

ACUTE AND CHRONIC ANTI-INFLAMMATORY STUDY OF ETHANOLIC EXTRACT OF *OCHNA OBTUSATA* LEAVES

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ABSTRACT

Since the leave extracts of *Ochna obtusata* is reportedly used to treat ulcer, asthma and bronchitis, an attempt was made to study the Anti-Inflammatory activity of leaves of *Ochna obtusata*. Ethanolic extracts was prepared and used for the evaluation. The anti-inflammatory activity of EEOO was studied by reported methods to check acute and chronic anti-inflammatory activity. The acute study was performed in rat model by forming five groups with six rats in each group. Group – I served as Negative control, Group – II served as positive control (Diclofenac sodium 10mg/kg p.o), Group – III (EEOO 100mg/kg p.o), Group – IV (EEOO 200mg/kg p.o) and Group – V (EEOO 400mg/kg

p.o) served as tests. The chronic study was also studied in the same way by replacing using Dexamethasone 2.5mg/kg p.o towards positive control.

KEYWORDS: *Ochna obtusata*, Ethanolic extract, Carreegeenan, Xylene, Cotton pellet.

INTRODUCTION

India flooded with rich culture of medicinal herbs and spices, includes about more than two thousand species and with a geographical area large enough with high potential abilities for Ayurvedic, Unani, Siddha traditional medicines but only few have been studied for their pharmacological and chemical potential medicinal value.^[1,2] Human beings have been using various parts of these plants for the diversified treatment of ailments for more than thousand years.^[3,4] WHO states that most populations rely on traditional medicines at present too for their psychological and physical health requirements, since it's difficult for them to afford the products of Western pharmaceutical industries,^[5] together with their side effects and lack of healthcare facilities.^[6] Herbal molecules are reported to be safe and are proved to overcome

pathogenic resistance due to their existence in a combined as well as pooled form with one or more molecule in the protoplasm of the plant cell.^[7,8]

Ochna is a genus comprising 86 species of evergreen trees, shrubs and shrublets belonging to the family Ochnaceae. These species are native to tropical woodlands of Africa or Asia while some species are distributed in tropical and subtropical zones throughout the World.^[9,10] This family is characterized by the presence of flavonoids and biflavonoids and terpenoids as main secondary metabolites,^[11,12] and several studies on other *Ochna* species were conducted and revealed that the phytochemical contained within this genus constitutes mainly glycosides, saponins, steroids, flavones and fatty acids.^[14]

Ochna obtusata, a member of the *Ochna* genus is a medium-sized tree found widely throughout the hilly tracts of South India.^[14] *Ochna obtusata* (Family - Ochnaceae). Habit - Small trees up to 8m tall. Trunk & Bark - Bark greyish, smooth; blaze pinkish. Branches and branchlets - Branchlets terete, lenticellate, glabrous Leaves - Leaves simple, alternate, distichous; stipules caducous and leaving scar; petioles ca. 0.4 cm long, planoconvex, glabrous, lamina 16 X 5 cms, elliptic-oblong to obovate, apex acute to rounded, base acute to rounded, margin serrate, shining above, chartaceous, glabrous beneath. 12 pairs, ascending towards apex; tertiary nerves slender, reticulo-percurrent Inflorescence/Flower: Inflorescence axillary or lateral racemes; flowers yellow; pedicels up to 2.5 cm long. Fruit - Drupe, 3-5 distinct drupes seated on the enlarged disk; Seed - 1 drupe Distribution - South Asia, in the Western Ghats - South Central and Maharashtra Sahyadris.

From the source of Literature documentation and relevant traditional approaches on plant drugs, the present investigation was carried to investigate and compare the acute and chronic Anti-inflammatory activities of ethanolic extract of *Ochna obtusata* leaves by selecting the respective models.

MATERIALS AND METHODS

Plant Material

The fresh leaves of *Ochna obtusata* were collected from the forest of Tirumala Hills and authenticated by Dr. Madhava Shetty, taxonomist, Department of Botany, Sri Venkateswara University; AP. A voucher specimen has been kept in our laboratory for future reference. Only fresh green leaves were chosen for the experiment.

