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Research Article

# ANTINOCICEPTIVE AND ANTIINFLAMMATORY EFFECT OF *CRINUM ASIATICUM* BULB EXTRACT

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**ABSTRACT**

The methanol extract of *Crinum asiaticum* bulb was evaluated for its antinociceptive properties on the pain induced by acetic acid and formalin in Swiss albino mice. The bulb extract at a dose of 1, 1.5 and 2 g/kg produced an inhibition of 40.71, 56.42 and 68.57% on pain induced by acetic acid and at a dose of 1.5g/kg & 2g/kg produced an inhibition of 60.0 and 67.0% on that induced by formalin. The anti-inflammatory 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 bulb extract at a dose of 1.5g/kg & 2g/kg showed the highest inhibition 52.56% and 47.37%, respectively, at the 3rd hour of administration whereas at a dose of 1g/kg showed 37.5% inhibition at the 4th hour. Diclofenac sodium at a dose of 40 mg/kg was used as a standard drug in the inhibition of acetic acid and formalin induced pain as well as in carrageenan-induced paw edema.

**Key words:** *Crinum asiaticum,* antinociceptive, anti-inflammatory, paw edema, carrageenan.

## INTRODUCTION

*Crinum asiaticum* is an evergreen herb locally known as Bara kanur in Bangladesh. It is widely distributed to China, Hongkong, India, Srilanka, Myanmar, Thailand, Malayasia, Ryukyu Islands & Mainland Japan[1,2,3]. Chittagong Hill tracts of Bangladesh are the main habitat of this herb.

Tribes of Chittagong Hilly areas use this plant as a remedy of pain, swelling carbuncle, piles, earache, arthritis, skin disease (leprosy), cold and cough disorders, vomiting, worms infestation, disuria, polyuria, bowel complains, throat disorder, colic, flautulance, and fever[1,2,3]. *Crinum asiaticum* (*C. asiaticum*) is used traditionally for various purposes. Leaves and root of this plant are used as emetic, diaphoretic and purgative.

Leaves of this herb smeared with castor oil and warmed is a useful remedy for repelling inflammations and swellings at the end of toes and fingers. Alternatively, bruised leaves of the herb mixed with castor oil can be used for this purpose. The herb is also useful to treat inflammed joints and sprains. Slightly warmed juice of the leaves with a little salt is used for earache and other ear complaints. Roasted bulb is used as rubefacient in rheumatism. The bulbs are powerfully emetic and are used to produce vomiting in poisoning especially antiaries. Bruised leaves act as an efficient insect repellent[1,2,3]. Juice of the fresh bulb at a dose of 2 to 4 drachms is very effective in emetic for children.

Despite all these traditional uses of *C. asiaticum,* very few scientific evaluations of this plant have been documented so far. Present study targets to evaluate a few of the pharmacological properties namely anti-inflammatory and antinociceptive activities of *C. asiaticum* bulb extract in animal model.

## MATERIAL AND METHODS:

The bulbs of *C. asiaticum* were collected from Chittagong Hill tracts, Bangladesh, in the month of January 2009. The plant was identified and authenticated taxonomically by Dr. Shaikh Boktear Uddin, Assistant Professor, Department of Botany, University of Chittagong. A specimen of the plant was preserved in Bangladesh National Herbarium under the Plant Accession No. 34545.

### Preparation of Plant Extract

The fresh bulbs of *C. asiaticum* (Syn: *Crinum amabile*) were washed with distilled water immediately after collection. The collected bulbs were chopped into small pieces, air dried at room temperature for about 10 days and ground into powder (536.46 gm) to store in an airtight container. The resulting powder was macerated in 3 L pure methanol (99% Anal-R, Aldrich, Germany) for 7 days at room temperature with occasional stirring. Methanol extract, after 7 days, was filtered through a cotton plug and finally with a Whatman No. 1 filter paper. The extract was concentrated under reduced pressure below $50^0$C through rotatory vaccum evaporator. The concentrated extracts were collected in an Eggplant Flask and allow to air dry for complete evaporation of methanol. The whole process was repeated three times and finally, 35 gm of brownish colored, concentrated bulb extract was obtained (yield 6.5 % w/w) which was kept in refrigerator at $4^0$C.

