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Full Length Research Paper

Analgesic and anti-inflammatory effects of *Crinum asiaticum* leaf alcoholic extract in animal models

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This study investigated the analgesic and anti-inflammatory effects of *Crinum asiaticum* (Amaryllidaceae) leaf ethanolic extract. Analgesic effect was investigated in acetic acid induced writhing model and formalin induced licking model in swiss albino mice. Anti-inflammatory effect was conducted in carrageenan-induced paw edema model of albino rat. Data were analyzed by one-way analysis of variance (ANOVA) followed by *post hoc* multiple comparison test. In analgesic study, *C. asiaticum* extract inhibited 42.34±3.20% of acetic acid induced pain at higher dose of 2.0 g/kg body weight. The effect was statistically significant ($p<0.001$) compared to the positive control, diclofenac sodium (10 mg/kg). The extract reduced the formalin induced pain 22.60±1.39% in early phase and 27.11±0.87% in late phase at the same dose of 2.0 g/kg and the reductions were significant ($p<0.01$) compared to the positive control morphine (0.5 mg/kg). In a time-dependent inhibition of carrageenan-induced paw edema model, the extract promoted the inhibitions of paw edema 51.60±2.50% at the 1st h and 40.80±0.52% at the 4th h of administration. These inhibitions were also significant ($p<0.01$) in comparison to those promoted by diclofenac sodium. No mortality was observed in acute toxicity test. The study concludes that *C. asiaticum* leaf extract has potential analgesic and anti-inflammatory effects to be recorded as plant-derived complementary medicine.

Key words: *Crinum asiaticum*, anti-inflammatory, analgesic, Carrageenan, formalin.

INTRODUCTION

Important pharmacological properties, convenience to users, economic viability and low toxicity of plant-derived medicines from various plants have notably attracted and increased the interest of scientists since last couple of decades (Prashant et al., 2008). This revived interest to plant-derived medicines is mainly due to the current widespread perception that green medicine is safe and dependable than the costly synthetic drugs most of which have adverse effects (Jigna and Sumitra, 2006). This belief and perception led to explore a lot of new indigenous herbal medicines.

Crinum asiaticum (family: Amaryllidaceae) is an evergreen herbaceous plant of small to moderate size with greenish-feathery leaves locally known as Bara kanur in Bangladesh. Bangladeshi hilly areas especially the Chittagong hill tracts randomly habitat this herb. It is also found in China, Hongkong, India, Srilanka, Myanmar, Thailand, Malaysia, Ryukyu Islands and Mainland Japan (Ghani, 1998; Zhanhe and Alan, 2000). In Southeast Asian countries, *C. asiaticum* has a considerable medicinal reputation as a potent folk medicine in the treatment of injury and inflamed joints (Burkill, 1966). In early times, it had been used in Indonesia and in Malay Peninsula as an antidote for wounds from poisoned arrows. Anti-inflammatory effect of *C. asiaticum* by the inhibition of inducible nitric oxide

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synthase (iNOS) and the release of PGE2, IL-6, and IL-8 has also been recorded (Kim et al., 2008). Researchers also isolated many alkaloids (Kogure et al., 2011; Chen et al., 2011) few of them are completely new from this plant extract. Others evidenced the cytotoxic (Sun et al., 2009) and anti-platelet effect (Singh et al., 2011) of *C. asiaticum.*

In Bangladesh, different tribal groups have been using this plant for pain, swelling, carbuncle, piles, earache, arthritis, leprosy, cold and cough disorders, vomiting, worms infestation, disuria, polyuria, throat disorder, colic, flatulence and fever (Ghani, 1998; Zhanhe and Alan, 2000). Different parts, roots and leaves, of *C. asiaticum* have been widely used as emetic, diaphoretic and purgative. Leaves are smeared with castor oil and warmed to make a useful remedy for repelling inflammations and swellings at the end of toes and fingers. Alternatively, bruised leaves of this herb mixed with castor oil are also used for such cases. Slightly warmed juice of the leaves with a little salt is used for ear-ache and other ear complaints. Bruised leaves also act as an efficient insect repellent (Ghani, 1998; Zhanhe and Alan, 2000). Asmawi et al. (2011) studied in vivo antinociceptive activity of chloroform, petroleum ether and methanol extract of *C. asiaticum* although chloroform extract was especially attended.

