ANTI-INFLAMMATORY EFFICACY OF CURCUMA ZEDOARIA ROSC ROOT EXTRACTS

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ABSTRACT
Anti-inflammatory activity of Curcuma zedoaria Rosc was studied in albino rats by using Carrageenan and Histamine induced hind paw edema method. The paw edema was induced by the subplantar injection of above inflammasens, and oedema volume was recorded using a plethysmometer. Curcuma zedoaria Rosc are traditionally used in treatment of inflammation. Petroleum ether, chloroform and methanol root extracts of C. zedoaria were administered orally half hour before inducing inflammation. All extracts showed significant p<0.001 anti-inflammatory activity except methanol extract, when compared to control with standard drugs (Indomethacin 10 mg/kg,i.p and Rumalaya forte 200 mg/kg). Amongst these extracts petroleum ether 200 and chloroform 400 mg/kg extracts of Curcuma zedoaria showed maximum anti-inflammatory activity on 2nd to 6th hours.

Key words: Curcuma zedoaria Rosc, Carrageenan, Histamine, Anti-inflammatory.

INTRODUCTION
Curcuma zedoaria Rosc, also known as white turmeric, zedoaria or gajutsu2 is a perennial rhizomatous herb that belongs to the Zingiberaceae family. The plant is indigenous to Bangladesh, Sri Lanka and India. In India it is known by its several vernacular names, the most commonly used ones being: Dravi-a (Sanskrit), Kacura (Hindi) and Kachora (Kannada).2,4

It is used traditionally for the treatment of menstrual disorders, dyspepsia, vomiting and for cancer.3 Rural people use the rhizome for its rubefacient, carminative, expectorant, demulcent, diuretic and stimulant properties while the root is used in the treatment of flatulence, dyspepsia, cold, cough and fever.1

Curcuma zedoaria is a reach source of essential oils, starch, curcumin arabin and gums etc. The enzyme, phospholipase A2, is known to be responsible for the formation of mediators of inflammation such as prostaglandins and leukotrienes which by attracting polymorphonuclear leucocytes to the site of inflammation would lead to tissue damage probably by the release of free radicals. Phospholipase A2 converts phospholipids in the cell membrane into arachidonic acid, which is highly reactive and is rapidly metabolized by cyclooxygenase (prostaglandin synthase) to prostaglandins, which are major components that induce pain and inflammation.5,6

The early phase of acute inflammation involves cellular influx associated with the release of mediators like histamine and prostaglandins (PGEs).7 All these mediators produce inflammation when injected subcutaneously in the rat paw.8 The present study is therefore an attempt to assess the efficacy of different extracts of Curcuma zedoaria Rosc root extracts on anti-inflammatory activity induced by carrageenan and histamine in rat paw oedema model.

MATERIALS AND METHODS:

Plant material:
The matured roots of Curcuma zedoaria Rosc were collected from Odakali, near to Cochin, Kerala. The plant materials were authenticated from National Botanical Research Institute Lucknow (NBRI). All the roots were Shade dried at room temperature until they were free from moisture pulverized in electric grinder.

The powder was obtained and extracted separately by continuous hot extraction process using soxhlet extractor with different solvents in increasing order of polarity from petroleum ether, chloroform, and methanol.9 The extracts were concentrated under reduced pressure and dried.

Experimental animals:
Healthy albino rats of either sex (Wistar strain) weighing 150-160 g were used for present study. The animals had free access to food and water and were maintained under controlled temperature (27±2°C) and 12 h: 12 h light and dark cycle. Initial body weight of each animal was recorded.

Acute toxicity studies:10
The roots of each extracts of Curcuma zedoaria Rosc, at different doses (175, 550, 1500, 2000 and 5000 mg/kg) were administered orally to normal rats. During the first four hours after the drug administration, the animals were observed for gross behavioral changes.

The parameters such as hyperactivity, grooming, convulsions, sedation, hypothermia, body weight and mortality were observed up to 14 days. No mortality observed with oral administration of all the extracts even at the highest dose (5000 mg/kg). Institutional Animal Ethical Committee (IAEC) had approved the experimental protocol and care of animals was taken as per the guidelines of CPCSEA, Department of animal welfare, Government of India.

