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Abstract

The methanolic leaf extract of *Rhynchosystylis retusa* (L.) Blume was evaluated for analgesic and anti-inflammatory activities in mice. The analgesic activity was studied using acetic acid induced writhing and the anti-inflammatory activity was studied on carrageenan and formaldehyde induced paw edema. It was observed that the extract showed 28.84% and 35.81% inhibition of acetic acid induced writhing at doses of 200 mg/kg and 400 mg/kg, respectively, 7.80%, 8.67% and 14.32% mean inhibition of carrageenan induced paw edema at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg, respectively, and significant ($p < 0.01$, $p < 0.001$) anti-inflammatory activity of formaldehyde induced mice paw edema at doses of 200 mg/kg and 400 mg/kg. It can be concluded that the extract exerts dose dependent analgesic and anti-inflammatory activities.

Keywords: *Rhynchosystylis retusa* (L.) Blume; Analgesic; Anti-inflammatory.

Introduction

Inflammation is a disorder mediated by enzyme activation, mediator release, cell migration, edema, fever, tissue breakdown and increase in a number of electrolytes. The prostaglandins production is increased due to cell and tissue responses involved in inflammation (Vane and Bolting, 1995; Perianayagam et al., 2006; Gupta et al., 2006; Nonato et al., 2010). The long-term administration of non-steroidal anti-inflammatory drugs (NSAIDs) may induce gastro-intestinal ulcers, bleeding, and renal disorders due to their non-selective inhibition of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzymes. The selective COX-2 inhibitors cause gastro-intestinal toxicity and adverse cardiovascular effects (Nonato et al., 2010). Nevertheless, the existing anti-inflammatory agents are toxic, expensive and not available by the rural people. Therefore, the phytochemical study for the development of new anti-inflammatory agents is increasing day by day. (Dharmasiri et al., 2003; Li et al., 2003).

Pain is a general problem of people throughout the world. Pain is a primary response of body injury, inflammation, cancer etc. It can also occur for brain or nerve injury. With many pathological conditions, tissue injury is the immediate cause of the pain, and this result in

the local release of a variety of chemical agents which act on the nerve terminals, either activating them directly or enhancing their sensitivity to other forms of stimulation (Rang et al., 1993).

Rhynchosystylis retusa (L.) Blume (Family: Orchidaceae, Syn. *Epidendrum retusum* L., Common name: Foxtail Orchid, Sanskrit name: Banda and Rasna) is a medium sized monocotyledon plant and grows in Bangladesh. The fresh leaves or its extracts traditionally is used to treat rheumatic disease, ear pain, blood dysentery, skin diseases and external inflammations (Hossain, 2011; Silja et al., 2008; Jonathan and Raju, 2005). Various preparations of this plant are also traditionally used to cure asthma, tuberculosis, epilepsy, vertigo, palpitation, kidney stone and menstrual disorders (Hossain, 2011). It is reported that the plant showed significant antibacterial activity against *Bacillus subtilis* and *Escherichia coli* (Hossain, 2011). Although, this plant is traditionally applicable for the treatment of several diseases, but to our knowledge, there are no systematic scientific studies on it. This paper deals with the analgesic and anti-inflammatory activities of *Rhynchosystylis retusa* (L.) Blume in mice model.

Materials and Methods

Plant and extract

Rhynchosystis retusa (L.) Blume leaves were collected from Panchagar district of Bangladesh on 3rd April, 2010 and identified by National Herbarium of Bangladesh, where a voucher specimen was conserved under the reference number DACB Accession Number 35584. The leaves were washed with water and shade dried for 20 days. The leaves were powdered by using blender. Dried powdered leaves (50 g) were extracted by MeOH at room temperature (300 ml × 72 hrs × 3 times). Solvent from each sample was filtered, and evaporated off under reduced pressure in a rotary evaporator to obtain 3 g of crude extract. A yield of 6% of extract was obtained.

Animals

Swiss albino mice of either sex, weighing 22-28 g, obtained from the Animal Resource Division, International Center for Diarrheal Disease and Research, Bangladesh (ICDDR,B), were used throughout the experiments. All animals were kept in standard environment condition, had free access to standard food (ICDDR,B formulated) and water *ad libitum*.

