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Anti-inflammatory and Analgesic activity of mature leaves methanol extract of *Clerodendrum inerme* L. (Gaertn)

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Abstract: *Clerodendrum inerme* is a hedge plant belongs to the Verbenaceae family, traditionally used for ornamental purpose in home gardens. In the present study, anti-inflammatory and analgesic effect of methanol extract of *Clerodendrum inerme* (MECI) was evaluated in animal models. The anti-inflammatory activity of MECI exhibited sub-chronic (cotton pellet-induced granuloma) models of inflammation was found to be significant. In addition, the extract also showed significant analgesic activity in acetic acid induced writhing. Therefore, the anti-inflammatory and analgesic activity observed in the present study with MECI could be attributed largely to its antioxidant and lysosomal membrane stabilizing effects.

Keywords: *Analgesic activity, Anti-inflammatory, Clerodendrum inerme*

Introduction

Inflammation is the complex biological response of vascular tissues to harmful stimuli including pathogens, irritants, or damaged cells. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue [1]. The process of inflammation is necessary for healing of wounds, however if not controlled, lead to onset of diseases like vasomotor rhinnorhoea, rheumatoid arthritis and atherosclerosis [2]. Inflammation is characterised by classical signs edema, erythrema, pain, heat, and subsequently loss of function. Inflammatory models are of two types, acute and chronic inflammatory model. Acute models are designed to test drugs that modulate erythema, changes in vascular permeability, leukocyte migration and chemotaxis, phagocytosis poly-morpho nuclear leucocytes and other phagocytic cells, measurement of local pain, antipyretic activity and local analgesic action [3]. Chronic models are designed to find drugs that may modulate the disease process and these include sponge and pellet implants and granuloma pouches which deposit granulation tissue, adjuvant induced arthritis and mono-articular arthritis which have an immune etiology [4].

The genus *Clerodendrum* (Family: Verbenaceae), contains many plant species that are being used in various health care systems for the treatment of various disorders including life threatening diseases [5]. The *Clerodendrum inerme* L. Gaertn commonly known as Kashmir bouquet is a biennial hardy plant and grown as a hedge plant along home gardens throughout the India. The therapeutic studies showed that organic extracts of *C. inerme* revealed strong uterine stimulant activity when tested in female rats and rabbits [6], and also showed strong anti-hemolytic activity in human adults with inhibition of phospholipase [7]. The methanolic leaf extracts of *C. phlomidis* and *C. inerme* showed antispasmodic activity in mouse as well as antifungal activity [8] and anti-carcinogenic effects on hamster [9]. The insecticidal activity results proved that *C. inerme* leaf powder and petroleum ether and ether extracts inhibited the growth and development of *Aedes aegypti*, *Culex quinquefasciatus* and *Culex pipiens* larvae [10, 11, 12]. The ether extract of *C. inerme* was found to possess insecticidal, ovicidal, growth inhibition and morphogenetic effects against various life stages of a noxious lepidopteron insect-pest [13, 14]. The present study was designed to evaluate the anti-inflammatory and analgesic activity of methanol extract of *Clerodendrum inerme* (MECI) in rat and mice respectively.

Materials and Methods

Animals

Healthy and same age male albino rats (170-180 g) and Swiss male mice (25-30 g) were used for present investigation. Animals were breed and reared in the Departmental Animal House (Reg. No. CPCSEA-233) under laboratory conditions of temperature (24°±2°C) and relative humidity (65±5%). Animals were fed with standard pellet food (Amrit animal feed, Navmaharastra Chakan

Oil Mills, Sangli, Maharastra, India) and water *ad libitum*.

Plant material and Extraction process

The mature and succulent leaves of *Clerodendrum inerme* L. (Gaertn) were collected in and around Kolhapur city, Maharashtra at morning (0700-0800 h). The collected leaves were brought to the laboratory, washed with distilled water to remove dust and other contaminants. The clean leaves were air dried for 5-6 days at room temperature (28 ± 2ºC) until all the moisture content was evaporated and dried leaves were pulverized using domestic grinder. The powder of *C. inerme* was extracted with methanol for 10 h by soxhlet procedure, which was repeated for 3 times to ensure the complete extraction of chemical constituents from the leaves [15]. The extract was filtered through Whatman (No. 1) filter paper and concentrated by a rotatory evaporator under low pressure. Dark-green residue obtained was stored in glass vials and kept in a refrigerator (4ºC) until use.

Anti-inflammatory activity in rats

Cotton pellet granuloma was induced as described by Bailey [16]. Two cotton pellets (10 mg) were implanted on either side (one on each side) of the ventral region of rats. In preliminary experiments, 0.01ml of 0.01% formalin revealed optimum sub-chronic inflammatory status of granulomata in rats. Cotton pellets implanted rats were randomly divided into 5 groups (5 rats/ group). The group I rats were received phosphate buffer saline (PBS) used as control and group II rats were received 0.01 ml of 0.01% formalin to induce sub-chronic inflammatory status. The III, IV and V rats received the sterilized cotton pellets soaked in 0.01 ml of 0.01% formalin with 0.25%, 0.5%and 1.0% of MECI respectively. The rats were anaesthetized and granulomas were removed after 48 hours and used for determination of granular tissue formation. Granular tissue formation was studied by drying cotton pellets at 60°C for 6 h or till the weight of the pellet remains constant. The dry weight was calculated after deducting cotton pellet weight and taken as a measure of granular tissue formation.