Test for Anti-inflammatory Activity:
These extracts were tested for anti-inflammatory activity by carrageenan11 and histamine12 (inflamagens) induced rat paw edema method.

Different groups of animals were taken for experiment as follows: group

Group-I: Control: Inflamagens+Normal Saline (p.o)

Standard group:
Group-II (SD-I): Standard: Inflamagens +10 mg/kg Indomethacin (i.p)
Group-III (SD-II): Inflamagens+ Rumalaya forte 200 mg/kg (p.o)

Test groups:
Group-IV (PEE): Inflamagens+Petroleum ether extract 200 mg/kg (p.o)
Group-V (PEE): Inflamagens+Petroleum ether extract 400 mg/kg (p.o)
Group-VI (CE): Inflamagens+Chloroform extract 200 mg/kg (p.o)
Group-VII (CE): Inflamagens+Chloroform extract 400 mg/kg (p.o)
Group-VIII (ME): Inflamagens+Methanol extract 200 mg/kg (p.o)
Group-IX: (ME): Inflamagens+Methanol extract 400 mg/kg (p.o)
Table I: Effect of C. zedoaria Rosc root extracts on carrageenan induced paw edema in rats

<table>
<thead>
<tr>
<th>Treatment Groups (n=6)</th>
<th>Dose mg/kg</th>
<th>1st hour</th>
<th>2nd hour</th>
<th>3rd hour</th>
<th>4th hour</th>
<th>5th hour</th>
<th>6th hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>saline</td>
<td>0.550±0.050</td>
<td>0.883±0.130</td>
<td>0.967±0.966</td>
<td>0.900±0.063</td>
<td>0.817±0.060</td>
<td>0.716±0.030</td>
</tr>
<tr>
<td>SD-I</td>
<td>10</td>
<td>0.250±0.022 a</td>
<td>0.316±0.030 a</td>
<td>0.367±0.033 a</td>
<td>0.367±0.042 a</td>
<td>0.300±0.025 a</td>
<td>0.283±0.030 a</td>
</tr>
<tr>
<td>SD-II</td>
<td>200</td>
<td>0.267±0.021 a</td>
<td>0.283±0.030</td>
<td>0.366±0.021 a</td>
<td>0.383±0.016 a</td>
<td>0.367±0.028 a</td>
<td>0.333±0.033 a</td>
</tr>
<tr>
<td>PEE</td>
<td>200</td>
<td>0.250±0.022 a</td>
<td>0.383±0.031 a</td>
<td>0.417±0.031 a</td>
<td>0.367±0.033 a</td>
<td>0.350±0.023 a</td>
<td>0.317±0.031 a</td>
</tr>
<tr>
<td>PEE</td>
<td>400</td>
<td>0.350±0.034 a</td>
<td>0.383±0.031 a</td>
<td>0.450±0.034 a</td>
<td>0.417±0.031 a</td>
<td>0.350±0.022 a</td>
<td>0.317±0.017 a</td>
</tr>
<tr>
<td>CE</td>
<td>200</td>
<td>0.333±0.022 a</td>
<td>0.367±0.022 a</td>
<td>0.433±0.033 a</td>
<td>0.467±0.033 a</td>
<td>0.300±0.031 a</td>
<td>0.350±0.024 a</td>
</tr>
<tr>
<td>CE</td>
<td>400</td>
<td>0.317±0.030 a</td>
<td>0.366±0.033 a</td>
<td>0.450±0.042 a</td>
<td>0.417±0.047 a</td>
<td>0.383±0.040 a</td>
<td>0.350±0.022 a</td>
</tr>
<tr>
<td>ME</td>
<td>200</td>
<td>0.433±0.021 a</td>
<td>0.700±0.044 a</td>
<td>0.883±0.147 a</td>
<td>0.816±0.030 a</td>
<td>0.783±0.166 a</td>
<td>0.60±0.044 a</td>
</tr>
<tr>
<td>ME</td>
<td>400</td>
<td>0.483±0.047 a</td>
<td>0.800±0.089 a</td>
<td>0.883±0.161 a</td>
<td>0.850±0.056 a</td>
<td>0.667±0.056 a</td>
<td>0.617±0.083 a</td>
</tr>
</tbody>
</table>

\[N=6, \text{Values are Mean±S.E.M. } \text{ap}<0.001, \text{bp}<0.01, \text{cp}<0.05 \text{ (significant) statistical analysis was done by one way analysis of variation (ANOVA) followed by Dunnet’s test.}\]

