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ANTINOCICEPTIVE AND ANTIINFLAMMATORY PROPERTIES OF THE METHANOL EXTRACT OF *URTICA CRENULATA* STEM

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The methanol extract of Urtica crenulata stem was evaluated for its antinociceptive properties on the pain induced by acetic acid and formalin in Swiss albino mice. The methanol extract of U. crenulata at a dose of 1 g/kg produced an inhibition of 32.3% on pain induced by acetic acid and at a dose of 1.5 g/kg produced an inhibition of 31.0% for that induced by formalin. The antiinflammatory activity of the same extract was estimated volumetrically by measuring the mean increase in hind paw volume of carrageenan-induced Wistar albino rat with the help of plethysmometer. Oral administration of stem extract at a dose of 1 g/kg showed no significant effect in the reduction of carrageenan-induced paw oedema. Diclofenac sodium at a dose of 40 mg/kg was used as a standard drug in the inhibition of acetic acid induced pain and carrageenan-induced paw oedema whereas morphine was used as the same in formalin-induced pain inhibition.

Keywords: *Urtica crenulata, Antinociceptive, Antiinflammatory, Paw oedema, Carrageenan*

INTRODUCTION

Inflammation is a complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants that lead to local accumulation of plasmatic fluid and blood cells. It is a protective attempt by the organism to remove the injurious stimuli as well as to initiate the healing process for the tissue (Ferrero-Miliani *et al*. 2007). In the absence of inflammation, wounds and infections are not healed and progressive destruction of the tissue compromises the survival of the organism. However, an unchecked inflammation can lead to a variety of diseases, such as hay fever, atherosclerosis, and rheumatoid arthritis. Most of the drugs used presently for the management of pain and inflammation possess some side and toxic effects (Ahmad, Khan and

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Rasheed 1992). For example, steroidal drugs usually upset the hormonal balance in different endocrine system. Salicylates, phenylbutazone, and indomethacin frequently produce ulcers and dyscrasia (Ahmad, Khan and Rasheed 1992). It is therefore inevitable to search for new, less toxic and more effective antiinflammatory and antinociceptive agents.

Urtica crenulata Roxburgh (Syn: *Laportea crenulata,* Gaud), locally known as *Agnichutra,* is an evergreen shrub (Hooker 1879; Kirtikar and Basu 1993) that is widely distributed in Bangladesh, India, Srilanka, and the Malay Islands (Kirtikar and Basu 1993; Hasan and Haque 1993). It is 3.7 m tall, semi woody with elliptic, oblong or obovate-lanceolate with rarely present rhombic leaf. The plant contains formic acid, mucilage, ammonia, carbonic acid, protein, calcium, phosphorus, iron, magnesium, and beta-carotene, along with vitamins A, C, D, and B complex. Recently a new triterpenoid 2α, 3β, 21β, 23, 28-penta hydroxyl 12-oleanene and two known compounds, beta-sitosterol and beta-sitosterol 3-beta-D-glucopyranoside have been isolated from the roots of *U. crenulata* Gaud (Khan *et al.* 2007).

It is widely used by the tribal communities of Chittagong Hill tracts in the treatment of bleeding from nose and/or mouth, excessive gas in the stomach, constipation, weakness, asthma, gout, mumps, whooping cough, and chronic fever (Bhattacharya 1990; Kirtikar and Basu 1993; Hasan and Haque 1993). The root of the plant is very helpful in the treatment of urinary tract infection and is said to reduce susceptibility to rheumatic problems and colds. The traditional use of various parts of *U. crenulata* and its different species have been documented and described (Kirtikar and Basu 1993; Hasan and Haque 1993), but very few pharmacological studies (e.g. antipyretic effect of root extract) have been performed to evaluate the scientific basis of the use of this plant (Khan *et al.* 2007). In this context, the present study aims to evaluate the antinociceptive and antiinflammatory properties of *U. crenulata* stem extract in experimental animal models.

METHODS

Collection of Plant

The stems of *U. crenulata* were collected from Chittagong Hill tracts, Bangladesh, in the month of January 2008. The plant was identified by taxonomist, Dr. Md. Yousuf, from Industrial Botany Research Division, Bangladesh Council for Scientific and Industrial Research (BCSIR) Laboratories, Chittagong. The specimen is preserved in the Bangladesh National Herbarium (plant accession no. 6159).

Preparation of Plant Extract

The fresh stems of *U. crenulata* were washed with distilled water immediately after collection. The collected stems were chopped into small pieces and air dried at room temperature for about 10 days. The pieces were then ground into powder form and stored in an airtight container. 850 g of powder was macerated in 5 L pure methanol for 7 days at room temperature with occasional stirring. After seven days, methanol extract was filtered off through a cotton plug and finally with a Whatman No. 1 filter paper. The extract was concentrated under reduced pressure at temperature below 50°C through a rotatory vacuum evaporator. The concentrated extracts were collected in a petri dish and allowed to air dry for complete evaporation of methanol. The whole process was repeated 3 times and finally 45 g of blackish–green, concentrated extract was obtained (yield 5.3%w/w), which was kept in refrigerator at 4°C.

