Anti-Inflammatory Activity of *Chrozophora rottleri* Extracts on Carrageenan-Induced Rat Paw Edema

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Abstract. The present study was intended to evaluate Anti-inflammatory activity of *C. rottleri* extracts (Hydroalcoholic, Methanol, Ethyl acetate and Hexane). The Anti-inflammatory activity of *C. rottleri* extracts at doses of 125mg/kg, 250mg/kg and 500mg/kg using carrageenan induced rat paw edema model compared with standard drug (Indomethacin). The selected plant extracts significantly inhibited paw edema along with the standard drug Indomethacin. Of all extracts, methanol extract produced significant effect on reduction of increased paw thickness, hydro alcoholic and ethyl acetate extracts produced moderate percentage inhibition and hexane extract produced low level of percentage inhibition in reducing paw edema on carrageenan induced rats. In all extracts, methanol extract at a dose of 500mg/kg showed more percentage inhibition i.e. 53.47 ± 2.19 . From the results obtained during the study it is concluded that *C. rottleri* having the bioactive molecule responsible for Anti-inflammatory activity by individually or by combination of different bio-active compounds present in it. Further is necessary for isolation and characterization of bioactive molecules which are responsible for the selected plant biological activities.

1.0 Introduction

The uses of plants in traditional medicine have been wide spread around the world since ancient time and still plants have unidentified bioactive molecules those are recently discovered and developed novel drugs from plants in recent times like anti-inflammatory, digestive, antinecrotic, neuroprotective, antimicrobial and hepatoprotective [1-5]. Recently, medicinal plants have received much attention as a source for treatments of several diseases based on their traditional uses because of their less side effects compared to chemical therapeutics which are long term administration is required for treatments of chronic diseases causing various side effects. In this point of view, many researchers scientifically reporting the important bioactive molecules from plants based on their traditional uses by evaluating their biological activities [6-8]. Therefore, the present study was carried out to evaluate the Anti-inflammatory potential of the *C. rottleri* aerials parts and further experiments are in progress in order to isolate and characterize secondary metabolites responsible for the anti-inflammatory activity and elucidate the mechanism of action.

Chrozophora rottleri have been using in traditional medicine by native medical practitioners for the treatment of various diseases in Saudi Arabia, Pakistan and India (e.g. against jaundice and purifying blood). In India and Sudan, powdered stems or whole plants are applied to wounds to improve healing. In Nepal, juice of the fruit is given in cases of cough and colds, purifying agent (leaf) and laxative (seed), having bioactive components. The fruits yield a purplish blue dye, which is used in East Africa to dye mats. The leaves are very much beneficial in treating skin diseases and also used as a depurative agent. The seeds are used as cathartic like Ghodtapde and credited with purgative properties [9-14].

2.0 Materials and Methods

2.1 Chemicals

Indomethacin, Sodium Carboxy Methyl cellulose (Na CMC) and Carrageenan were purchased from Sigma chemicals, USA. All other chemicals used were of analytical grade.

2.2 Preparation of Extracts

The plant materials were collected from Jagityala and Tirupathi, Andhra Pradesh. The aerial parts were separated, dried under shade and powdered. The coarse powder was extracted with 70% v/v ethanol, methanol, ethyl acetate and hexane separately in a Soxhlet apparatus. The liquid extracts were filtered and evaporated under reduced pressure by using rotary evaporator (Buchi R-210) until a soft mass obtained and then four extracts were used for further investigation.

2.3 Selection of Animals

Adult Wistar rats (National Institute of Nutrition, Hyderabad, India) of either sex weighing 200-250gm were used in the studies. The animals were maintained under standard laboratory conditions at an ambient temperature of $23\pm 2^{\circ}\text{C}$ having $50\pm 5\%$ relative humidity with 12-h light and dark cycle. The use and care of the animals in the experimental protocol has been approved by the local Institutional Animal Ethics Committee (Regd. No. 516/01/A/CPCSEA) following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

2.4 Acute toxicity studies

Acute toxicity study was conducted according OECD Guide lines No.423. After fasting overnight, mice were administered with extracts of *C. rottleri* in a single dose up to the highest dose of 2000 mg/kg orally. The animals were observed continuously for 1 h and then hourly for 6 h and finally after every 24 h up to 15 days for any toxicological symptoms or mortality.

2.5 Acute inflammation model, carrageenan induced rat paw Oedema [15, 16]

Animals were divided into XV groups (each contains 6 rats). The rats were given doses orally with extracts at different dose levels 18 h and 2 h prior to the induction of carrageenan subcutaneously (SC) into the subplantar tissue of the hind paw of each rat, 0.1 ml of 1% carrageenan suspension.

