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Evaluation of anti-inflammatory activity of ethanolic extract of leaves of *Clerodendrum serratum* Linn. in experimental animals

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Abstract
The present study was conducted to evaluate the anti-inflammatory activity of *Clerodendrum serratum* Linn. The anti-inflammatory activity was done by *in-vivo* model i.e. the carrageenan induced rat paw edema model. The standard drug diclofenac sodium (500mg/kg) has significantly reduced paw oedema volume by0.15±0.02 at 1st hr, 0.18±0.01 at2nd hr, 0.25 ±0.02 at 3rd hr and 0.3 at±0.02 at 4th hr, thus standard drug has exhibited time dependent reduction in oedema volume. The ethanolic extract with a dose of 200mg/kg b.w has significantly reduced oedema volume by 0.18±0.03, 0.25 ±0.0.02, 0.28. ±0.01, 0.35±0.03 at 1st, 2nd, 3rd and 4th hrs respectively which was found to be a time dependent effect. At the end of 4 hours in test group treated with 200mg/kg b.w of ethanolic extract showed inhibitory effects of 37.5% respectively.

Keywords: anti-inflammatory, ethanolic

Introduction
Disease has been an integral part of man from the beginning of his existence. The subject of drugs is also as old as disease and the search for remedies to combat it is perhaps equally old and for more than millennium, herbal medicine has been extensively used, apparently safely and effectively to alleviate various symptoms of disease. Inflammation is a local response of living mammalian tissues to the injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation represent such components of inflammation [1]. The treatment of inflammation is depends on nonsteroidal or steroidal anti-inflammatory agents [2]. Nonsteroidal antiinflammatory drugs (NSAID) reduce the pain and inflammation by blocking the cyclooxygenase enzyme (COX), and thus the production of prostaglandin [3], but long-term administration of NSAID may induce gastrointestinal ulcers and renal disorders due to their non-selective inhibition of both isoforms of COX enzyme, the constitutive (COX-1) and the inducible (COX-2) isoforms [4]. On other hand COX-2 inhibitors have been associated with cardiovascular side effects [5]. Therefore, developing new agents with more potential antiinflammatory activities and with lesser side effects is, at present, of great interest.
Clerodendrum serratum Linn. belongs to family Verbenaceae is a small perennial shrub growing in moist deciduous forests and occasionally in plains of peninsular India and the Western and Eastern Himalayas up to 1,400 feet above sea level. Therefore, the present study has been taken to validate the traditional claims associated with this plant and to carryout evaluation of anti-inflammatory activity of ethanolic leaf extract of *Clerodendrum serratum* Linn. in experimental animals. A literature review reveals anti-inflammatory property of *Clerodendrum serratum* Linn. So, the present study was carried out to evaluate the antiinflammatory activity of ethanolic extract.

Material & Methods
Preparation of plant material
The healthy leaves of *Clerodendrum searratum* Linn. were collected from Ekant forest park, Bhopal, India.

The plant was identified and authenticated by Dr. Ziaul Hassan, Professor of Botany, Saifia Science College, Bhopal, India. A voucher specimen No.305/Bot/Saifia/11 has been submitted to the Department of Botany of Saifia Science College, Bhopal, India for further reference. The collected leaves of *Clerodendrum searratum* Linn. Were thoroughly washed in running tape water and then shade dried. The completely shade dried leaves were homogenised to coarse powder and stored in air tight containers till further use.

Extraction process

A quantity of 100gm of powdered leaves of *Clerodendrum searratum* Linn. was extracted successively by Soxhelet apparatus with 500 ml of methanol (solvent) for a span of 72 hours. The temperature of methanol was kept at 80±5ºc.The extract was filtered using Whatman's No.1 filter paper. The filtered extract was evaporated and concentrated in water bath at a temperature of 40ºc.The extract was preserved in air tight container till further use.

Drugs and Chemicals

Carrageenan was purchased from Himedia. Diclofenac sodium tablets were purchased from rajshree medical store, Bhopal. All other chemicals used for this study were of analytical grade.

