Global Journal of Pharmacology 4 (1): 48-50, 2010
ISSN 1992-0075

Evaluation of Anti-Inflammatory Activity of *Clerodendron infortunatum* Linn. Extract in Rats

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Abstract: Inflammation is a response of vascularized living tissue to the local injury. The severe side effects of steroidal and non-steroidal anti-inflammatory drugs evoked us to search for new anti-inflammatory drugs from the indigenous source. The methanol extract of leaves of *Clerodendron infortunatum* Linn. (MECI) was evaluated for anti-inflammatory activity against the carrageenan, histamine and dextran induced rat paw edema. The methanol extract (250 and 500 mg/kg body weight) exhibited significant activity ($p < 0.01$) against all phlogistic agent used in dose dependant manner. All these effects were compared with reference drug phynylbutazone.

Key words: *Clerodendron infortunatum* · Anti-inflammatory · Carrageenan · Histamine · Dextran · Paw edema · Phenylbutazone

INTRODUCTION

Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. It is the body response to inactivate or destroy the invading organisms, to remove irritant and set the stage for tissue repair. It is triggered by the release of chemical mediators from injured tissue and migrating cells [1]. Drugs from plant origin are used in India for treatment of many diseases in traditional system of medicine. *Clerodendron infortunatum* Linn. belonging to family Verbenaceae, have been used in Indian folk medicine in the treatment of bronchitis, asthma, fever, burning sensation, disease of blood, inflammation and epilepsy [2]. Traditionally, the plant is used as an antipyretic and antihelmentic. Leaves of the plant are prescribed for tumour, certain skin diseases and scorpion sting [3]. Previous phytochemical investigation of the plant revealed the presence of alkyl sterols [4] and 2,-(3, 4-dehydroxyphenyl) ethanol 1- *O*-α-2 rhamnopyranosyl-(1→3)-β-D-(4-*O*-caffeoyl) glycopyranoside (acteoside) [5]. The present study was undertaken to evaluate the anti-inflammatory activity of methanol extract of leaves of *Clerodendron infortunatum* Linn. in rats.

MATERIALS AND METHODS

Plant Material: The plant *Clerodendron infortunatum* Linn. was collected during November 2008 from the forest region of Midnapore, West Bengal, India. The taxonomical identification of the plant was done by Botanical Survey of India, Shibpur, India. The voucher specimen (PMU-4/JU/2008) has been preserved in Pharmacology Research Laboratory, Jadavpur University, Kolkata for future reference.

Preparation of Extract: The leaves of the *Clerodendron infortunatum* was dried under shade and then powered by mechanical grinder. The powder plant material was extracted with 80% methanol using Soxhlet extraction apparatus. The solvent was completely removed under reduced pressure and semisolid mass was obtained (Yield 13.5% w/w) stored in a vacuum dessicator for further use. Preliminary phytochemical screening of the plant extract exhibited the presence of flavonoid, tannin and saponin.

Animal Used: Wistar strain rats of either sex (150-180 g) were maintained in identical laboratory conditions (25- 30°C temperature and relative humidity of 55-65%

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with alternate light and darkness 12 hours each) and fed with commercial pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. All procedures described were reviewed and approved by the Jadavpur University animal ethical committee (ref no. 367001/C/CPCACA).

Carrageenan-Induced Rat Paw Edema: Rats were divided into four groups (n=6). Acute inflammation was produced by sub planter administration of 0.1 ml of 1% w/v carrageenan in normal saline in the right hand paw of the rats. The paw volume was measured at 0-h and 3-h after carrageenan injection by using plethysmometer [6,7]. Animals of group I received normal saline (3 ml/kg b.w., intraperitoneal, i.p) and served as saline control. The groups II and III received methanol extract of *Clerodendron infortunatum* (250 and 500 mg/kg b.w., i.p, respectively) and group IV received reference drug phenylbutazone (100 mg/kg b. w., i.p). Animals of all groups were treated with the extract and reference drug 1 hour before the administration of carrageenan.

Mediator-Induced Inflammation: The anti-inflammatory activity of the extract was measured with phlogistic agents (*viz*. Histamine, Dextran) which act as mediator of inflammation. The paw edema was introduced in rats by sub plantar injection of freshly prepared histamine (1 mg/ml) and dextran (1 mg/ml) solution [8] and paw edema was measured as mentioned earlier.

Statistical Analysis: All data were expressed as the mean ± SEM. The results were analyzed for statistical significance (P<0.01) by One-way (ANOVA) followed by Dunnett's test using computerized Graph Pad InStat version 3.05, Graph pad software, U.S.A.