Apart from this, the bulb of *C. asiaticum* is also useful in inflamed joints and sprains. Roasted bulb is used as rubefacient in rheumatism. The bulbs are powerfully emetic and are used to produce vomiting in poisoning especially antiaries. Juice of the fresh bulb at a dose of 2 to 4 drachms is very effective in emesis of children. Very recently we have published the antinociceptive and anti-inflammatory effects of the *C. asiaticum* bulb extracts in animal model (Rahman et al., 2011). As part of our continuous investigation on the pharmacological actions of indigenous medicinal plants, we reported here the analgesic and anti-inflammatory effects of *C. asiatcum* leaf ethanolic extract in animal models.

MATERIALS AND METHODS

Reagents and drugs

Acetic acid, morphine hydrochloride and absolute ethanol (99.5%) were purchased from Sigma-Aldrich, Munich, Germany. Morphine was dissolved in saline solution just before use. Diclofenac sodium (powder form) was kindly donated by GlaxoSmithKline Ltd., Bangladesh. A commercially available (Sigma-Aldrich, Munich, Germany) concentrated formalin solution was diluted with saline to the appropriate concentration (2.5%). Carrageenan (lambda form, FMC Marine Colloids Division, Sigma-Aldrich, Poole, UK) was used for paw edema test.

Collection of plant material

C. asiaticum were collected from Chittagong, Bangladesh. The plant was taxonomically identified by Dr. Shaikh Boktear Uddin, Associate Professor and Taxonomist, Department of Botany, University of Chittagong, Bangladesh. A voucher specimen with the accession no. 34545 was preserved for further documentation.

Preparation of extract

Fresh leaves of *C. asiaticum* were washed with distilled water immediately after collection. The leaves were chopped into small pieces, air dried at room temperature (25±2)°C for about 10 days and ground into powder by Moulinex Blender (AK-241, Moulinex, France, 40 to 80 mesh) to store in an airtight container. The resulting powder (600g) was extracted in absolute ethanol for 10 days at room temperature. Extract was evaporated using rotary evaporator (RE200 Sterling, UK) to dryness to give 86.9 g (yield 14.4 % w/w) of blackish-green pastes which were preserved at 4°C to use in further.

Experimental animals

Six-week-old swiss albino mice of both sexes weighing 25 to 30 g and seven-week-old wistar albino rats of the both sexes weighing 150 to 200 g were obtained from animal house of Bangladesh Council of Scientific and Industrial Research (BCSIR) laboratories, Chittagong. The animals were housed individually in stainless steel wire meshed plastic cages in a temperature (25±2)°C and humidity (55 to 60%) room with a 12 h light-dark cycle. The animals were supplied with standard rat pellet diet and drinking water *ad libitum* during the entire period of the study. Maintenance of animals and experimentations were carried out according to the regulations of the Institutional Animal Ethics Committee (05- 2010/Animal).

Assay for analgesic effect

Acetic acid induced writhing test

The analgesic activity of *C. asiaticum* extract was measured by the acetic acid induced writhing test in swiss albino mice as described by Koster et al. (1959). Briefly, the inhibition of writhing produced by the extract was determined by comparing with the inhibition produced with positive control group. Diclofenac sodium at a dose of 10 mg/kg BW was used as standard analgesic agent (positive control). Intraperitoneal injection of (1%) acetic acid at a dose of 2.3 ml/kg was used to create pain sensation. The number of writhing and stretching was counted over 20 min (20 min after the application of acetic acid). The extract (1.0 and 2.0 g/kg BW) and distilled water (negative control) were administered orally 30 min before acetic acid injection as treatment and control. Five animals were taken in each group. The percent of analgesic action was determined by the following formula:

$$\% \text{ Analgesic effect} = \frac{\text{Mean writing count (control group-treated group)}}{\text{Mean writhing count of control group}} \times 100$$