Experimental animals

Studies were carried out using Wistar Albino rats of four months of both sexes weighing 140-200 grams. They rats were provided by Sapience Bioanalytical Research Labaratory. The animals were grouped in polypropylene cages with not more than six animals per cage and maintained under standard laboratory conditions (Temperature 25±2°C, relative humidity 60% ±15% and with dark & light circle 12hrs /14hrs). They were allowed free access to standard dry pellet (Hindustan lever, Kolkata, India) and water *ad libitum* (CPCSEA, 2003). The animals were acclimatized to laboratory conditions before commencement of the experiment. All animal experiments were carried out as per the guidelines of CPCSEA and were approved by institutional ethical committee vide approval no. 1413/PO/a/11/CPCSEA.

Test material

The ethanolic extract of *Clerodendrum serratum* Linn. were evaluated for anti-inflammatory activity.

Acute toxicity studies

Acute toxicity studies were carried out on Wistar aibino rats of both sexes according to the OECD guidelines (423). The doses of plant extracts selected for the study were 100 mg/kg, 200 mg/ kg, 500 mg/ kg, 1000 mg/ kg and 2000 mg/kg body weight were administered to separate groups of mice (n = 5) after overnight fasting. Subsequent to administration of drug extract, the animals were observed closely for the first 3 h for any toxic manifestations such as increased locomotor activity, salivation, clonic convulsion, coma and death. Subsequent observations were made at regular intervals for 24 h. The animals were observed for a further week (Ghose 2005). Signs of toxicity, body weight, feed and water consumption of each animal was observed every day for 14 days. The ethanolic extract did not show any signs of toxicity up to the dose of 2000 mg/kg p.o. From the acute toxicity test we selected 200mg/kg dose ($1/10^{th}$ of maximum tolerable dose) for subsequent pharmacological screening.

Experimental design

In the experiment, a total number of 42 rats were used. The rats were divided into 7 groups comprising of 6 animals in each group.

Group I (Normal control)

The rats were treated with distilled water

Group-II (Negative control group)

The rats were injected with 0.1ml of carrageenan on left hind paw.

Group-III (Standard group)

Animals were treated with Diclofenac sodium (10 mg/kg, p.o.) and also injected with 0.1ml carrageenan.

Group IV (Test group)

Animals were treated with a single dose of ethanolic extract 200mg/kg p.o. of *Clerodendrum serratum* Linn. daily for seven days and were also injected with 0.1 ml of carrageenan on 7^{th} day after 1h of last dose of ethanolic extract.

Animals were treated with a single dose of n-hexane extract 200mg/kg p.o. of *Clerodendrum serratum* Linn. daily for seven days and were also injected with 0.1 ml of carrageenan on 7^{th} day after 1h of last dose of ethanolic extract.

Carrageenan induced rat paw edema

Carrageenan induced rat paw edema was done by the method of Winter *et al*. (1962). Inflammation was induced by injection of 0.1 ml of freshly prepared carrageenan (1%) ethanolic suspension in normal saline underneath the plantar tissue of the right hind paw of rats. The 4^{th} group of rats were administered with 200mg/kg p.o. ethanolic extract of *Clerodendrum serratum* Linn. The normal control group received vehicle (Distilled water, 10 ml/kg, p.o.). The negative control group recived Sodium carboxyl methyl cellulose (0.5% CMC) in distilled water at 10 mL/kg body weight and standard group received Diclofenac sodium (10 mg/kg, p.o.). 1h after drug treatment, paw edema was induced by the injection of carrageenan (An edematogenic agent). The paw volume was measured by a Plethysmometer at 0 h, 1 h, 2 h, 3h, and 4h after the carrageenan injection. Edema was expressed as mean increase in paw volume relative to control animals. The percentage inhibition of edema was calculated by the following equation:

$$\% \text{ inhibition of edema} = \frac{1\text{-}Vt}{Vc} \times 100$$

Where Vc is the edema volume in the control group and Vt is the edema volume in tested groups.