### Experimental Animals and Diets

Swiss albino mice of both sexes weighing between 25 to 30 gm and Wistar Albino rats of the either sex weighing between 150-200 gm obtained from animal house of Bangladesh Council for Scientific and Industrial Research (BCSIR) laboratories, Chittagong were used for present study. The animals were acclimatized to room temperature $(28\pm5)^0$C with a relative humidity of 55±5 % in a 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 supplied standard pellet diet and water *ad libitum.* All animal experimentations were carried out with the guidelines of Institutional Animal Ethics Committee (IAEC).

### Assay for antinociceptive activity

### Acetic acid induced writhing test

For writhing test, 1% (v/v) acetic acid solution (2.3 ml/kg body weight) was injected intraperitoneally to mice (weighing 25-30 gm) and the number of writhing and stretching was counted over 20 minutes[4]. The methanol extract of *C. asiaticum* (2gm/kg), reference analgesic drug diclofenac sodium (40 mg/kg) and distilled water were administered orally 30 minute before acetic acid injection.

### Formalin test

The procedure was similar to that described previously by Gaertner *et al.*[5] (1999). 20 µL of 2.5% formalin (0.92% formaldehyde) made in phosphate buffer was injected under the right hind paw surface of experimental mice. Each mouse was placed individually in a cage and observed from 0 to 5 min followed by the injection of formalin to analyze 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 indicative of pain.

**Assay for anti-inflammatory activity**

Anti-inflammatory activity of *C. asiaticum* bulb extract was assessed using carrageenan induced paw edema model in the hind paw of rat by the reported method[6]. According to Winter, 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 1st, 2nd, 3rd and 4th hour after carrageenan injection and the paw edema was determined using plethysmometer (7150 UCG Basil, Italy). *C. asiaticum* bulb extract (1.0, 1.5 and 2 g/kg), standard anti-inflammatory drug diclofenac sodium (40 mg/kg), and distilled water were administered orally to treated, positive control and control groups one hour before the subplantar injection of carrageenan.

**Statistical analysis**

Values for analgesic activity were expressed as "mean increase in latency after drug administration ±SEM" in terms of seconds whereas values for anti-inflammatory activity were expressed as "mean increase in paw volume ±SEM". The significance of difference between means was determined by student's t-test values of $p<0.05$ were considered significant and $p<0.01$ and $P<0.001$ as highly significant.

**RESULT AND DISCUSSION**

Analgesic activity of *C. asiaticum* bulb extract was assessed using acetic acid induced writhing response model. Table 4 shows the pain behavior of writhing response of mice, which was presented as cumulative abdominal stretching response.

When 1 % (v/v) acetic acid solution (2.3 ml/kg body weight) was injected intraperitoneally in mice, the control animal showed 79.3 writhing count / 20 minutes. But, administration of diclofenac sodium caused significant reduction of writhing count, from 70 to 16.5. On the other hand, *C. asiaticum* bulb extract reduced the writhing count from 70 to 22. The effect of bulb extract and diclofenac sodium was analyzed statistically by Student's t test. The treatment of animals with methanol bulb extract of *C. asiaticum* (2gm/kg) and diclofenac sodium was found significant ($P<0.001$) compared with control group (Table 4). The percentage inhibition of analgesic activity was calculated by using following formula-

$$\% \text{ Analgesic activity} = \frac{\text{Mean writhing count (Control group- Treated group) x100}}{\text{Mean writhing count of control group}}$$

The degree of inhibition, at a dose 2g/kg, of bulb extract was found 68.57 % which was almost nearer to the effect showed by standard analgesic drug diclofenac sodium (76.43 %) (Fig.1). The methanol extract at a dose of 1.5 g/kg and 2.0g/kg inhibited the effect of formalin by 55% and 63%, respectively (Fig.2). Morphine did not exert any significant effect.