Preparation of Plant Extract

The collected leaves were dried at room temperature, pulverized by a mechanical grinder and sieved to obtain coarse powder. Extraction of Coarsely powdered leaves of *Ochna obtusata* was performed by solvent extraction with pet-ether, chloroform, methanol, ethanol and water. After filtration these were concentrated under reduced pressure yielding 1.08, 12.36, 15.28, 15.45, and 16.25.

Animals used

Adult Wistar albino rats (25±5gms) of either sex were utilized for the study. The animals were housed under conditions of controlled temperature ($25 \pm 2^{\circ}\text{C}$), humidity ($50 \pm 5\%$) and were acclimatized to 12 hr cycles of light and dark. Moreover the animals were kept in specially constructed cages to prevent coprophagia during the experiment. All the studies were carried out in accordance with the guidelines for care and use of experimental animals and approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute Toxicity Studies

Acute oral toxicity studies were performed as per the OECD guidelines. Healthy Wistar rats were used for the study. The animals were grouped into six groups containing six animals in each group. The doses were administered orally at the doses form 200 – 2000 mg/kg. There were no signs of any toxicity and mortality was observed up to 2000mg/kg.

Anti-Inflammatory Activity

Carrageenan Induced Rat Paw Edema method: The animals were grouped into five Groups of Six rats in each group. Group – I served as Negative control where the rats received normal diet and distilled Water. Group – II served as Positive Control and received Diclofenac Sodium (10mg/kg P.O). Group – III, Group IV and Group–V received EEEO (100mg/kg, 200mg/kg and 400mg/kg b.w) The animals were previously treated with the extract and 30 minutes after the treatment with the extract; freshly prepared Carrageenan solution (1% in normal saline solution) was injected under the sub planter region of the left hind paw to induce Oedema. The paw volume was measured using plethysmograph every hour till 5 hours after injection and compared with the standard treated group. The percentage inhibition of oedema was calculated by the formula.

$$\text{Percentage inhibition of oedema} = (A-B)/A \times 100$$

Where, A represents the paw volume of the control group and B represents the paw volume of the test drug treated group.

Xylene induced ear oedema method: The animals were grouped the same way as done in the case of Carrageenan. The extracts were administered to Group III, IV and Group V with dosage of 100mg/kg, 200mg/kg and 400mg/kg. Group I and Group II served as Negative and Positive Controls. Group I received distilled water and Group II received Dexamethasone as reference anti-inflammatory drug. Ear oedema was induced by applying carefully a drop of xylene (0.03 mL) to the anterior and posterior surfaces of the right ear. The left ear remained untreated and was considered as control. One hour after xylene application, the animals were killed under ether anaesthesia and 6 mm punches were made in the right and left ears of each mouse using a borer. Each ear punch was weighed and differences between the weight of the right and left ear punches of mice were recorded.

Cotton pellet-induced granuloma in rats: Five groups with six animals in each group were taken for the study and Grouped in the same way as per the prior two methods. Granulomatous lesions were induced by surgically inserting sterile cotton pellets (15 ± 1 mg) subcutaneously in both axilla regions of each rat following a single incision which was later closed by interrupted sutures. After implantation of pellets, the plant extracts (100, 200 and 400 mg/kg) were orally administered once a day for 7 consecutive days. Dexamethasone (2.5 mg/kg, p.o.) was also given daily to standard group while the control group received the same volume of distilled water (10 ml/kg). On day 8, the cotton pellets were dissected out under ether anaesthesia, cleaned of extraneous tissue, weighed and dried at 50°C to a constant weight. The mean weights for different groups were determined. The increase in dry weight of the pellets was taken as the measure of the granuloma formation.

Statistical Analysis: Data of Paw thickness was analyzed by using One-Way ANOVA followed by post hoc test Dunnett's test using Graph pad Prism-5 software. The results were expressed as Mean \pm S.E.M. $P < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Preliminary Phytochemical Tests: Since various solvents were used to prepare the extracts, they were tested to confirm the presence of various phytochemicals. Employing standard phytochemical tests the Ethanolic extracts were found to have flavonoids, anthraquinones, saponins, tannins, polyphenols and alkaloids in sufficient quantity and hence tested for its

anti-inflammatory activity. The presence of phytoconstituents in the extract is believed to stabilize the levels of disturbances caused by the Induction of inflammatory agents.