Formalin test

The procedure was similar to that described by Gaertner et al. (1999). The plant extract (1.0 and 2.0 g/kg), positive control morphine (0.5 mg/kg) and distilled water (negative control) were administered orally 30 min before formalin injection. Twenty microliter (20 µL) of 2.5% formalin (0.92% formaldehyde) made in phosphate buffer (pH 7.4) 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 as the first phase and 15 and 40 min as the late phase followed by the injection of formalin to

Table 1. Effect of *C. asiaticum* extract (CAEx) on the different phases of formalin induced licking response in mice.

Treatment	Duration of paw licking (s)			
	1st phase	%Inhibition	2nd phase	(%) Inhibition
Control	70.8±1.65	-	158.6±3.61	-
CAE 1 g/kg	67.4±1.72[a]	16.9±1.73[a]	140.6±1.50[b]**	13.3±1.47[a]
CAE 2 g/kg	45.8±1.39[c]**	22.6±2.22[c]**	115.6±0.87[c]**	27.1±1.60[c]**
Mph. 0.5 mg/kg	27.2±1.07[d]**	55.4±2.50[d]**	100.4±4.03[d]**	36.7±2.23[d]**

Data are shown as mean ± SD of five animals in each group. Mph: Morphine. Superscript letters in the table are significantly different (**p<0.01) from each other. Data were analyzed by one-way ANOVA followed by Tukey's *post hoc* test for multiple comparisons, p<0.05).

analyze the formalin induced pain. The length of time the animal spent for licking the injected paw was counted with a chronometer and was considered as indicative of pain. The percentage of inhibition was calculated by the following formula:

$$\% \text{ Inhibition} = \frac{L_{control} - L_{treated}}{L_{control}}$$

Where, L represents licking.

Assay for anti-inflammatory effect

The anti-inflammatory activity of the extract was determined by using carrageenan-induced paw edema test in the hind paw of rat as reported previously (Winter et al., 1962). Briefly, the initial volume of right hind paw of each rat was measured using plethysmometer (7150, UGO Basile, Italy) and then 0.1 ml of 1 % (w/v) carrageenan was subcutaneously injected into the subplantar region of right hind paw in order to induce acute inflammation. The extract (1.0 and 2.0 g/kg), standard anti-inflammatory drug diclofenac sodium (10 mg/kg, positive control) and distilled water (negative control) were administered orally half an hour before the subplantar injection of carrageenan to treated, positive control and normal control groups, respectively. The volume of right hind paw was measured at 1st, 2nd, 3rd and 4th h after the carrageenan injection. The inhibition of paw edema was calculated according to the following formula:

$$\% \text{ inhibition} = \frac{(Ct - Co)\ control - (Ct - Co)\ treated}{(Ct - Co)\ control} \times 100$$

Where, Ct is the right hind paw thickness volume (in mm^3) at time t; Co is the right hind paw thickness volume (in mm^3) before carrageenan injection.

Acute toxicity test

Wistar albino rats maintained under standard laboratory condition were used for acute toxicity study. A total of five animals received a single oral dose (1.0, 2.0, 3.0 and 4 g/kg BW) of the extract. Animals were kept over-night fasting prior to administration. After administration of the extract, food was withheld for further 3 to 4 h. Animals were observed individually once during the first 30 min after dosing, periodically during the first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. Once daily cage side observation including changes in skin and fur, eyes and mucous membrane, respiratory and circulatory rate, autonomic and CNS changes were observed (Zaoui et al., 2002).

Statistical analysis

Values for analgesic activity were expressed as "mean increase in latency after drug administration ± SD" in terms of seconds whereas values for anti-inflammatory activity were expressed as "mean increase in paw volume ±SD". Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test for multiple comparisons. The values were considered significantly different at p< 0.05.