Table II: Effect of C. zedoaria Rosc root extracts on histamine induced paw edema in rats

<table>
<thead>
<tr>
<th>Treatment Groups (n=6)</th>
<th>Dose mg/kg</th>
<th>1st hour</th>
<th>2nd hour</th>
<th>3rd hour</th>
<th>4th hour</th>
<th>5th hour</th>
<th>6th hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>saline</td>
<td>0.567±0.056</td>
<td>0.800±0.052</td>
<td>1.212±0.307</td>
<td>1.083±0.040</td>
<td>0.967±0.056</td>
<td>0.900±0.577</td>
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<tr>
<td>SD-I</td>
<td>10</td>
<td>0.416±0.016 c</td>
<td>0.550±0.341 b</td>
<td>0.683±0.047 a</td>
<td>0.700±0.036 a</td>
<td>0.683±0.065 b</td>
<td>0.667±0.066 c</td>
</tr>
<tr>
<td>SD-II</td>
<td>200</td>
<td>0.367±0.044 b</td>
<td>0.416±0.030 a</td>
<td>0.583±0.054 b</td>
<td>0.617±0.030 a</td>
<td>0.425±0.047 b</td>
<td>0.583±0.030 b</td>
</tr>
<tr>
<td>PEE</td>
<td>200</td>
<td>0.417±0.031 a</td>
<td>0.517±0.032 a</td>
<td>0.567±0.043 a</td>
<td>0.567±0.042 a</td>
<td>0.483±0.047 a</td>
<td>0.483±0.048 a</td>
</tr>
<tr>
<td>PEE</td>
<td>400</td>
<td>0.367±0.033 a</td>
<td>0.467±0.042 a</td>
<td>0.583±0.048 b</td>
<td>0.617±0.040 a</td>
<td>0.583±0.05 b</td>
<td>0.517±0.031 a</td>
</tr>
<tr>
<td>CE</td>
<td>200</td>
<td>0.383±0.030 a</td>
<td>0.483±0.040 a</td>
<td>0.566±0.033 a</td>
<td>0.616±0.040 a</td>
<td>0.516±0.047 b</td>
<td>0.433±0.021 a</td>
</tr>
<tr>
<td>CE</td>
<td>400</td>
<td>0.417±0.054 a</td>
<td>0.533±0.056 a</td>
<td>0.650±0.068 a</td>
<td>0.667±0.056 a</td>
<td>0.567±0.033 a</td>
<td>0.500±0.063 a</td>
</tr>
<tr>
<td>ME</td>
<td>200</td>
<td>0.517±0.031 a</td>
<td>0.783±0.047 a</td>
<td>1.017±0.047 a</td>
<td>0.983±0.075 a</td>
<td>0.800±0.073 a</td>
<td>0.750±0.042 a</td>
</tr>
<tr>
<td>ME</td>
<td>400</td>
<td>0.500±0.036 a</td>
<td>0.733±0.042 a</td>
<td>0.983±0.054 a</td>
<td>1.033±0.080 a</td>
<td>0.900±0.036 a</td>
<td>0.800±0.077 a</td>
</tr>
</tbody>
</table>

\[N=6, \text{Values are Mean±S.E.M. } \text{ap}<0.001, \text{bp}<0.01, \text{cp}<0.05 \text{ (significant) statistical analysis was done by one way analysis of variation (ANOVA) followed by Dunnet’s test.}\]

Fig. 1: Root extracts of C. zedoaria Rosc percentage inhibition against carrageenan and histamine induced paw edema

Percentage inhibitions were calculated. C –C_1/C X 100. C= Control group and C_1= drug treated groups
Before the experiment, food was withdrawn overnight but adequate water was given to the rats. Dose selected were 200 mg and 400 mg/kg for each extract. The animals were divided into nine groups of 6 animals each. All the doses were given orally half an hour before the administration of carrageenan (Sigma chemical co. St. Louis MO, USA) and histamine into the plantar side of the left hind paw. The paw was marked with ink at the level of lateral malleolus and immersed in mercury up to the mark in the plethysmometer. 

