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Malik Tafazul Rashid
Molecular Biology and Seed Technology Laboratory, Govt. Motilal Vigyan Mahavidyalaya (MVM), Bhopal, Madhya Pradesh, India

Sajad H Wani
Biotechnology division, Central Institute of Temperate Horticulture, Old Air Field Rangreth, Srinagar, Jammu & Kashmir, India

Shabir A Lone
Govt. Degree College Boys Ananthnag, Kashmir, Jammu & Kashmir, India

AS Yadav
Molecular Biology and Seed Technology Laboratory, Govt. Motilal Vigyan Mahavidyalaya (MVM), Bhopal, Madhya Pradesh, India

Correspondence
Malik Tafazul Rashid
Molecular Biology and Seed Technology Laboratory, Govt. Motilal Vigyan Mahavidyalaya (MVM), Bhopal, Madhya Pradesh, India

Phytochemical investigation and Antiinflammatory activity of aqueous extract of leaves of *Clerodendrum serratum* Linn.

Malik Tafazul Rashid, Sajad H Wani, Shabir A Lone and AS Yadav

Abstract
The present investigation was carried out to find the effect of aqueous extract of leaves of *Clerodendrum serratum* Linn. for antiinflammatory activity in rodents. The aqueous 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. The standard drug diclofenac sodium (500mg/kg) has significantly reduced paw oedema volume by 42.30% at 1st 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 significantly reduced oedema volume by 11.53%, 15.15%, 23.25% and 28.57.% and 19.14% 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 aqueous extract showed inhibitory effects of 28.57% respectively.

Keywords: *Clerodendrum serratum* Linn., diclofenac, carrageenan, paw edema

Introduction
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 inflamemation (Mitchell and Cotran, 2000) [1]. Oedema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow (Ialenti *et al*., 1995) [2]. Several experimental models of paw oedema have been described. Carrageenan-induced paw oedemais widely used for determining the acute phase of inflamemation. Histamine, 5-hydroxytryptamine and bradykinin are the first detectable mediators in the early phase of carrageenan-induced inflammation (Di and Willoughby, 1971) [3] whereas prostaglandins are detectable in the late phase of inflammation (Salvemini *et al*., 1996) [4]. Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone. All of these drugs possess well known side and toxic effects. Moreover, synthetic drugs are very expensive to develop and whose cost of development ranges from 0.5 to 5 million dollars. On the contrary many medicines of plant origin had been used since long time without any adverse effects. Exploring the healing power of plants is an ancient concept. For centuries people have been trying to alleviate and treat disease with different plant extracts and formulations (Cowan, 1999) [5]. It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper drugs. Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs. Screening of the plants for their biological activity is done on the basis of either their chemotaxonomic investigation or ethnobotanical knowledge for a particular disease. Identification of a particular compound against a specific disease is a challenging long process. Importance of the plant lies in their biologically active principles. There are two types of plant chemicals, primary metabolites such as sugars, proteins, amino acids, chlorophylls etc.

The other category of chemicals is called secondary metabolites, which includes alkaloids, terpenoids, saponins and phenolic compounds. These chemicals exert a significant physiological effect on the mammalian system. A lot of references are available in the field of ethnomedicinal plants used as anti-inflammatory drugs. Bagul *et al*. *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. The leaf and root of this plant have great medicinal value. Ethnopharmacological and ethnobotanical knowledge are percolating down to these days among the tribal population, but much of this information is empirical at best, and lacks preclinical scientific validations. Therefore, the present study has been taken to validate the traditional claims associated with this plant and to carryout phytochemical investigation and evaluation of anti-inflammatory activity of aqueous leaf extract of *Clerodendrum Serratum* Linn.

Material & Methods

Preparation of plant material

The healthy leaves of *Clerodendrum serratum* 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 serratum* 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 serratum* 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*. 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 aqueous 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. Signs of toxicity, body weight, feed and water consumption of each animal was observed every day for 14 days. The aqueous 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 aqueous 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 aqueous 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) [6]. Inflammation was induced by injection of 0.1 ml of freshly prepared carrageenan (1%) aqueous 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. aqueous 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:

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

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 carrageenan 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 aqueous 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 aqueous extract with a dose of 200mg/kg b.w has significantly reduced oedema volume by 11.53%, 15.15%, 23.25% and 28.57.% and 19.14% 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 aqueous extracts showed inhibitory effects of 28.57% 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 aqueous 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	-
Group III	0.11±0.025	0.15±0.02a*	0.18±0.01a**	0.25±0.02a**	0.3±0.02a**	46.42
Group IV	0.10±0.024	0.23±0.0.2	0.28±0.03b*	0.33±0.02a**b*	0.40±0.03a**	28.57

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.

Discussion

The anti-inflammatory effects of aqueous leaf extract of *Clerodendrum serratum* Linn. with a selected dose of 200mg/kg b.w were evaluated on carrageenan (acute) induced inflammation in rats. The injection of carrageenan to the hind paw of rats is a common model to study inflammation and inflammatory pain. From the result, it is clear that aqueous leaf extracts 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. The results of our study are in agreement with the studies conducted by Singh *et al*. 2012 [7] who evaluated anti-inflammatory activity of ethanolic root extract of *Clerodendrum serratum* Linn. using carrageen ininduced oedema model and cotton pellet model in experimental mice, rats and rabbits at concentrations of 50,100and 200mg/kg Surendrakumar 1988 [8]. He also reported that *Clerdendrum phlomidis* significantly decreasing paw oedemas induced by carrageenan in rats at a dose of 1g/kg. Bhangare *et al*. 2012 also assessed anti-inflammatory activity in rats by Granuloma pouch method. Their study shown that Low Dose (LD) of *Clerodendrum serratum* Linn. root and High Dose of stem Show anti-inflammatory (23%) and anti-allergic activity (21%) equivalent to Dexamethasone (21%). The alcoholic extract of roots of *Clerodendrum serratum* Linn. showed a significant anti-inflammatory activity in carrageenan and also in the cotton pellet model in experimental mice, rats and rabbits (Narayanan *et al*. 1999) [10].