The drug effects were estimated by comparing the maximal paw edema response during 6 h in the drug as extract treated group with that of vehicle treated group as control. Group I normal rats treated with Drug vehicle (1% Sodium CMC) and served as normal control and Group II rats were treated with Indomethacin 1.3×10^{-5} moles/kg b.wt. Remaining groups were treated with the selected plant extracts at different dose levels all the doses were administered orally according to the body weight of the animals.

To evaluate this model, the percentage increase in paw thickness was plotted against the time (Hour) and the maximal paw edema response induced during the 6 h was determined. The results showed the ability of the model in detecting that the time course changes in the paw size was associated with carrageenan induced rat paw edema. The paw edema was constantly increased during 4 h and reached peak of edema at 4th hour. At the 5th and 6th hour, the edema was gradually reduced. The percentage increase in paw edema thickness was calculated by using the following formula.

$$\% \text{ Increase in paw thickness} = \frac{Y_t - Y_o}{Y_o} \times 100$$

Y_t = Paw thickness at time (1, 2, 3, 4, 5 and 6th) after injection

Y_o = Paw thickness at 0 hr (before injection)

Data were expressed in terms of mean values + S.E.M

The % increase in paw edema response during 6 hr was determined. The percentage inhibition of paw edema thickness is calculated using the formula.

$$\text{Percentage Inhibition} = 100 \times (1 - Y_1/Y_o)$$

Y_1 = Average increase in paw thickness in groups treated with test extracts.

Y_o = Average increase in paw thickness in control

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 are expressed as Mean $\pm$ S.E.M. $P < 0.001$ was considered as significant.

3.0 Results

3.1 Acute toxicity study

Acute toxicity studies in mice revealed that the extracts up to 2000 mg/kg produced no sign of toxicity or mortality.

3.2 Anti-inflammatory activity

The role of test extracts on carrageenan induced acute inflammation model was evaluated at concentrations of 125, 250 and 400 mg/kg. Paw oedema was reduced by extracts in a dose dependent manner till the end of the sixth hour. In carrageenan administered animals the severe swelling was reached during 3 h and the swelling was maintained until the fourth hour. The Indomethacin treated groups decreased paw edema significantly throughout the period of study. However, the animals treated with test extracts showed considerable inhibition on swelling as compared to carrageenan administered animals. The results (Table 1 and figure 1) revealed that all the test extracts protected the rats from carrageenan induced inflammation and the test extracts showed a moderate anti-inflammatory activity.

Indomethacin significantly inhibited the maximal paw edema response by 68.26 ± 0.63 and total edema response (AUC) was inhibited by 67.42 ± 1.05 . Hydroalcoholic extract of *C. rottleri* at 125 mg/kg, 250 mg/kg and 500mg/kg doses, 15.30 ± 1.82 , 27.38 ± 1.16 and 49.65 ± 1.88 respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total edema response (AUC) was inhibited by 25.30 ± 2.19 , 31.56 ± 2.28 and 48.90 ± 2.20 respectively over 6 h when compared to the control group treated with drug vehicle.

Methanolic extracts of *C. rottleri* at 125 mg/kg, 250 mg/kg and 500mg/kg doses significantly inhibited the maximal paw edema response by 68.26 ± 0.63 , 15.03 ± 4.06 , 29.07 ± 0.39 and 53.47 ± 2.19 respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total edema response (AUC) was inhibited by 67.42 ± 1.05 , 27.13 ± 4.27 , 39.06 ± 4.79 and 52.46 ± 3.24 respectively.

Ethyl acetate extract of *C. rottleri* significantly inhibited the maximal paw edema response by 13.94 ± 3.54 , 24.63 ± 3.57 and 41.17 ± 2.00 respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total edema response (AUC) was inhibited by 22.06 ± 3.80 , 35.49 ± 3.74 and 46.10 ± 2.10 respectively over 6 h when compared to the control group treated with drug vehicle.

Hexane extract of *C. rottleri* significantly inhibited the maximal paw edema response by 12.72 ± 2.73 , 21.21 ± 2.85 and 38.93 ± 1.72 respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total edema response (AUC) was inhibited by 19.02 ± 1.71 , 32.04 ± 2.08 and 44.60 ± 3.65 respectively over 6 h when compared to the control group treated with drug vehicle.

Table 1: Effect of *C. rotleri* Extracts on Carrageenan Induced rat paw edema.

