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Phytochemical Investigation and *in vitro* Antinociceptive Activity of *Clerodendrum indicum* Leaves

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Abstract: The crude ethanolic extracts of *Clerodendrum indicum* Linn. leaves were investigated for possible antinociceptive activity using acetic acid induced writhing model in mice. Phytochemical analysis was also carried out according to the standard procedures to identify the presence of different phytoconstituents in the ethanolic extract of the plant leaves. The study results showed 38.91 and 55.24% inhibition of writhings in the tested mice when ethanolic extract of *Clerodendrum indicum* Linn. leaves at doses of 250 and 500 mg kg^{-1} body weight was given intraperitoneally, respectively. The study results were also compared with antinociceptive activity of the standard drug, Diclofenac sodium (68.37% inhibition) used at 25 mg kg^{-1} body weight. At the above doses, the crude ethanolic extract of the plant showed significant antinociceptive activity in dose dependent fashion in acetic acid-induced writhing model in mice. The inhibition of writhings was calculated in respective to control group and it was found that p-values (<0.0001) obtained in all cases were extremely statistically significant. However, the phytochemical analysis showed the presence of alkaloid, steroid, saponin, tannin, reducing sugar and gum. The results suggest that crude ethanolic extracts of *Clerodendrum indicum* leaves possess significant antinociceptive properties justifying its folkloric use as analgesics and further research is necessary to isolate the principle phytochemical constituent(s) responsible for this activity.

Key words: *Clerodendrum indicum,* antinociceptive, ethanolic extract, phytochemical

INTRODUCTION

Medicinal plants are important sources for new chemical compounds with significant pharmacological effects (Farnsworth, 1989). They act as natural reservoir of many analgesic or anti-inflammatory agents. In Bangladesh, the plants are used in traditional medical practice for the treatment of various diseases (Rashid *et al*., 1997). In the present study, *Clerodendrum indicum* Linn. (English name: Skyrocket, Bengali name: Bamunhati, Family: Verbenaceae) was included. It is a shrub about 2.5 m high with leaves stalked, 6-23 cm long, 1-5 cm wide, lanceolate, base narrowed and subentire. The plant is widely distributed throughout Southeast Asia, India, Nepal, Bhutan, Sri Lanka and southern China (Manandhar and Manandhar, 2002). The plant extract has traditional uses in serofulous infection, buboes problem, venereal infections and skin diseases. In addition to this, it has been employed as a vermifuge and febrifuge (Rehman *et al*., 1997). Rheumatism, asthma and other inflammatory diseases can also be treated with the root and leaf extracts of *Clerodendrum indicum* (Shrivastava and Patel, 2007).

Moreover, the juice of leaves has wider applications in hepatic eruptions and pemhigus (Rahman *et al*., 2000). The methanolic extract of the plant has been shown to inhibit lipid peroxidation in bovine brain (Kumar and Muller, 1999). The stem and root of the plant contain two flavonoidal compounds such as pectolinarigenin and hispidulin. The petroleum ether, chloroform and ethyl acetate extracts of the stem and root of the plant showed significant antibacterial activities against twelve pathogenic bacteria (Rahman *et al*., 2000).

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Ravindranath *et al.* (2003) also isolated clerodendrone, a novel hydroquinone diterpenoid from *Clerodendrum indicum*. The inflammatory response constitutes complex biological activities like various types of enzyme activation, mediators release, fluid extravasations, cell migration, tissue break down and repairs (Vane and Botting, 1995). It was also suggested that the mechanism of action of analgesic activity may be due to the blockade or release of endogenous substances such as lipoxygenase and/or cyclo-oxygenase (Gupta *et al*., 2007) that stimulate pain nerve endings (Lompo *et al*., 2007).

Currently many NSAIDs have been developed but none of them are not clearly safe (Rang *et al*., 2003) and show wide ranges of adverse effects. Due to adverse reactions of these synthetic drugs, herbal medicines are now getting much attention worldwide for the discovery of new therapeutic agents. As a part of our continuing studies on the medicinal plants of Bangladesh, it was our interest to investigate whether, *Clerodendrum indicum* has any effect on pain and inflammation.

MATERIALS AND METHODS

Collection of plant materials: The leaves of *Clerodendrum indicum* Linn. were collected from Baldha Garden, Dhaka, Bangladesh on 2009 and its aerial part was identified taxonomically by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (Accession No. DACB 31184).

Preparation of plant extracts: The leaves were dried by shed drying for about one week and then grounded into a coarse powder (1.0 kg) with the help of a suitable blender. Two hundred and fifty grams of powered material was extracted with 850 mL of 80% ethanol for 17 days with occasional shaking and stirring upto 2 inch height above the sample surface as it could sufficiently cover the sample surface. Then, it was filtered through whatman filter paper. After filtration the remaining portion of the plant extract was given for re-extraction for 7 days with another 250 mL of ethanol. The liquid extract was then concentrated on a water bath to give a greenish black type residue of 3.2 g (yield 2.13%).

Experimental animals: The experiments were carried out on 20 swiss albino mice aged 4 to 5 weeks and weighing 25 to 30 g of both sexes collected from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDRB). They were housed under room temperature, relative-humidity and light/dark cycle and were fed ICDDRB formulated rodent food and water *ad libitum*. Before starting of the experiment, all the mice were subjected to acclimatize for one week under standard experimental conditions (relative humidity 55 to 65% room temperature 25±2.0°C and 12 h light/dark cycle).

Chemicals and standard drug: Acetic acid and ethanol were collected from LOBA Chemicals Pvt. Ltd., India. Diclofenac sodium was obtained from Incepta Pharmaceuticals Ltd., Bangladesh. All the chemicals used in the entire study were of analytical grade.