Changes in paw volume after the injection of carrageenan corresponding to oedema occurred rapidly and in a biphasic manner (Hargreaves *et al*., 1988) [11] The biochemical mechanism for the inflammatory reaction induced by carrageenan in animals, however, is not clear. On the other hand, chemical mediators such as histamine, serotonin, prostaglandins and kinin are presumed to be involved in the occurrence and development of inflammation (Matsuda *et al*., 1997) [12].

The acute inflammation is produced when water and plasma increases in tissues during arachidonic acid metabolism via cyclooxgenase (COX) and lipooxgenase (LOX) enzyme pathways (Moura *et al*., 2005) [13]. The first phase of inflammation is characterized by the release of histamine and serotonin that begins immediately after injection and last for one hour. The second phase is characterized by the bradykinin release via prostaglandins mediator, oxygen-derived free radicals and cyclooxgenase that begin after one

hour and last for three hours (Gracia *et al*., 1999 and Panthong *et al*., 2004) [14, 15]. The later phase that begins more than four hours was reported to be sensitive to most clinically effective anti-inflammation agents.

From the results, it is clear that the swelling of rat's hind paw induced by carrageenan on the treated groups were decreased when compared to control group started from 0 minute to 60 minutes. Between this first hour, the compounds that present in the extracts may involve in the reduction of histamine and serotonin release as first phase of acute inflammation. The second phase that started from 90 minutes to 240 minutes showed a decreasing in swelling of rat hind paw. Thus, the compounds of plant extracts may also involve inhibiting the prostaglandin production.

The onset of inflammation and increased prostaglandin production are usually related to COX-2 expression. After injection of carrageenan, substantial induction of COX-2 was observed after three hours with enhanced level of thromboxane B2 (TxB2) and local oedema (Seibert *et al*., 1994) [16]. Therefore, the ability of plant extracts in oedema inhibition may resulted from the action on COX-2. It can therefore be postulated that EAT inhibited the production of second phase of chemical mediators such as prostaglandin and bradykinin, and or antagonized the actions of these chemical mediators (Otterners *et al*., 1982) [17]. Diclofenac sodium was used as a positive control because it strongly inhibits both COX-1 and COX-2 at therapeutic concentrations, and shows some preference for COX-1.

The possible mechanism which involved in the reduction of oedema volume may come from the inhibition or antagonism of actions of chemical mediators such as histamine (phase 1) and prostaglandins (phase 2) via COX-2. The mechanism identified for the inhibition of histamine release was related to calcium concentration (Lee *et al*. 2006) [18]. The aqueous leaf extracts of *Clerodendrum serratum* Linn. exhibits promising anti-inflammatory effect in acute inflammation model. The effect is almost comparable to that of the standard drug. However, the exact mechanism of action for its pharmacological activity has not been determined yet. Studies are underway to evaluate the mediators and pathways involved in the inflammatory activity. Also, it is worthwhile to isolate the bioactive compounds which are responsible for those activities.

Plants contain an array of secondary metabolites which include phenolic compounds, (Phenols, flavonoids, coumarins), nitrogen compounds (Alkaloids, nitosoamines and other components). Many of such naturally occurring compounds such as polyphenolics, diterpenoids, triterpenoids are reported to ameliorate inflammatory response. The qualitative screening of phytochemicals from *Clerodendrum serratum* Linn. showed high phenolic content. Total flavonoid and flavanol concentration was also significantly high. Flavonoids also have biochemical effects including inhibition of enzymes such as lipoxygenase, cycloxygenase, etc. and have been found to have anti-inflammatory activity in both proliferative and exudative phases of inflammation. It has also been demonstrated that flavonoids inhibit the expression of isoforms of inducible nitric oxide synthase, cyclooxygenase and lipooxygenase which are responsible to cause diminished formation of pro-inflammatory mediators (Prostaglandins, leukotrienes, reactive oxygen species, nitric oxide) as well as other mediators of the inflammatory process such as cytokines, chemokines or adhesion molecules. The diverse mode of action of *Clerodendrum serratum* Linn. may be attributed possibly due to the high polyphenolic and flavonoid content present in the extract. The results of our study indicate that the aqueous, alcoholic, ethylacetate and n-Hexane leaf extracts of *Clerodendrum serratum* Linn. demonstrated highly potent anti-inflammatory effects mediated by suppression of release of inflammatory mediator, NO, prostaglandins, leukotrienes and pro-inflammatory cytokines. Since pro inflammatory cytokines upregulate the expression of such genes (NF-kB) that are involved in inflammatory response and cause coinduction of iNOS and COX2, the two pivotal enzymes related to NO and eicosanoid production in inflamed tissues, *Clerodendrum serratum* Linn. can be presumed to act through inhibition of NF-κB signaling pathway leading to reduced expression of interdependent cytokine and NO production and in turn lead to decreased COX enzyme activity.

Conclusion

Thus, it can be concluded that the aqueous extract of leaves of *Clerodendrum serratum* Linn. possess anti-inflammatory activity. Further studies involving the purification of the chemical constituents of the plant and the investigations in the biochemical pathways may result in the development of a potent anti-inflammatory agent with low toxicity and better therapeutic index.

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