Acetic acid induced writhing method

The analgesic activity of the samples was evaluated using acetic acid induced writhing method in mice (Zulfiker *et al.*, 2010). In this method, acetic acid is administered intraperitoneally (i.p.) to the experimental animals to create pain sensation. As a positive control, indomethacin was used. The plant extract was administered orally (200 mg/kg and 400 mg/kg body weight) to the Swiss Albino mice after an overnight fast. Test samples (aqueous suspensions of the MeOH extract with 1% Tween 80) and vehicle (1% Tween 80 in H₂O) were administered orally 30 minutes prior to intraperitoneal administration of 0.7% v/v acetic acid solution (0.1ml/10g) and indomethacin was administered 15 minutes prior to acetic acid injection. Then the animals were placed on an observation table. Each mouse of all groups was observed individually for counting the number of writhing they made in 10 minutes commencing just 5 minutes after the intraperitoneal administration of acetic acid solution. Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete

writhing was considered as half-writhing. Accordingly, two half-writhings were taken as one full writhing. The number of writhes in each treated group was compared to that of a control group, while indomethacin (10 mg/kg) was used as a reference substance (positive control).

Carrageenan induced mice paw edema method

Anti-inflammatory activity was assessed by the method described by Okokon and Nwafor with slight modification (Okokon and Nwafor, 2010). Swiss albino mice of weighing 22-28 g (collected from the animal house of ICDDR,B, Bangladesh) were divided in 5 groups (n=5). Group-I received 0.5% carboxymethyl cellulose (CMC) suspension (control), group-II received indomethacin (reference drug 10 mg/kg, p.o.), and group-III, IV and V received extracts (100 mg/kg, 200 mg/kg and 400 mg/kg, p.o.), respectively. Subsequently, 1 h after treatment, 0.05 ml of 1% suspension of carrageenan in normal saline was injected into the sub-planter region of left hind paw to induce edema. The paw edema was measured initially at 0, 1, 2, and 3 hr after carrageenan injection. The difference between the initial and subsequent values gave the actual edema which was compared with control. The inhibition of inflammation was calculated using the following formula: % inhibition = 100 (V_c-V_t/V_c), where V_c represents mean edema in control and V_t mean edema in group treated with drug and test extract.

Formaldehyde-induced hind paw edema

The test was performed according to the technique described by Amresh *et al.* (2007). Pedal inflammation was induced by injecting 0.05 ml of 4% formaldehyde solution below the plantar aponeurosis of the right hind paw of the mice. The paw edema was recorded immediately prior to compound administration (0 h) and then at 1.5, 24 and 48 h after formaldehyde injection. Vehicle (distilled water 10 ml/kg, p.o.), extract (200 mg/kg and 400 mg/kg, p.o.) and standard drug, aspirin (300 mg/kg, p.o.) were administered 1 h prior to formaldehyde injection.

Statistical analysis

All data were expressed as mean ± SEM using one-sample T Test. Comparison with control was made using one way ANOVA followed by Dunnett's multiple comparison tests. Significance level was p < 0.05.

Results

Acetic acid induced writhing method

Table 1 shows the effect of extract on acetic acid induced writhing in mice. The extract (200 mg/kg and 400 mg/kg) showed significant reduction ($p < 0.05$ and $p < 0.01$) of writhing induced by the acetic acid after oral

administration in a dose dependant manner. After oral administration of extract (200 mg/kg and 400 mg/kg body weight), the percent inhibition was 28.84% for 200 mg/kg, and 35.81% for 400 mg/kg. The reference drug indomethacin was found slightly potent than the extract.

Table 1: Effect of extract on acetic acid induced writhing in mice.

Groups	Treatment	Dose, Route	No. of writhing	Percent inhibition
Group-I	1% Tween 80 in water	0.4 ml/mouse, p.o.	43.00±3.97	-
Group-II	Indomethacin	10 mg/kg, p.o.	25.20±1.88**	41.40
Group-III	Extract	200 mg/kg, p.o.	30.60±4.19*	28.84
Group-IV	Extract	400 mg/kg, p.o.	27.60±1.12**	35.81

Results are represented as Mean ± SEM, (n=5), * $p < 0.05$, ** $p < 0.01$, Dunnett's test as compared to control.

Carrageenan induced paw edema

The effect of extract (100 mg/kg, 200 mg/kg and 400 mg/kg) in carrageenan induced paw edema in mice is shown in Table 2 and 3. The extract (100 mg/kg, 200 mg/kg and 400 mg/kg) prevented the formation of edema induced by carrageenan and thus showed significant anti-inflammatory activity ($p < 0.05$, $p < 0.01$, $p < 0.001$). The extract (100 mg/kg, 200 mg/kg and 400 mg/kg) reduced the edema induced by

carrageenan with the mean inhibition of 7.80% for 100 mg/kg, 8.67% for 200 mg/kg and 14.32% for 400 mg/kg after 4 hrs of extract administration as compared to the control vehicle treated group. Indomethacin at 10 mg/kg inhibited the edema by 9.30%. On carrageenan induced acute inflammation model, extract (100 mg/kg, 200 mg/kg and 400 mg/kg) produced better inhibition of paw edema. The inhibition is increased by increasing dose of the extract.

Table 2: Anti-inflammatory activities of extract and Indomethacin on carrageenan-induced edema in the left hind limb of mice.