Preliminary phytochemical screening: Preliminary phytochemical analysis of the ethanolic extract of *Clerodendrum indicum* was carried out based on the standard methods to identify the presence of alkaloid, steroid, flavonoid, tannin, reducing sugar, saponin and gum (Trease and Evans, 1989).

Experimental model: The antinociceptive activity of *Clerodendrum indicum* leaves extract was measured by acetic acid induced writhing method as previously described (Whittle, 1964) with minor modifications. In the writhing model, 1% acetic acid was used to induce pain through intraperitoneal administration. For experiments with *Clerodendrum indicum* Linn. leaves extract, mice were randomly divided into four groups of five mice each where Group-1 served as control and was administered 1% Tween solution in water (10 mg kg^{-1} body weight). On the other hand, Diclofenac sodium (25 mg kg^{-1} body weight) was administered to Group-2. In addition to these, Groups 3 and 4 received ethanolic extract of *Clerodendrum indicum* leaves at doses of 250 and 500 mg kg^{-1} body weight, respectively before intraperitoneal administration of acetic acid. A period of 5 min was given to each mouse to make sure the bioavailability of acetic acid and the number of abdominal contractions (writhings) was observed for 5 min after stimulation for a period of 10 mins.

Statistical analysis: The percentage inhibition of writhing was obtained using the following formula:

$$\% \text{ inhibition} = \frac{\text{Mean No. of writhing (control)-Mean No. of writhing (test)}}{\text{Mean No. of writhing (control)}} \times 100$$

The results of the experiment were expressed as Means±Standard Error of Mean (SEM). Student's t-test (GraphPad Software) was used to determine a significant difference between the control and experimental groups where p values of less than 5% ($p<0.05$) was chosen as the level of significance.

RESULTS

Preliminary phytochemical screening: The results of phytochemical investigation of the ethanolic extract of

Table 1: Results of preliminary screening of ethanolic extract of the leaves of *Clerodendrum indicum*

Extracts	Alkaloid	Steroid	Flavonoid	Tannin	Reducing sugar	Saponin	Gum
EECI	+	+	-	+	+	+	+

+: Present and -: Absent

Table 2: Effect of ethanolic extract of the leaves of *Clerodendrum indicum* on acetic acid-induced writhing in mice

Treatment	Writhing (Mean±SEM)	Inhibition of writhing (%)	95% **CI	p-value
1% Tween solution in water (10 mg kg^{-1} body weight)	36.26±0.14	-	-	-
Diclofenac sodium (25 mg kg^{-1} body weight)	11.47±0.14	68.37*	24.34 to 25.24	<0.0001
Ethanol extract (250 mg kg^{-1} body weight)	22.15±0.09	38.91*	13.73 to 14.49	<0.0001
Ethanol extract (500 mg kg^{-1} body weight)	15.61±0.21	55.24*	20.05 to 21.25	<0.0001

Values are expressed as Mean±SEM, SEM = Standard error of mean, n = No. of mice, % = Percentage. *Significant at 5% significance level. *p <0.05 vs. control, **CI = Confidence interval

Clerodendrum indicum leaves are summarized in Table 1. Phytochemical study of ethanolic extracts of *Clerodendrum indicum* revealed the presence of alkaloid, steroid, saponin, tannin, reducing sugar and gum.

Antinociceptive activity: In acetic acid-induced writhing model, the crude ethanolic extract of *Clerodendrum indicum* leaves showed a significant dose-dependent decrease in the number of writhings. Even at the lowest dose of the plant extract tested (250 mg extract kg^{-1} body weight), the extract showed 38.91% inhibition of writhings in the experimental animals compared to the standard drug, Diclofenac sodium at 25 mg kg^{-1} body weight (68.37% inhibition) and the reduction was extremely statistically significant ($p<0.0001$). However, the crude ethanolic extract, when administered at a dose of 500 mg extract kg^{-1} body weight, significantly reduced the number of writhings (55.24% inhibition; $p<0.0001$), which was comparable to the inhibition observed with a standard antinociceptive drug, Diclofenac sodium at 25 mg kg^{-1} body weight (68.37% inhibition). The results are shown in Table 2.

DISCUSSION

It is reported that acetic acid stimulates the production of prostaglandin which ultimately causes writhing (Satyanarayana *et al*., 2004) and acetic acid writhing model is also used to assess peripherally acting analgesic drugs (Eddy and Leimbach, 1953). In the present study, the ethanolic extract of *Clerodendrum indicum* leaves attenuated the acetic acid induced writhing which may suggest that ethanolic extract of the medicinal plant has peripheral antinociceptive potentials. It was also revealed that saponins possessed analgesic activities (Amabeoku and Kabatende, 2011). Various flavonoids (Rahman *et al*., 2011a; Zakaria *et al*., 2006) and polyphenolic compound such as tannin have been reported to possess several pharmacological potentials, including antinociceptive activity (Rahman *et al*., 2011b).

Thus, the presence of saponins and tannins in the crude plant extract may play as important contributors to the antinociceptive activities of *Clerodendrum indicum*. From the experiment, it is clearly evident that the ethanolic extract of the plant may contain some active phytoconstituents which are responsible for the inhibition of prostaglandin synthesis or may have particular mechanism to work against pain produced by acetic acid. Finally, it may be concluded that crude ethanolic extract *Clerodendrum indicum* possesses a significant antinociceptive activities and the results tend to corroborate the traditional use of this plant in the treatment of pain. However, further investigations are required to identify the active constituent(s) and to verify the therapeutic merits of the active constituent(s).

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