Carrageenan induced paw edema model indicated that subplantar injection of carrageenan in experimental rats showed a time-dependent increase in paw thickness (Table 1). This increase was observed at 1st h and was maximal at 4h after administration of carrageenan injection in the control group. Methanolic bulb extract of *C. asiaticum* (2 g/kg) produced 33.06%, 50.55%, 52.56%, 51.02% inhibition of paw edema at 1st, 2nd, 3rd and 4th h after carrageenan injection respectively (Table 2 and Fig. 3). The percent of inhibition for other concentration are also delineated (Table 2 and Fig. 3). The effect was found statistically non-significant, compared to control, at 1st h ($P > 0.05$), significant at 2nd h ($P<0.05$) & 4h ($P<0.05$) and very significant at 3h ($P<0.01$) after carrageenan injection.

On the other hand, carrageenan-induced inflammation was significantly ($P<0.05$) reduced in all phases of the experiment by treatment with reference anti-inflammatory drug diclofenac sodium (40 mg/kg). Diclofenac sodium produced 38.70%, 45.67%, 58.32%, 60.88% anti-inflammatory effect at 1st, 2nd, 3rd and 4th, respectively, after carrageenan injection (Table 2).

Medicinal plants indeed have been an indispensable arm in ameliorating common inflammation, pain sensation as well as nonciception[7]. The bulb of *C. asiaticum* had been used traditionally in the rheumatoid arthritis and cold since long but no work has been done to confirm its analgesic and anti-inflammatory activity albeit the antipyretic effect of petroleum ether and chloroform soluble fractions of ethanol extract of its roots has been observed recently[8].

Acetic acid induced abdominal constrictions are useful experimental tools in the testing of new analgesic drugs[9] because the abdominal injection of acetic acid in mice has been attributed to the release of arachidonic acid, which results the synthesis of prostaglandin via the cyclooxygenase (COX) enzyme[10]. 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 cause visceral writhing stimuli in mice[11,12,13].

Therefore, it has been suggested that the inhibition of prostaglandin synthesis is remarkably an efficient antinociceptive mechanism in visceral pain[14]. Since methanol extract in this study showed very significant inhibition ($P<0.001$) (Table 4) in acetic acid induced pain, it may be predicted as the analgesic effect of extract. The extract was then tested against other model 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 (Fig. 2). The activity of the extract in this model suggests the activation of opioid receptors in their action mechanism[5].

The analgesic effect of the extract, therefore, may be due to its action on visceral nociceptors sensitive to acid, to the inhibition of the synthesis of the arachidonic acid metabolite[15]. Anti-inflammatory activity through carrageenan induced paw edema is a suitable test for evaluating anti-inflammatory properties for natural drugs because it shows very promising sensitivity, particularly in the acute phase of inflammation, in detecting orally active anti-inflammatory agents[16]. Development of edema in the paw of rat after injection of carrageenen is indeed a biphasic event[17], of which the initial phase observed during the first hour is attributed to the release of histamine and serotonin whereas the second one of edema is due to the release of prostaglandins, protease, and lysosome[18,19,20]. This leads to a dilation of the arterioles and venules and to an increased vascular permeability. As a consequence, fluid and plasma proteins are extravagated, and edema forms[21].

The mediators, including histamine, 5-HT, the kinins and their complements, have become the recent focus of attention as the metabolites of arachidonic acid (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, edema, and cellular filtration. The COX products, particularly prostaglandin E2 (PGE2), contribute to increased blood flow through a vasodilatation action, but the lipoxygenase (LOX) pathway is necessary for vascular leakage and edema consequently on cellular infiltration.