RESULTS

The extract showed a dose-dependent analgesic actions on acetic-acid induced writhing response of abdominal stretching (Figure 1). This analgesic effect in terms of writhing response was significant (p< 0.001) at all the doses of extract in comparison to the control group. However, the activity was lower as compared to diclofenac sodium. Acetic acid induced writhing 71.8±1.24 was reduced to 41.4±1.43 and 59.6±0.81 at 20 min counts with the treatment of 2 and 1 g/kg BW of extract showing analgesic actions 42.34±3.20% and 16.99±1.99%, respectively.

The results of the present study reveal that the extract significantly (p<0.01) reduced the licking times in both phases (Table 1). In the first phase licking time 70.8±1.65 was reduced to 67.4±1.72 by 1 g/kg and to 45.8±1.39 by 2 g/kg promoting the analgesic action 16.9±1.73 and 22.6±2.22%, respectively. In the second phase, the licking time 158.6±3.61 was reduced to 140.6±1.50 by 1 g/kg and to 115.6±0.87 by 2 g/kg promoting the analgesic effects 13.3±1.47% and 27.1±1.60% respectively. The percent (%) inhibition for positive control (morphine, 0.5 mg/kg) was higher than the extract in both the phases but the actions were statistically significant (p<0.01). Figure 2 shows a time-dependent increase of rat hind paw edema with the administration of carrageenan along with extract and diclofenac sodium. However, paw edema was inversely

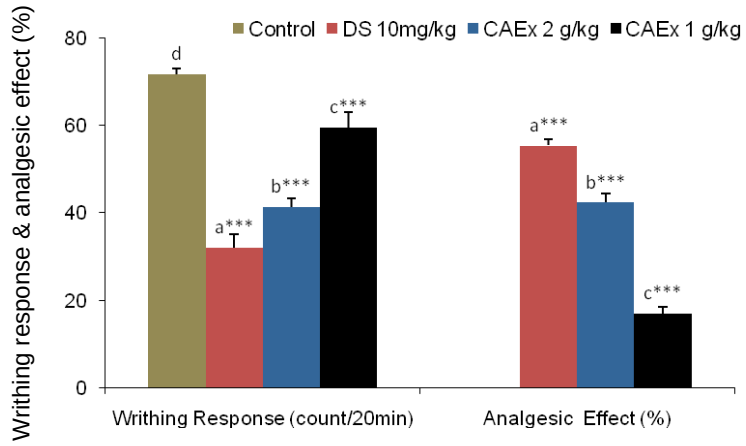

Figure 1. Effect of *C. asiaticum* extract (CAEx) on acetic acid-induced writhing response in mice. Data are shown as mean ± SD of five animals in each group. DS denotes diclofenac sodium. Superscript letters on the bar graph are significantly ($p< 0.05$) different from each other. Data were analyzed by one-way ANOVA followed by Tukey's *post hoc* test for multiple comparisons.

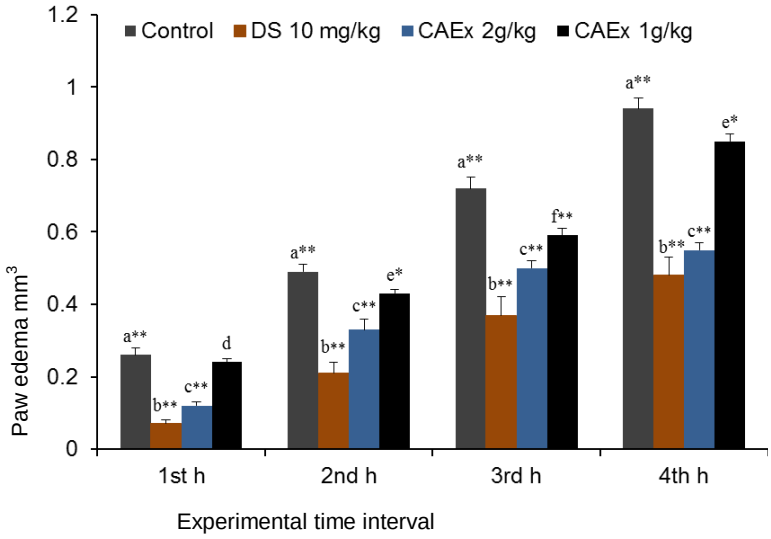

Figure 2. Effect of *C. asiaticum* extract (CAEx) on the carrageenan-induced paw edema of albino rats. Data are shown as mean ± SD of five animals in each group. Superscript letters on the bar graph are significantly ($p< 0.05$) different from each other. Data were analyzed by one-way ANOVA followed by Tukey's *post hoc* test for multiple comparisons.