Effect of the EEOO on Carrageenan induced paw oedema in rats: The inhibitory action of EEOO is shown in Table No 1. The study confirms the anti-inflammatory activity of EEOO. Carrageenan in an early phase releases serotonin and histamine in the surrounding tissue which is later followed by the release of bradykinin, protease, prostaglandin and lysozymes. Though the doses 100 and 200 mg/kg were found to inhibit the oedema, 400 mg / kg b.w. was found to have strong anti-inflammatory action as it showed a significant decrease in the oedema after a period of 5 hours of Carrageenan administration. The extract maintained the suppression of the inhibition throughout the duration of the study which may be by hindering the process of inflammation.

Effect of the EEOO on Xylene induced ear oedema in mice: The effect of the EEOO on xylene induced ear oedema in mice is recapitulated in Table No 2. Application of xylene induces acute neurogenous edema which is associated with Substance P, which when released from the sensory neurons in the periphery causes plasma extravasations and vasodilatation which leads to swelling of the ear, suggesting the role of xylene in neurogenous inflammation. Administration of the plant extracts (100, 200 and 400mg/kg), 1 h after xylene application, significantly ($P < 0.01$) inhibited the development of ear oedema in mice in a dose dependent manner. The inhibition produced by 400 mg/kg of the extract was similar to that produced by dexamethasone. The inhibition may reduce the release of substance P or other inflammatory mediators such as histamine, kinin and fibrinolysin or antagonize the actions.

Effect of the EEOO on Cotton pellet-induced granuloma in rats: Table No 3 shows the effect of EEOO on Cotton Pellet induced granuloma in rats. Granuloma formation is the result of leukocyte accumulation and the administration of low doses of plant extract of *Ochna obtusata* and it was found to be less effective in reducing leukocyte migration to the areas of Inflammation. The administration of plant extract in a dose dependant manner showed a significant inhibition of granuloma in rats. The inhibition produced by 400mg/kg of the extract was similar to that produced by dexamethasone which confirms that the leukocyte migration was reduced to a better extent thereby showing a significant anti-inflammatory activity.

Table 1: Effect of EEEO on Carrageenan induced paw oedema in rats.

Group	Doses (mg/kg)	Volume of mercury displaced in ml at various time intervals in hours.					
		0	1	2	3	4	5
Control	0	0.39±0.008	0.49±0.017	0.61±0.14	0.66±0.015	0.68±0.015	0.70±0.014
Diclofenac Sodium	2.5	0.25±0.010	0.29±0.012	0.28±0.007	0.25±0.007	0.25±0.009	0.21±0.004
<i>O.obtusata</i>	100	0.37±0.008	0.45±0.010	0.55±0.009	0.47±0.008	0.47±0.005	0.45±0.006
<i>O.obtusata</i>	200	0.28±0.008	0.34±0.005	0.39±0.006	0.37±0.006	0.37±0.004	0.37±0.004
<i>O.obtusata</i>	400	0.31±0.004	0.38±0.005	0.42±0.005	0.29±0.007	0.32±0.006	0.27±0.007

Table 2: Effect of EEEO on Xylene induced Ear oedema in rats.

Group	Doses (mg/kg)	Oedema (mg)	Inhibition (%)
Control	0	3.88±0.329	-
Dexamethasone	2.5	2.67±0.348	31.23
<i>O.obtusata</i>	100	3.39±0.267	12.54
<i>O.obtusata</i>	200	3.05±0.239	21.39
<i>O.obtusata</i>	400	2.67±0.234	28.82

Table 3: Effect of EEEO on Cotton Pellet induced granuloma in rats.

Group	Doses (mg/kg)	Oedema (mg)	Inhibition (%)
Control	0	33.88±0.329	-
Dexamethasone	2.5	21.67±0.348	36.04
<i>O.obtusata</i>	100	27.19±0.623	19.75
<i>O.obtusata</i>	200	25.24±0.741	25.50
<i>O.obtusata</i>	400	22.78±0.711	32.75

CONCLUSION

In conclusion, these results indicated that EEEO do not have any toxicity and hence the extract was found to possess potent anti-inflammatory activity in acute (Carrageenan induced Paw oedema and xylene induced ear edema) and chronic (cotton pellet granuloma) inflammation models, thereby indicating the possibility of developing EEEO as a safe and potent anti-inflammatory substance.

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