It is possible that the methanol extract of bulb contains the active constituents that exhibit its anti-inflammatory action probably by means of either inhibiting the synthesis, release or action of inflammatory mediators like histamine, serotonin, prostaglandin, protease, and lysosome.

From our observation we assume that different active secondary metabolites are present in crude extracts of *C. asiaticum* bulb and perhaps some of these compounds may operate in a synergistic manner. Moreover, the anti-inflammatory effect of the *C. asiaticum* crude extract is almost near to the effect of standard anti-inflammatory drug diclofenac sodium. From this observation, it can be suggested that if the compound responsible for anti-inflammatory effect could be isolated from crude extract, it might show very potent anti-inflammatory effect even better than diclofenac sodium.

**Table 1: Evaluation of anti-inflammatory activity of *Crinum asiaticum* bulb extract by carrageenan induced paw edema model**

| Group | Treatment | Dose | Paw edema ($mm^3$)(Ct -Co) | | | |
|---|---|---|---|---|---|---|
| | | | 1st hr | 2nd hr | 3rd hr | 4th hr |
| Control | Distilled water | 2 ml | 0.37± 0.05 | 0.64± 0.06 | 0.76± 0.07 | 0.88 ± 0.11 |
| Positive control | Diclofenac Sodium | 40 mg/kg | *0.23± 0.04 | *0.35±0.07 | **0.32±0.03 | **0.35±0.04 |
| Sample treated | *Crinum asiaticum* Bulb extract | 2gm/kg | NS0.25± 0.05 | **0.31±0.06 | *0.36± 0.10 | **0.43± 0.07 |
| | | 1.5gm/kg | *0.27±0.007 | *0.36±0.012 | *0.4±.01 | *0.48±.01 |
| | | 1 gm/kg | *0.29±.017 | *0.44±.028 | *0.49±.02 | *0.55±.015 |

Here,All values are expressed as mean ± SEM (n=5) NS = Not significant (P > 0.05) compared with control.** P < 0.01 compared with control (Student's t- test).* P<0.05 significant compared with control (Student's t-test).

**Table 2: % Anti-inflammatory activity of *Crinum asiaticum* bulb extract and Diclofenac Sodium**

| Group | Treatment | Dose | % Inhibition of Paw edema $\frac{(Ct-Co)\ control - (Ct-Co)\ treated}{(Ct-Co)\ control} \times 100$ | | | |
|---|---|---|---|---|---|---|
| | | | 1st hr | 2nd hr | 3rd hr | 4th hr |
| Control | Distilled water | 2 ml | - | - | - | - |
| Positive control | DiclofenacSodium | 40 mg/kg | 38.70% | 45.67% | 58.32% | 60.88% |
| Sample treated | *C. asiaticum*bulb extract | 2gm/kg | 33.06% | 50.55% | 52.56% | 51.02% |
| | | 1.5gm/kg | 27.02% | 43.75% | 47.37% | 45.45% |
| | | 1.0gm/kg | 21.62% | 31.25% | 35.52% | 37.5% |

Here, Co is the paw thickness volume (in $mm^3$) before carrageenan injection, Ct is the paw thickness volume (in $mm^3$) at time t, (Ct -Co) is paw edema.

**Table 3: Effect of *Crinum asiaticum* bulb extract on acetic acid induced writhing response**

| Rat No. | Writhing Count/ 20 minutes | | | | |
|---|---|---|---|---|---|
| | Control | Diclofenac Sodium (40mg/kg) | Bulb Extract | | |
| | | | 2 (gm/kg) | 1.5 (gm/kg) | 1.00 (gm/kg) |
| 1 | 70 | 12 | 26 | 36 | 48 |
| 2 | 69 | 16 | 27 | 29 | 36 |
| 3 | 66 | 20 | 10 | 30 | 40 |
| 4 | 75 | 18 | 25 | 27 | 42 |
| MEAN | 70 | 16.5 | 22 | 30.5 | 41.5 |
| SEM | 1.87 | 1.71 | 4.02 | 1.93 | 2.5 |
| % Analgesicactivity | - | 76.43% | 68.57% | 56.42% | 40.71% |