increased with the increase of extract dose. Reduction of paw edema was significant ($p<0.05$) at the doses of 1.0 and 2.0 g/kg compared to positive control diclofenac sodium (10 mg/kg). The extract also showed a potent anti-inflammatory action 51.6 ±2.50 and 40.80±0.52% by 2 g/kg at the 1st and 4th h of administration (Figure 3). Lower to moderate anti-inflammatory actions were observed for the 1 g/kg of *C. asiaticum* extract. No acute toxicity was observed for different doses from 1.0 to 4.0 g/kg of extract.

DISCUSSION

The study was carried out to investigate the analgesic effects of *C. asiaticum* leaf extract in acetic induced writhing model and formalin induced licking model of mice. Acetic acid induced writhing in mice attributed the visceral pain to screen new analgesic drugs (Hasan et al., 2010) because of the effectiveness of this method to evaluate peripherally active analgesics. The crude extract of *C. asiaticum* showed significant analgesic action

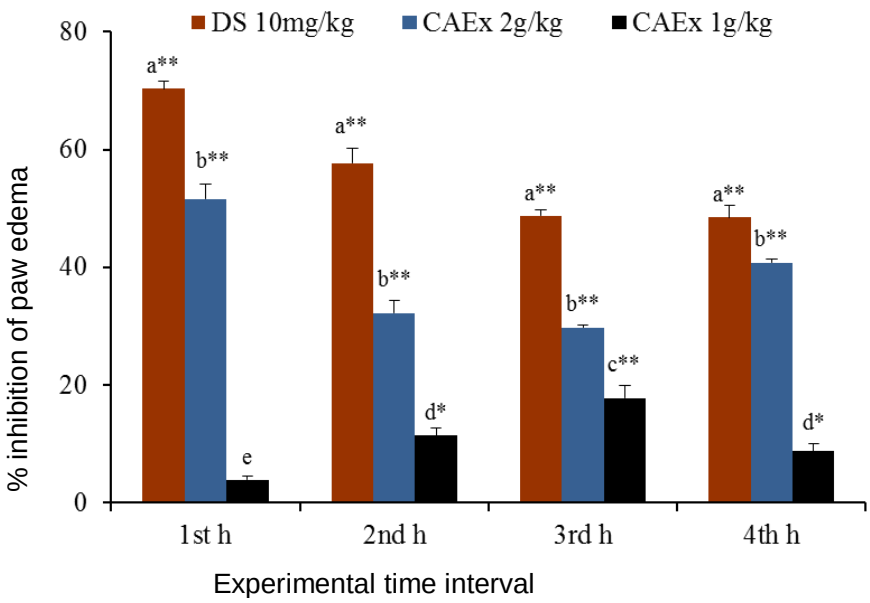

Figure 3. Percentage inhibition (%) of paw edema in time–dependent doses of *C. asiaticum* extract (CAEx). Data are shown as mean ± SD of five animals in each group. Values with superscript letters on the bar graph are significantly ($p<0.05$) different from each other. Data were analyzed by one-way ANOVA followed by Tukey's *post hoc* test for multiple comparisons.

compared to positive control diclofenac sodium.