The paw volume was measured after (1 h) injection carrageenan and then every hour till 6 h of each group. The difference between the initial and subsequent reading gave the actual edema volume. The average paw swelling is calculated by comparing the normal group with control. Standards and all treated groups compared with the control. Percent inhibition of inflammation was calculated by using the formula,

\[
\% \text{ inhibition} = 100 \left( \frac{V_t - V_c}{V_c} \right) \times 100
\]

Where 'Vc' represents edema in control.

'Vt' is the edema in group treated with extracts.

**Histamine-Induced Edema:**

For the study of Histamine – induced paw edema in the animals were treated exactly the same method as carrageenan induced model but instead of carrageenan, here 0.1 ml of 1% w/w histamine in normal saline was used.

**RESULTS**

**Acute toxicity**

The acute toxicity study revealed non toxic nature of all the extracts at a higher dose of 5 g/kg body weight.

**Anti-inflammatory activity**

The effect of petroleum ether and chloroform extracts of *C. zedoaria Rosc* on carrageenan induced edema in albino rat is shown in Table 1. The results obtained indicate that both above extract had significant anti-inflammatory activity in albino rats, when compared with reference standards *(p< 0.001).* The potency was found to be inversely proportional to the time taken for reduction in the paw volume. The petroleum ether extract of *C. zedoaria Rosc* reduced edema 56.70% near to standard group II induced by carrageenan on oral administration 200 mg/kg when compared with untreated control group. Petroleum ether 400 and chloroform extracts 200, 400 mg/kg shows 52.18, 50.90 and 51.99% standard groups- I and II shows 60.6 and 56.95% inhibition respectively.

The effect of petroleum ether and chloroform extract of *C. zedoaria Rosc* on histamine-induced edema in rats is shown in Table 2. The histamine induced inflammation is significantly *(p< 0.001)* inhibits the paw volume at 200 mg/kg dose of petroleum ether extract. Petroleum ether extract 400, chloroform extracts 200 and 400 mg/kg shows 42.41, 44.47 and 38.41% inhibition respectively. Standard group- I and II inhibits paw edema 34.13 and 44.86% respectively. Methanol groups showed non significant results.

**DISCUSSION**

Due to the increasing frequency of intake of NSAID’s and their reported common side effects, there is need to focus on the scientific exploration of herbal drugs having fewer side effects. So, there is a continuous search for indigenous drugs, which can provide relief to inflammation. Carrageenan induced inflammation is a biphasic phenomenon. The first phase of edema is attributed to release of histamine and 5-hydroxytryptamine. Plateau phase is maintained by kinin like substances and second accelerating phase of swelling is attributed to prostaglandin like substances.

The knowledge of these mediators involved in different phases is an important for interpreting mode of drug action, in this study the petroleum ether and chloroform extract of *C. zedoaria Rosc* (200 and 400 mg/kg) showed significant reduction of paw edema at 2 h or more after carrageenan injection, suggesting that curcumin produces an anti-edematous effect during the second phase, similarly to indomethacin. Therefore, our results confirm that the mechanism of the anti-inflammatory effect of curcumin involves reduction of prostaglandins through inhibition of cyclooxygenase. The antiedematous effect of methanol extract of *C. zedoaria* showed a delayed onset (6 h), In addition, the efficacy of petroleum ether and chloroform extract of *C. zedoaria* was comparable to that of Indomethacin and Rhumahly fort with a longer duration of action showed significant reduction in paw edema volume in carrageenan and histamine induced inflammation.

Thus it can be concluded that petroleum ether and chloroform root extracts of plant *C. zedoaria* possess significant anti inflammatory activity in rats. Further studies involving the isolation and 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 a low toxicity and better therapeutic index.

**ACKNOWLEDGEMENT**

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**REFERENCES**