of endogenous substances, which then excite the pain nerve endings; the abdominal constriction is related to the sensitization of nociceptive receptors to prostaglandins [14]. Methanolic extract of *L. interrupea* may prevent biosynthesis of prostaglandin or may block nociceptive receptors for prostaglandin.

The extract at doses of 200-800mg/kg protected the test mice from thermal stimulus significantly which was evidenced from tail immersion tests. There are several types of ion channels in the skin that respond to temperature. They are all transmembrane proteins in the plasma membrane that open to let in both calcium ions and sodium ions and TRPV2 is one of them which become active more than 52^{o}C temperature. Activation of this channel causes pain. The experiment was performed at 55^{o}C, so it can be said that the plant extract may block TRPV2 ion channel. However exact mechanism of action not clear.

CONCLUSION

From the finding and above discussion it can be inferred that methanolic extract of *L. interrupea has* analgesic activity though the mechanism of action as analgesic is not clear. The activity was related to the dose and these results corroborate the traditional use of the plant in folk medicine to remove muscle pain. So it can be demanded that the plant is a good candidates of active compounds for analgesic therapy. As known as there are no reports on investigation to identify the active components present in *L. interrupea.* Further investigation was required for isolation, identification and characterization of different active compounds of leaves extract, their mode of action and therapeutic ranges.

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