Acetic acid induced writhing method is very sensitive and able to detect analgesic effects of compound(s) at dose level that may appear to be inactive in other methods like tail flick test. Pain sensation in acetic acid induced writhing method elicited by triggering localized inflammatory response resulting release of free arachidonic acid from tissue phospholipid (Ahmed et al., 2006) via cyclooxygenase (COX), and prostaglandin biosynthesis (Duarte et al., 1988). In other words, the acetic acid induced writhing has been associated with increased level of prostanoids especially PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products (Dhara et al., 2000). The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability (Zakaria et al., 2008). This prostaglandin synthesis is inhibited, through a peripheral mechanism of pain inhibition, by the agents which reduce the number of writhing will rendering the analgesic effect (Ferdous et al., 2008).

The significant pain reduction of *C. asiaticum* extract might be due to the presence of analgesic principles, achieved by the chemical constituents of the extract, acting with the prostaglandin pathways. It was found that the extract of *C. asiaticum* was possessed with some phytochemical metabolites especially the alkaloids (Chen et al., 2007) which were reported to have a role in analgesic activity primarily by targeting prostaglandins (Rajnarayana et al., 2001). *C. asiaticum* were also documented to present huge alkaloids (Sun et al., 2009; zhang et al., 2009) while alkaloids and tannins are well known for their ability to inhibit pain perception (Uche et al., 2008; Vanu et al., 2006).

Formalin test is a useful vehicle, particularly for the screening of novel compounds, since it encompasses inflammatory, neurogenic and central mechanisms of nociception (Lee et al., 2000). The test is sensitive for various classes of analgesic drugs of two distinct phases, reflecting different types of pain. The early phase (initial pain) conducted by our study reflects a direct effect of formalin on nociceptors (neurogenic pain) while centrally acting narcotics inhibit both the phases and peripherally acting drugs inhibit only the late phase of formalin induced pain (Hunskaar and Hole, 1987; Elisabetsky et al., 1995). Our results show that the inhibition in the first phase is higher for low doses whereas the reverse is observed for higher doses indicating that low doses of *C. asiaticum* extract is more prominent in neurogenic pain than in tissue injury, but, the higher doses attain the peripheral pain inhibition more than the neurogenic pain. This result could also be explained by the fact that the centrally acting drugs such as narcotic (morphine) inhibits the initial phase more than the late phase while a peripherally acting drug only inhibits the late phase. However, the effects of the extract used in this study were reflected through the comparable reduction, with analgesic drug morphine, of the time spent in licking and biting of the injected paw after the administration of C. asiaticum extract.

Carrageenan-induced rat paw edema model is a suitable test for evaluating anti-inflammatory properties for

natural drugs because of its sensitivity in detecting orally active anti-inflammatory agents particularly in the acute phase of inflammation (Di Rosa et al., 1971). Development of edema in the paw of rat after injection of carrageenan is a biphasic event (Vinegar et al., 1969). The initial phase observed during the first hour is attributed to the release of histamine and serotonin. The second phase of edema is due to the release of prostaglandins, protease, and lysosome (Asongalem et al., 2004; Silva et al., 2005). This leads to a dilation of the arterioles and venules to an increased vascular permeability which consequently makes edema (Ozaki, 1990). Although, the mediators including histamine, 5-HT, the kinins and their complements, have become the recent focus of attention as they are 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, 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 lipooxygenase (LOX) pathway is necessary for vascular leakage and edema consequently on cellular infiltration. In our study, carrageenan-induced inflammation was significantly ($P<0.05$ and $P<0.01$) reduced in all phases of the experiments by treatment with ethanol extract. These results are consistent with those of Asmawi et al. (2011) who basically focused on the chloroform extract of *C. asiaticum* although the methanol extract was briefed. Whatever the mechanism, it is assumed that at least some of the above discussed mediators, either partially or completely, are subjects of inhibition by the extracts of *C. asiaticum*. No toxicity or abnormality in acute toxicity test supports the safe use of the extract.

Conclusion

The analgesic and anti-inflammatory effect of the extract in animal models established the scientific basis of the folkloric use of the leafy parts of *C. asiaticum*. Although the mechanisms of such studies are not studied here but it is assumed that the bioactive compounds of the crude extract might have a synergistic role in analgesia and anti-inflammation. Isolation of active metabolites and mechanism behind these effects could be suggested for further study.

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