Experimental Animals and Diets

Swiss albino mice of both sexes weighing between 25 to 30 g and Wistar albino rats of the either sex weighing between 150–200 g obtained from the animal house of BCSIR Laboratories, Chittagong were used for the present study. The animals were acclimatised to room temperature (28±5°C) with a relative humidity of 55±5% in standard wire meshed plastic cages for 4 to 5 days prior to commencement of the experiment. During the entire period of study the animals were fed with standard pellet diet and water *ad libitum.* In this study, all the animal

experimentation was carried out according to the guidelines of the Institutional Animal Ethics Committee (IAEC).

Assay for antinociceptive activity

Acetic acid induced writhing test

For writhing test, 1% (v/v) acetic acid solution (3.3 mL/kg body weight) was injected intra peritoneally to mice (weighing 25–30 g) and the number of writhing and stretching was counted over 20 minutes (Koster, Anderson and de Beer 1959). The methanol extract of *U. crenulata* (1g/kg), standard analgesic drug diclofenac sodium (40 mg/kg) and distilled water were administered orally 30 min before acetic acid injection.

Formalin test

The procedure is similar to that described previously by Gaertner, Müller and Roos (1999). 20 μL of 2.5% formalin (0.92% formaldehyde) made in phosphate buffer was injected under the right hind paw surface of the experimental mice. Each mouse was placed individually in a cage and observed from 0 to 5 min followed by the injection of formalin to analyse the first phase of formalin-induced pain (neurogenic pain). The length of time the animal spent licking the injected paw was timed with a chronometer and was considered as the indication of pain.

Assay for antiinflammatory activity

The antiinflammatory activity of *U. crenulata* stem extract was assessed using carrageenan induced paw oedema model in the hind paw of rat (Winter, Risley and Nuss 1962). Acute inflammation was induced in albino rats by subplantar injection of 0.1 mL of 1% (w/v) carrageenan after measuring the initial right hind paw volume of each rat. The volume of right hind paw was measured at 1, 2, 3 and 4 h after carrageenan injection and the paw oedema was determined using plethysmometer (7150 UCG Basil, Italy). *U. crenulata* stem extract (1 g/kg), standard antiinflammatory drug diclofenac sodium (40 mg/kg), and distilled water were administered orally to treated, positive control and control groups 1 h before the subplantar injection of carrageenan.

RESULTS AND DISCUSSION

Antinociceptive activity of *U. crenulata* stem extract was determined using the acetic acid induced writhing response model. Table 1 shows the pain behavior of writhing response, which was presented as cumulative abdominal stretching response. With the administration of 1% (v/v) acetic acid solution (3.3 mL/kg body weight) in mice, the control animal showed 79.3 writhing count/20 min. Intraperitoneal injection of diclofenac sodium caused significant reduction in writhing count, from 79.3 to 39.5 whereas *U. crenulata* stem extract made it 53.7 from 79.3. The effect of stem extract and diclofenac sodium was analysed statistically by Student's t-test. The treatment of animals with methanol stem extract of *U. crenulata* (1 gm/kg) and diclofenac sodium was found to be significant (p<0.001) compared with control group (Table 1).

Table 1: Effect of *U. crenulata* stem extract on acetic acid induced writhing response.

Treatment	**Dose**	**No. of writhing (counts/20 min)**	**Analgesic activity (%)**
Control (distilled water)	2 ml	79.3 ± 2.40	–
Diclofenac sodium	(40 mg/kg)	39.5 ± 2.96**	50.2
Stem extract	(1 gm/kg)	53.7 ± 3.53**@	32.3
Student's t-test	t calculated	10.5	6.0
	t tabulated	5.98	5.9
	degrees of freedom	6.0	6.0
	p value	<0.001	<0.001

Notes: All values are expressed as mean ± SEM (n=4)
** p<0.001 significant compared to control
@ p<0.01 significant compared to positive control (Student's t-test)

The percentage of analgesic activity was calculated using the following formula:

$$\text{Analgesic activity (\%)} = \frac{\text{Mean writhing count (control group – treated group) x 100}}{\text{Mean writhing count of control group}}$$

The degree of stem extract inhibition was found to be 32.3% while that of standard analgesic drug diclofenac sodium was 50.2% (Table 1). The methanol extract also demonstrated a significant analgesic action against the early phase of formalin-induced pain (Fig. 1). Methanol extract at 1.5 gm/kg inhibited the effect of formalin by 31.0%. Morphine was almost inefficient to exert a significant protective effect.