The animals were fasted for 24 h prior to the experiment. A mark was made on the right hind paw just beyond the tibio-tarsal junction to ensure that the paw volume, as measured with a plethysmograph, was measured consistently every time it was dipped in the mercury (Hg) column up to the fixed mark. The initial volume was noted for each rat by the mercury (Hg) displacement method. After 0 h, 1 h, 2 h, 3h,

and 4h of carrageenan administration, the paw volumes of all groups were measured using a plethysmograph.

Results

Intraperitoneal injection of carragenean to rats caused an inflammatory reaction and in presence of our test extracts inhibitory effects were observed one hour before the injection of carrageenan. These effects were observed at four time points (1, 2, 3and 4 hours). In carregenean administered animals the severe paw swelling observed reached maximum at 4th hour. The ethanolic extract of *Clerodendrum serratum* Linn. With a selected dose of 200mg/kg b.w have exhibited a significant reduction in paw oedema volume in carrageenan induced paw oedema in rats at different time intervals. Results are tabulated in Table 1. The standard drug diclofenac sodium (500mg/kg) has significantly reduced paw oedema volume by 42.30% at1st hr, 45.45% at 2nd hr, 41.86% at 3rd hr and 46.42% at 4th hr, thus standard drug has exhibited time dependent reduction in oedema volume. The ethanolic extract with a dose of 200mg/kg b.w has exhibited significant reduction in paw volume by 30.76%, 24.24%, 34.88% and 37.50% at 1st, 2nd, 3rd and 4th hrs respectively which was found to be a time dependent effect. At the end of 4 hours in test group treated with 200mg/kg b.w of ethanolic extract showed inhibitory effects of 35.5% respectively.

Our data revealed that in carrageenan administered animals (Group II) swelling reached its maximum by the end of 4th hour and in case of standard treated group swelling was inhibited to 46.42% at the end of 4th hour in comparison to carrageenan treated group.

Table 1: Effect of Ethanolic extract of *Clerodendrum serratum* on carrageenan induced paw edema in rats

Groups	Paw volume (mm) (Mean ± SEM)					% Inhibition
	0hr	1 hr	2 hr	3hr	4hr	
Group I	0.14±0.011	0.14±0.011	0.14±0.011	0.14±0.011	0.14±0.011	
Group II	0.11±0.13	0.26±0.2	0.33±0.02	0.43±0.02	0.56±0.03	-
GroupIII	0.11±0.025	0.15±0.02a*	0.18±0.01a**	0.25±0.02a**	0.3±0.02a**	46.42
GroupIV	0.11±0.45	0.18±0.03	0.25±0.02	0.28±0.01a**	0.35±0.03a**	37.5

All values are mean ± SEM, n = 6. $^{*}p<0.05$, $^{**}p<0.01$

a) Significance difference as compared to group-I (Inducer control).

b) Significance difference as compared to group-II (standard).

Statistical analysis

Results are expressed as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison test. *P* =.05 was considered statistically significant.

Discussions

Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and devoid of apparent systemic effect. It induce oedema is a biphasic response which is shown to be mediated by histamine and serotonin during first 1h. After which increased vascular permeability is maintained by the release of kinins upto 2.5 h, followed by the release of kinins and finally through the release of bradykinin, prostaglandin and lysosomes from 2.5 to 6 h. The later phase is reported to be sensitive to most of the clinically effective antiinflammatory agents[7]. The mediators appear to be prostaglandins, the release of which is closely associated with migration of leucocytes into the inflamed site [8]. The Carrageenan induced paw edema model in rats is known to be sensitive to cyclo-oxygenase (COX) inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory drugs (NSAID) [9, 10]. This method was chosen for this study since it is the most prominent experimental model in search for new antiinflammatory drugs and evaluation of anti-inflammatory effect of natural products [11, 12]. We found that the administration of the ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions at the dose of 200 mg/kg, p.o reduced significantly the carrageenan-induced paw oedema.

The injection of carrageenan produces a typical biphasic oedema associated with the production of several inflammatory mediators such as histamine, serotonin, bradykinin, prostaglandins, nitric oxide, and cytokines [15]. According to the result of our study, the ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions were able to effectively inhibit the oedema in the later phases, suggesting that these compounds inhibit different chemical mediators of inflammation.

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