Acetic acid induced writhing in mice

A group of mice were injected intra-peritoneal (ip) with 0.lml/l0mg of 0.3 % (v/v) acetic acid. The mice exhibiting the writhing movements (stretching of hind limbs and bending of trunk) were selected for the study. These mice were randomly divided into 5 groups (5 mice/ group). These mice were administered with MECI (125, 250 and 500 mg/kg, po) and diclofenac sodium (10 mg/kg, po) 1-h prior to acetic acid injection. The numbers of writhings movements were counted for 30 minutes following acetic acid injection [17]. Both experimental data are expressed as mean ± SEM. Statistical analysis was carried out by using one-way ANOVA followed by Dunnett's test. The values at $P<0.05$ were considered as significant.

Results and Discussion

The extraction process, percentage of yield by methanol was found to 2.1% (w/w). MECI treatment inhibited granulatory phases of inflammation and its inhibitory effect was significant and dose related (Table 1).

Table 1: Effect of *C. inerme* extract on cotton-pellet granuloma in rats

Treatments and dose	Granuloma weight (mg)	
	Wet	Dry
Phosphate Buffer Saline (control)	132.7 ± 8.2	81.6 ± 6.8
Formalin (0.01%)	264.6 ± 12.8	146.2 ± 10.2
Formalin (0.01%)+ MECI (0.25%)	228.4 ± 9.4	120.0 ± 6.2
Formalin (0.01%)+ MECI (0.5%)	182.8 ± 6.8	92.8 ± 5.4
Formalin (0.01%)+ MECI (1.0%)	132.8 ± 6.2	68.6 ± 3.8

Significance level $P<0.05$ was calculated by comparing with control group.

Table 2: Effect of *C. inerme* extract on acetic acid-induced writhing in mice in mice

Treatments and dose (mg/kg)	Number of writhing			
	0-10 min	10-20 min	20-30 min	Total
Vehicle (mg/kg, po)	20.42±2.1	23.33±1.2	10.00±1.4	53.75±3.2
Diclofenac sodium (10)	09.60±0.8	12.64±1.2	04.16±0.7	26.40±2.4
MECI (125)	12.60±0.8	16.24±1.2	05.16±1.0	34.00±2.7
MECI (250)	10.00±1.0	15.21±1.1	04.10±0.6	29.31±2.2
MECI (500)	08.20±1.1	12.0±0.7	03.64±0.4	23.84±2.7

Significance level $P<0.05$ was calculated by comparing with vehicle treated group.

Pre-treatment with MECI (125, 250 and 400 mg/kg) prevented acetic acid induced writhing movements in mice. Inhibitory effect of diclofenac sodium (10 mg/kg) on acetic acid induced writhing was greater than MECI (500 mg/kg) effect (Table 2)..

Formaldehyde induced inflammation has been reported to be a useful model for screening of clinically effective anti-inflammatory agents [18]. Edema formation due to formaldehyde in rat and mice is a biphasic event. The initial phase of edema is attributed to the release of histamine and serotonin and the second phase of oedema is due to the release of prostaglandins, protease and lysosomal enzymes. Further, it has been demonstrated that the second phase is sensitive to the most clinically effective anti-inflammatory drugs [19].

Phytochemical studies have been made by various researchers to isolate and identify biologically active principle and other major chemical constituents from *C. inerme* species are steroids, flavonoids, phenolic compounds and diterpenoids [20, 21]. In the present experiments, MECI inhibited the inflammation in a dose related manner.

Therefore, it is likely that MECI might elicit its anti-inflammatory activity by inhibiting synthesis and release of prostaglandins, proteases and lysosomal enzymes like non-steriodal anti-inflammatory drugs [19]. During the repair process of inflammation, there is proliferation of macrophages, neutrophils, fibroblasts and multiplication of small vessels, which are the basic sources of forming a highly vascularised reddish mass termed granular tissue [22]. In sub-chronic rat model of inflammation (cotton pellet granuloma), MECI inhibited the granulatory phase of inflammation in a dose related manner. This inhibitory effect can be attributed to its multiple actions on targets like mediators of inflammation, lysosomal enzymes, oxidative stress and capillary permeability. There are documented reports that lysosomal enzymes play an important role in the development of acute and chronic inflammation [23]. Acetic acid-induced writhing is highly sensitive and documented model of visceral pain for screening of analgesic drugs [24]. Methanol extract of *C. inerme* reduced the acetic acid writhing movements in mice significantly, thereby indicating its analgesic activity. Since pain is an integral part of inflammation, the analgesic activity shall certainly a beneficial factor during inflammatory condition.

Conclusion:

Pharmacological activities in different animal models suggest that methanol extract of *C. inerme* (MECI) is a promising anti-inflammatory and analgesic agent and may be useful for the treatment of inflammatory conditions. However, studies are required on human subjects to prove its clinical efficacy as an anti-inflammatory-analgesic agent.

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