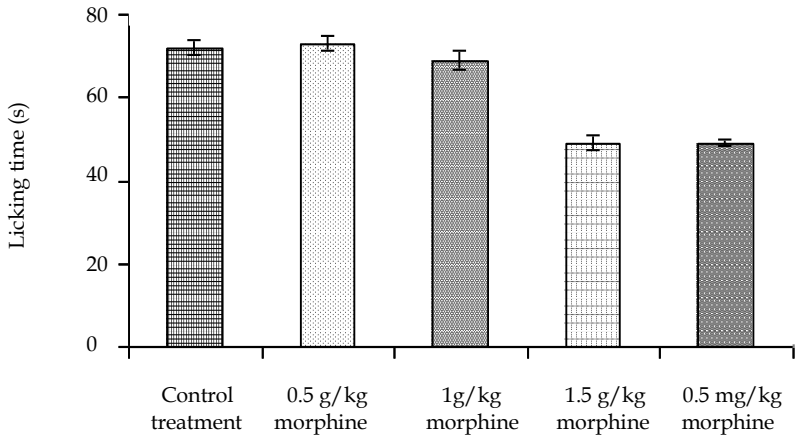

Fig. 1: Effect of ethanol extract of *U. crenulata* and morphine on formalin-induced pain in mice. n=5, $p<0.05$ compared with control.

Carrageenan induced paw oedema model showed (Table 2) that subplantar injection of carrageenan in rats caused a time-dependent increase in paw thickness where the maximal increase was observed at 4 h after carrageenan administration to the control group. However, carrageenan induced inflammation was significantly ($p<0.05$) reduced in all phases of the experiment for treatment with reference antiinflammatory drug diclofenac sodium (40 mg/kg). Diclofenac sodium produced 44.6%, 33.7%, 60.6%, and 59% antiinflammatory effect at 1 h, 2 h, 3 h and 4 h after carrageenan injection respectively (Fig. 2). On the other hand, no significant antiinflammatory effect was observed for *U. crenulata* stem extract (1 gm/kg). The extract produced 0.71%, 7%, 14.8%, and 28.3% effect at 1 h, 2 h, 3 h and 4 h after carrageenan injection respectively (Fig. 2). But neither effect was found to be statistically significant compared with the control group.

Table 2: Effect of *U. crenulata* stem extract on carrageenan induced paw oedema model.

Group	Treatment	Dose	Paw oedema (mm^3) (Ct-Co)			
			1st h	**2nd h**	**3rd h**	**4th h**
Control	Distilled water	2 mL	0.71 ± 0.05	0.82 ± 0.07	0.94 ± 0.09	1.03 ± 0.04
Positive control	Diclofenac sodium	40 mg/kg	0.39 ± 0.06*	0.55 ± 0.09*	0.37 ± 0.07*	0.42 ± 0.04*
Sample treated	*U. crenulata* stem extract	1 g/kg	0.70 ± 0.02	0.77 ± 0.03	0.80 ± 0.05	0.74 ± 0.1

Notes: All values are expressed as mean ± SEM (n=4)
$p<0.05$ significant compared to control

Medicinal plants indeed have been an indispensable arm in ameliorating common inflammation, pain sensation as well as nonciception. The root of *U. crenulata* had been used traditionally in alleviating rheumatoid arthritis and cold but no work has been done to test and confirm its analgesic and antiinflammatory activity although antipyretic effect of petroleum ether and chloroform soluble fractions of ethanol extract of its roots has been observed recently (Khan *et al*. 2007).

Acetic acid-induced abdominal constrictions are useful experimental methods in the testing of new analgesic drugs (Otterness and Bliven 1985). Abdominal injection of acetic acid in mice has been attributed to the release of arachidonic acid (AA), which results in the synthesis of prostaglandin via the cyclooxygenase (COX) enzyme pathway (Davies *et al*. 1984). The special nerve endings that sense pain is very sensitive to prostaglandin. When prostaglandin is released, the nerve endings respond to it through prostaglandin E2 (PGE2) receptor by picking up and transmitting the pain and injury messages to the brain and subsequently causing visceral writhing stimuli in mice (Ferreira, Nakamura and Abreucastro 1978; Seibert *et al*. 1994; Hosoi *et al*. 1999). Therefore, it has been suggested that the inhibition of prostaglandin synthesis is a remarkably efficient antinociceptive mechanism in visceral pain (Franzotti *et al*. 2002). Since methanol extract in this study showed a very significant inhibition ($p<0.001$) in acetic acid-induced pain, it may be predicted as the analgesic effect of extract. But it is also known that antihistaminic (Naik *et al.* 2000), myorelaxant, antiinflammatory substances (Koyama *et al.* 1997) and opioids such as codeine (Schowb and

