Evaluation of Anti-Inflammatory Effect of Ethanolic and Aqueous extracts of Curcuma Zedoaria Rosc Root

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Abstract
The present study investigates the anti-inflammatory activity of ethanolic and aqueous extracts of Curcuma zedoaria Rosc roots in albino rats by using Carrageenan and Histamine induced hind paw edema method. The medicinal values of Curcuma zedoaria root have been mentioned in ancient literature as useful in disorders of inflammation. Dried pulverized root of Curcuma zedoaria was extracted with ethanol by using soxhlet apparatus and aqueous extract was prepared by cold maceration. The paw edema was induced by subplantar injection of above inflammagens and oedema volume was recorded using a Plethysmometer. Ethanol 200 and 400 mg/kg root extracts showed significant p<0.001 anti-inflammatory activity on 2nd to 6th hours and aqueous root extract showed non-significant anti-inflammatory activity when compared with control group using standard groups (Indomethacin 10 mg/kg,i.p and Rumalaya forte 200 mg/kg p.o). It concludes that ethanolic root extract of Curcuma zedoaria Rosc augments that it shows good anti-inflammatory activity against carrageenan and histamine induced paw edema in rats.

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

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Introduction:
Nature has provided a complete store-house of remedies to cure all ailments of mankind [1] Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. The research into plants with alleged folkloric use as pain relievers and anti-inflammatory agents should therefore be viewed as a fruitful and logical research strategy in the search for new anti-
inflammatory drugs since existing synthetic molecule like nonsteroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors that increase the incidence of adverse cardiovascular and hepatotoxicity effects\[^2\]. So in order to overcome there is a need to focus on the scientific exploration of herbal drugs having fewer side effects. Curcuma zedoaria Rosc (CZ) also known as white turmeric, zedoaria or gajutsu\[^3-4\] 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 one being Kaccura (Sanskrit), Kacura (Hindi) and Kachura (Bengali)\[^5\]. It is used traditionally for the treatment of menstrual disorders, dyspepsia, vomiting\[^3\] and cancer\[^6\]. 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, cold, cough, inflammation and fever\[^3\]. 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 act by adhering 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\[^7-8\]. The early phase of acute inflammation involves cellular influx associated with the release of mediators like histamine and prostaglandins (PGEs)\[^9\]. All these mediators produce inflammation when injected subcutaneously in the rat paw\[^10\]. The present study is therefore an attempt to assess the efficacy of different root extracts of Curcuma zedoaria on inflammation induced by carrageenin and histamine in rat paw oedema model.

**Materials and Methods:**

**Plant material:**
The matured roots of Curcuma zedoaria (CZ) were collected from Cochin, Kerala, India. The plant materials were identified by Dr. A.K.S. Rawat, Scientist-E National Botanical Research Institute Lucknow, India. All the roots were shade dried at room temperature until they were free from moisture and pulverized in mechanical grinder. The powder obtained was extracted by continuous hot extraction process using soxhlet apparatus with universal solvent ethanol. The aqueous extract was prepared by cold maceration\[^11\]. The extracts were concentrated under reduced pressure and dried.

**Experimental animals:**
Albino rats of either sex (Wistar strain) weighing 150-170 g were used for the present study. Food and water were supplied ad libitum and the animals were kept in a 12-hour light-dark cycle. All the animals were maintained under controlled temperature (27±2°C). The experiment was conducted in accordance with the direction of Institutional Animal Ethics Committee (IAEC), CPCSEA, Government of India. (Resolution No. 31/7/2010-13). Due to painful condition imposed on animals the numbers of subjects used were restricted to the minimum six per group that allowed reliable statistical analysis of the results.

**Acute toxicity studies:**\[^12\]
Albino rats (150±40 g) received per oral (p. o.) extracts of Curcuma zedoaria in an increasing order of 175, 550, 1500, 2000 and 5000 mg/kg and an equivalent dose of vehicle was administrated to the control group. Extract treated and control group (5 animals each) were observed for 24 h under normal environmental conditions with free access to food and water. The gross behavioral changes were observed in animals such as hyperactivity, grooming,
convulsions, sedation, hypothermia and mortality up to 14 days.

**Anti-inflammatory Activity:**

_Curcuma zedoaria_ extracts were evaluated for anti-inflammatory activity against carrageenan and histamine (inflamagens) induced rat paw edema method.