Group (n=5)	Treatment	Dose (mg/kg)	Edema diameter (mm) after carrageenan			
			0 hr	1 hr	2 hr	3 hr
I	Control	10 ml/kg	2.94±0.02	3.0±0.03	3.04±0.04	3.12±0.06
II	Indomethacin	10	2.90±0.04	2.92±0.04	2.76±0.08*	2.62±0.08**
III	Extract	100	2.90±0.04	2.92±0.04	2.78±0.02*	2.74±0.02**
IV	Extract	200	2.88±0.04	2.90±0.04	2.76±0.02*	2.70±0.03**
V	Extract	400	2.92±0.04	2.76±0.12*	2.64±0.10**	2.44±0.14***

Each value is mean ± SEM, n=5 mice

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

One way ANOVA followed by Dunnett's multiple comparison tests.
Statistically significant when compared to control.

Table 3: Percentage inhibition of paw edema exhibited by extracts.

Treatment	Percentage inhibition at various time intervals			
	1 hr	2 hr	3 hr	Mean of % inhibition
Indomethacin 10 mg/kg	2.67	9.21	16.03	9.30
Extract 100 mg/kg	2.67	8.55	12.18	7.80
Extract 200 mg/kg	3.33	9.21	13.46	8.67
Extract 400 mg/kg	8.00	13.16	21.80	14.32

Formaldehyde-induced hind paw edema
 Extracts (200 mg/kg and 400 mg/kg, p.o.) significantly diminished the mean paw edema at 24 h (13.76% and 14.09%) ($p < 0.01$, and $p < 0.001$). The maximum inhibition of edema produced by extract (400 mg/kg, p.o.) was more

than that of aspirin (300 mg/kg, p.o., 9.40% at 24 h). Interestingly, the effect of extract persisted up to a period of 24 h in contrast to aspirin. The effect was significant only at 24 hr (Table 4).

Table 4: Effect of extract on formaldehyde induced hind paw edema in mice.

Treatment	Dose (mg/kg)	Formaldehyde induced hind paw edema (mm)					
		1.5 h	Percentage protection	24 h	Percentage protection	48 h	Percentage protection
Control	10 ml/kg	3.08±0.15	-	2.98±0.06	-	2.52±0.05	-
Aspirin	300	3.07±0.09	0.32	2.70±0.08*	9.40	2.51±0.06	0.40
Extract	200	3.02±0.04	1.95	2.57±0.04**	13.76	2.47±0.05	1.98
Extract	400	2.95±0.13	4.22	2.56±0.05***	14.09	2.45±0.07	2.78

Values are mean ± SEM, (n=5). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared to control.

Discussion

Acetic acid induced writhing is the main point for finding new peripherally active analgesic drugs (Hasan et al., 2010). Pain sensation in writhing method is due to abdominal constrictions associated with irritation of peritoneal cavity by acetic acid. Prolonged acetic acid induced irritation lead to increase levels of prostaglandins (PGE₂ and PGF_{2α}) biosynthesis via cyclooxygenase (COX) and lipoxygenase products in peritoneal fluids followed by increase levels of free arachidonic acid secretion from tissue phospholipid. These increase levels of prostaglandins and lipoxygenase products enhance inflammatory pain by increasing capillary permeability in peritoneal cavity of abdomen (Zulfiker et al., 2010; Zakaria et al., 2008). The analgesic agents reduced the number of writhing preferably by inhibition of synthesis of prostaglandins and lipoxygenase products (Ferdous et al., 2008). The crude extract showed significant analgesic action

compared to the reference drug indomethacin by reducing the number of acetic acid induced writhing in mice at doses of 200 mg/kg and 400 mg/kg. Thus, the results indicate that the significant pain reduction of the plant extract might be due to the presence of peripherally active analgesic principles.

The most widely used test to evaluate anti-inflammatory agents is the ability of a compound to inhibit paw edema induced by injection of a phlogistic agent. Carrageenan induced edema is used for acute inflammation test and is believed to be biphasic. The early phase (1-2 h) is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes and prostaglandins produced by tissue macrophages (Gupta et al., 2006). The extract (100 mg/kg, 200 mg/kg and 400 mg/kg) showed significant inhibitory activity over a period of 3 h in

carageenan induced inflammation. The activity was higher than that exhibited by the group treated with indomethacin 10 mg/kg. The highest percentage inhibition was found in the dose of 400 mg/kg with the mean percentage inhibition of 14.32% after 4 hours of extract administration. These results indicate that extract acts in both phases in dose dependent manner.

Conclusion

The methanolic leaf extract of *Rhynchosystylis retusa* (L.) Blume showed a significant analgesic activity in writhing test and a beneficial preventive effect in carrageenan and formaldehyde induced paw edema. However,

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