Dubost 1984; Aydin *et al*. 1999) are also able to reduce pain induced by acetic acid. Thus it becomes evident that this model of experimental pain is unable to indicate the mechanism of analgesic effects of the test substances. The extract was then tested against other models of experimental pain. It was assayed on the first phase of formalin-induced pain known as neurogenic pain. The methanol extract exhibited a significant analgesic activity against neurogenic pain. The activity of the extract in this model suggests the activation of opioid receptors in their action mechanism (Gaertner, Müller and Roos 1999). The analgesic effect of the extract, therefore, may be due to either its action on visceral nociceptors sensitive to acid, to the inhibition of the arachidonic acid metabolite synthesis (Vane 1987) or the inhibition, at the central level, of the transmission of painful messages.

Antiinflammatory activity through carrageenan induced paw oedema is a suitable test for evaluating antiinflammatory properties for natural drugs because it shows very promising sensitivity, particularly in the acute phase of inflammation, detecting orally active antiinflammatory agents (Di Rosa, Giroud and Willoughby 1971). The development of oedema in the paw of rat after injection of carrageenen is a biphasic event (Vinegar, Schreiber and Hugo 1969), of which the initial phase observed during the first hour is attributed to the release of histamine and serotonin whereas the second phase of oedema is due to the release of prostaglandins, protease, and lysosome (Crunkhon and Meacock 1971; Asongalem *et al*. 2004; Silva, Martins and Matheus 2005). This leads to dilation of the arterioles and venules and to an increased vascular permeability. As a consequence, fluid and plasma proteins are extravagated, and oedema forms (Ozaki 1990). The mediators, including histamine, 5-HT, the kinins, and their complements, have become the recent focus of attention as the metabolites of AA. Alone or in appropriate combination, AA products of COX pathway are capable of producing the characteristic signs of inflammation which subsequently produces vasodilatation, hyperemia, pain, oedema, and cellular filtration. The COX products, particularly PGE2, contribute to the increase in blood flow through a vasodilatation action, but the lipoxygenase (LOX) pathway is necessary for vascular leakage resulting in cellular infiltration and

oedema. The present study showed that the percent inhibition of inflammation is not effective, inferring an insignificant antiinflammatory effect of *U. crenulata* extract (Table 2) compared to that of diclofenac sodium (p<0.05).

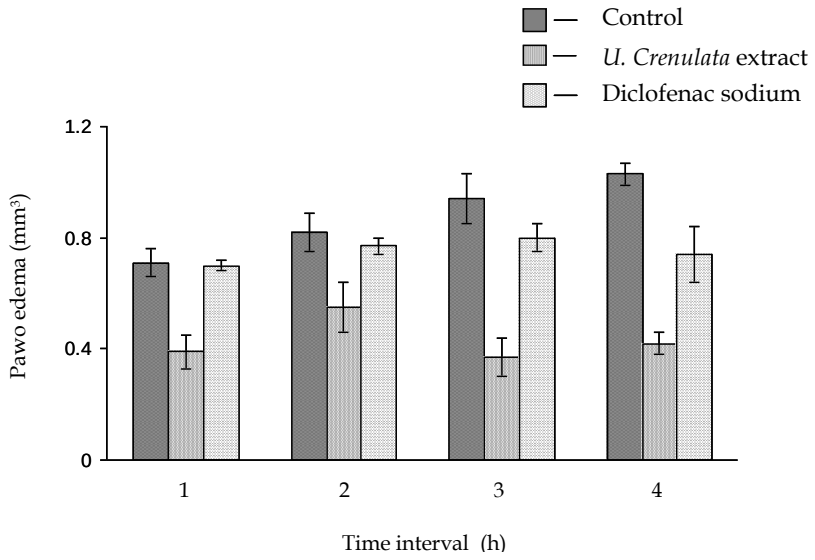

Fig. 2: Comparative effect of *U. crenulata* stem extract and diclofenac sodium in carrageenan induced paw oedema.

This study has shown that the methanol extract of *U. crenulata* stem possessed no significant anti-oedematogenic effect on paw oedema induced by carrageenan. This is the first report on the antiinflammatory properties of the methanol extract of *U. crenulata stem*.

CONCLUSION

The results of this study demonstrated that the methanol extract of *U. crenulata* stem 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 analgesic drugs source. However, further studies are still necessary to verify the above results in other experimental models to conclude whether the effect

observed is truly authentic for analgesic and antiinflammatory effect. Pharmacodynamic studies should be undertaken to establish the mechanism of action of the plant extracts. Phytochemical investigation is also proposed in order to isolate the active fraction and eventually the pure compound.

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