**Table 4: Effect of *Crinum asiaticum* bulb extract on acetic acid induced writhing response (Student's t-test, ***P<0.001 significant compared to control)**

| | Wreathing Counts/20 min | | | | | |
|---|---|---|---|---|---|---|
| Treatment | Control Distilled water | | Diclofenac Sodium | Stem extract | | |
| Dose | 2ml | | 40 (mg/kg) | 1.00 (gm/kg) | 1.5 (gm/kg) | 2 (gm/kg) |
| Mean±SEM | 70 ± 1.87 | | 16.5 ± 1.71 | 41.5±2.5 | 30.5±1.96 | 22 ±4.02 |
| | **Student's t test** | t-calculated | 21.1 | 9.13 | 14.58 | 10.82 |
| | | t-tabulated | 5.96 | 5.96 | 5.96 | 5.96 |
| | | Deg. of fred. | 6 | 6 | 6 | 6.0 |
| | | p-value | <0.001 | <0.001 | <0.001 | <0.001 |

Here, all values are expressed as Mean ± SEM (n=4), P<0.001 significant compared to control

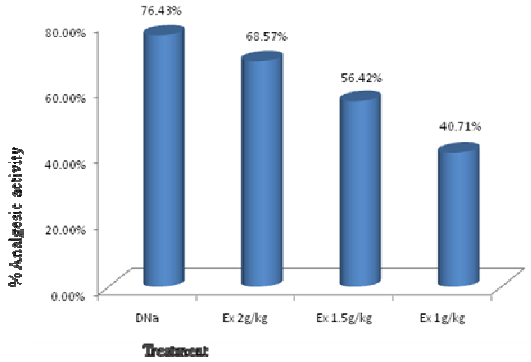


**Fig. 1: Comparative % analgesic activity of *C. asiaticum* bulb extract and commercially available analgesic drug Diclofenac Sodium**

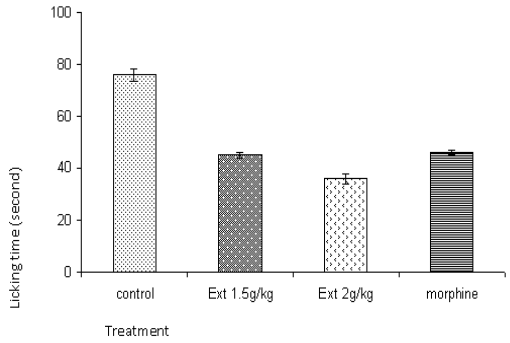


**Fig. 2: Effect of ethanol extract of *C. asiaticum* and morphine on formalin-induced pain in mice. n=5, P<0.05 compared with control**

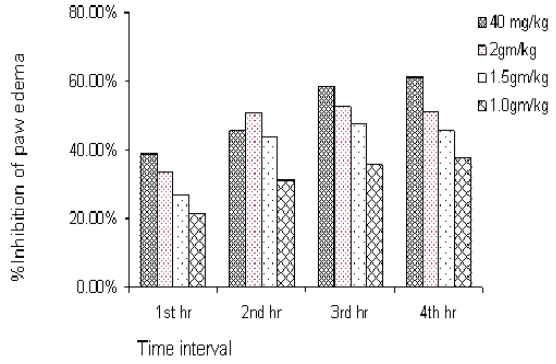


**Fig. 3: Comparative % anti-inflammatory activity of *C. asiaticum* bulb extract and diclofenac sodium**

## CONCLUSION

The results of the study demonstrate that the methanol extract of *C. asiaticum* bulb exerts potential analgesic and anti-inflammatory 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 nonciception andinflammation. Phytochemical investigation is also proposed in order to isolate the active fraction and eventually the pure compound.

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