Extract Name of the <i>C. rotleri</i>	Group/Drug	Dose	Percentage inhibition of the maximal paw edema during 6 hours	Percentage inhibition of total AUC paw edema during 6 hours
1%Na CMC	Group I	1mL	0.0	0.0
Indomethacin	GroupII	1.3×10^{-5} moles/kg	68.26 $\pm$ 0.63	67.42 $\pm$ 1.02***
Ehanol (70%)	Group III	125 mg/kg	15.30 $\pm$ 1.82	25.30 $\pm$ 2.19***
	Group IV	250 mg/kg	27.38 $\pm$ 1.16	31.56 $\pm$ 2.28***
	Group V	500 mg/kg	49.65 $\pm$ 1.88	48.90 $\pm$ 2.20***
	Group VI	125 mg/kg	15.03 $\pm$ 4.06	27.13 $\pm$ 4.27***
Methanol	Group VII	250 mg/kg	29.07 $\pm$ 0.39	39.06 $\pm$ 4.79***
	Group VIII	500 mg/kg	53.47 $\pm$ 2.19	52.46 $\pm$ 3.24***
	Group IX	125 mg/kg	13.94 $\pm$ 3.54	22.06 $\pm$ 3.80***
Ethyl acetate	Group XI	250 mg/kg	24.63 $\pm$ 3.57	35.49 $\pm$ 3.74***
	Group XII	500 mg/kg	41.17 $\pm$ 2.00	46.10 $\pm$ 2.10***
	Group XIII	125 mg/kg	12.72 $\pm$ 2.73	19.02 $\pm$ 1.71***
Hexane	Group XIV	250 mg/kg	21.21 $\pm$ 2.85	32.04 $\pm$ 2.08***
	Group XV	500 mg/kg	38.93 $\pm$ 1.72	44.60 $\pm$ 3.65***

A)

B)

Fig 1. Effect of *C. rottleri* extracts at 125, 250 & 500 mg/kg along with Indomethacin (1.3×10^{-5} moles/kg body wt) on a) the maximal and b) the total paw oedema in Carrageenan induced rats.

4.0 Discussion

The present study is an attempt to evaluate anti-inflammatory potential of *C. rottleri* extracts because people have been exploring the plants since the ancient times and have been trying to reduce the side effects and develop effective anti-inflammatory agents using different plant extracts and formulations [17]. Since the non-steroidal anti-inflammatory drugs (NSAIDs) present in the market induce several side effects like gastric ulcer [18] and hepatotoxicity [19] therefore there is a need for effective anti-inflammatory drugs with no or low side effects.

Carrageenan paw edema is a test used largely to study anti-inflammatory drugs both steroidal and non-steroidal since it involves several mediators [20]. This suitable test also has frequently been used to access the antiedematous effect of natural products [21]. The local injection of carrageenan-induced inflammatory process in the rat involves three phases through sequential release of several mediators. An initial phase, during the first 1.5 h, is caused by the release of histamine and serotonin [22], a second phase is mediated by bradykinin from 1.5 to 2.5 h and finally, a third phase, the mediator of which is suspected to be prostaglandins occurs from 2.5 to 6 h after carrageenan injection [23]. This third phase appears to be the most interesting phase in terms of inflammatory processes. Thus, the maximal vascular response as determined with leukocyte migration to the inflamed area, also reaches its maximum level in this third phase. It is well established that prostaglandins, by virtue of their activity as modulators of inflammatory responses, have a major role in the inflammatory mechanism [21]. The carrageenan-induced hind paw edema in rat is known to be sensitive to COX inhibitors, but not to 5-LO inhibitors, and has been used to evaluate the effect of non-steroidal anti-inflammatory agents which primarily inhibit the COX involved in prostaglandins synthesis. It has been demonstrated that the suppression of carrageenan-induced hind paw edema after the third-fourth hour correlates reasonably with therapeutic doses of most clinically effective anti-inflammatory agents [21].

In the present study, methanol extract (53.47 ± 2.19) has shown better inhibition of inflammation when compared to the other extracts (Table 1) and standard drug Indomethacin (68.26 ± 0.63) exhibited more inhibition of the paw edema during 6h as compared to tested plant

extracts. This may indicate that the active constituent(s) act by affecting the synthesis, release and action of inflammatory mediators involved in carrageenan induced edema. So it may be possible to suggest that anti-inflammatory activity of *C. rottleri* extracts is due to blockade of action of histamine and serotonin which are also involved in carrageenan induced edema. On preliminary phytochemical screening leaves of *C. rottlri* were found to contain different phytochemicals [24-26, 14, 12], which are probably responsible for its anti-inflammatory activity. The present efforts are helpful to isolate the active constituents from extracts of this species and allow to understanding their mechanism (s) of action and to design new therapeutic agents with potential anti-inflammatory effect.

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Conflict of interests

The authors declared no conflict of interest.

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