RESULTS

The anti-inflammatory activity of *Clerodendron infortunatum* against carrageen induced paw edema has been shown in Table 1 and the results were comparable to that of reference drug phenylbutazone. The methanol extract of *Clerodendron infortunatum* showed maximum inhibition of 49.64 and 65.63% at the dose of 250 and 500 mg/kg body wt. respectively after 3 hrs of the extract treatment against carrageenan induced paw edema (Table 1) whereas the reference drug produced 76.29% of inhibition at the dose 100 mg/kg body wt. In case of histamine induced paw edema, the methanol extract produced 45.85 and 58.02% of inhibition (Table 2) at the dose of 250 and 500 mg/kg body wt., respectively whereas the reference drug produced 71.22% of inhibition. In case of dextran induced paw edema, the methanol extract produced 39.65 and 57.90% of inhibition (Table 3) at the dose of 250 and 500 mg/kg body wt., respectively whereas the reference drug produced 69.14% of inhibition. The study revealed that after 3 h of carrageenan, histamine and dextran administration,

Table 1: Effect of *Clerodendron infortunatum* extract and phynylbuyazone on carrageenan induced paw edema in rats (n=6)

Treatment	Dose (mg/kg)	Paw volume (ml)	Percentage of inhibition	*P value
Carrageenan control		0.9066±0.03490	-	-
Extract	250	0.4566±0.02275	49.64%	< 0.01
Extract	500	0.3116±0.03468	65.63%	< 0.01
Phenylbutazone	100	0.215±0.01821	76.29%	< 0.01

*P<0.01 when compared with control group; statistically analysis evaluation by Dunnett's vs. control.

Table 2: Effect of *Clerodendron infortunatum* extract and phynylbuyazone on histamine induced paw edema in rats (n=6)

Treatment	Dose (mg/kg)	Paw volume (ml)	Percentage of inhibition	*P value
Histamine control		1.1233±0.04745	-	-
Extract	250	0.6083±0.03936	45.85%	< 0.01
Extract	500	0.4716±0.02469	58.02%	< 0.01
Phenylbutazone	100	0.3233±0.03721	71.22%	< 0.01

*P<0.01 when compared with control group; statistically analysis evaluation by Dunnett's vs. control

Table 3: Effect of *Clerodendron infortunatum* extract and phynylbuyazone on dextran induced paw edema in rats (n=6)

Treatment	Dose (mg/kg)	Paw volume (ml)	Percentage of inhibition	*P value
Dextran control		1.2150±0.03810	-	-
Extract	250	0.7333±0.03029	39.65%	< 0.01
Extract	500	0.5116±0.03229	57.90%	< 0.01
Phenylbutazone	100	0.3750±0.01945	69.14%	< 0.01

*P<0.01 when compared with control group; statistically analysis evaluation by Dunnett's vs. control

the methanol extract of *Clerodendron infortunatum* (MECI) exhibited statistically significant ($p < 0.01$) inhibition of paw volume at doses of 250 and 500 mg/kg body weight, respectively, which was less than that observed with standard drug phenylbutazone ($p < 0.01$) given at a dose of 100 mg/kg body wt.

DISCUSSION

It is evident that carrageenan induced edema is commonly used as an experimental model for inflammation and is belived to be biphasic; the first phase is attributed to the release of histamine, serotonin and kinin and the second phase is related to the release of prostaglandin and bradykinins [9, 10]. So the effect of the extract against inflammation produced by these individual mediators was studied. The extract effectively suppressed the inflammation produced by histamine and dextran. The methanol extract was found to possess tannins, saponin and flavonoid. So the anti-inflammatory activity of this plant may be presence of these chemical constituents. Flavonoids are known to inhibit the enzyme prostaglandin synthesis, more specifically the endoperoxide and reported to produce anti-inflammatory effect [11, 12]. Although preliminary biological study has revealed that MECI possesses significant anti-inflammatory activity, mechanisms underlying the observed pharmacological effects are not clear. The assessment of observed pharmacological effects with isolated individual chemical constituent merit further investigation for better understanding of the molecular mechanisms underlying anti-inflammatory activity of the MECI. However, the present study suggested that the methanol extracts of *Clerodendron infortunatum*, may be used as an herbal remedy for the management of inflammation. Although no sign of toxicity was observed after 3 h of administration of MECI at a dose of 500 mg/kg body weight, however, detailed toxicological study should be performed before exploiting this plant extract for therapeutic purpose.

It is concluded that the methanol extract of leaves of *Clerodendron infortunatum* possesses significant anti-inflammatory activity in dose dependent manner against the tested models.

ACKNOWLEDEMENT

The financial assistance of Department of Pharmaceutical Technology, Jadavpur University, kolkata, India is gratefully acknowledged. This work would not have been possible without the support of authority of Netaji Subhas Chandra Bose Institute of Pharmacy, Chakdaha, Nadia, India.

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