**Selection of animal groups were taken for experiment is as follows:**

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

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

- **Test groups:**
  - Group-IV (ETE): Inflamagens + Ethanol extract 200 mg/kg (p.o)
  - Group-V (ETE): Inflamagens + Ethanol extract 400 mg/kg (p.o)
  - Group-VI (AE): Inflamagens + Aqueous extract 200 mg/kg (p.o)
  - Group-VII (AE): Inflamagens + Aqueous extract 400 mg/kg (p.o)

The root extracts were tested for anti-inflammatory activity by carrageenan and histamine induced rat paw edema method. Overnight-starved Wistar albino rats were divided into seven groups of 6 animals each. Selected doses were 200 mg and 400 mg/kg for each extracts. All the extracts and herbal drug were given orally half an hour before the administration of carrageenan (Sigma chemical co, St. Louis MO, USA) suspension. The edema was induced by the subplantar injection of 0.1ml of 1% solution of carrageenan and the volume of the injected foot was measured periodically. 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 before and after (1h) of carrageenan injection and then every hour up to 6 hours of each group. The difference between the initial and subsequent reading reveals the actual edema volume. The average paw swelling is calculated by comparing with control, standards and all extract treated groups.

Percent inhibition of inflammation was calculated by using the formula,

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

Where 'Vc' represents edema in control. 'Vt' is the edema in group treated with extracts.

**Histamine–Induced Paw Edema:**

In the present study of Histamine–induced paw edema in rats were treated exactly with 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**

No toxic effects were observed at a higher dose of extracts and standard herbal drug 5 g/kg body weight. Hence, there were no lethal effects in any of the groups.

**Anti-inflammatory activity**

The effect of ethanol and aqueous extracts of _C. zedoaria_ on carrageenan induced edema in albino rat is shown in Table I. The results obtained indicate that both ethanol 200 and 400 mg/kg extract showed 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 ethanolic 200 and 400 mg/kg extract treated groups of _C. zedoaria_ reduced edema up to 43.62 and 44.48% respectively. Aqueous 200 and 400 mg/kg extract shows inhibit paw edema up to 18.66 and 18.16% respectively. When compared with control group
The effect of ethanolic and aqueous extract of *C. zedoaria* root on histamine-induced edema in rats is shown in Table II. The histamine induced inflammation significantly (p< 0.001) inhibits (37.72 and 36.35%) the paw edema at 200 and 400 mg/kg dose of ethanol extract and the aqueous extract of CZ 200 and 400 mg/kg, shows non significant inhibition 15.76 and 11.47% respectively when compared with control group.

**Discussion:**
The present study investigates the inflammation inhibitory effect of ethanolic and aqueous extract of *Curcuma zedoaria* Rosc root. The most commonly used animal model for acute inflammation is carrageenan and histamine-induced rat paw edema. The inflammation induced by carrageenan is biphasic in nature. The initial phase of edema has been attributed to release histamine and serotonin. The edema maintained during the plateau phase, attributes to kinin like substances [15] and the late phase is mainly mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandin produced in the tissue macrophages [16-17]. The knowledge of these mediators involved in different phases is an important for interpreting mode of drug action. In this study the ethanol extract of CZ (200 and 400 mg/kg) showed significant reduce the paw edema at 2 h or more after carrageenan injection where as the aqueous extract (200 and 400 mg/kg) showed non significant reduction, suggesting that curcumin produces an anti-edematous effect during the second phase, similar to Indomethacin. In addition, the efficacy of ethanolic extract of CZ was comparable to that of Indomethacin with a longer duration of action showed significant reduction in paw edema volume equal to Rumalaya forte in carrageenan and histamine induced inflammation. More prominent inhibitory effect were observed in the late phase reaction indicating that this anti-inflammatory activity could be due to inhibitory effect on bradykinin, leukotrienes, polymorphonuclear cells and macrophage infiltration. These studies provide, a basis for further detailed investigations on therapeutic efficacy of the root extracts of this plant. Further study in the direction of elucidating the mechanism of anti-inflammatory activity should be carried out in future.

**Acknowledgements:**
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**Table-I:** Effect of *C. zedoaria* root extracts on carrageenan induced paw edema in rats

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Dose mg/kg</th>
<th>Paw edema (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st hour</td>
</tr>
<tr>
<td>Control</td>
<td>saline</td>
<td>0.550±0.050</td>
</tr>
<tr>
<td>SD-I</td>
<td>10</td>
<td>0.250±0.022a</td>
</tr>
<tr>
<td>SD-II</td>
<td>200</td>
<td>0.267±0.021a</td>
</tr>
<tr>
<td>ETE</td>
<td>200</td>
<td>0.400±0.043b</td>
</tr>
<tr>
<td>ETE</td>
<td>400</td>
<td>0.383±0.047b</td>
</tr>
<tr>
<td>AE</td>
<td>200</td>
<td>0.475±0.149</td>
</tr>
<tr>
<td>AE</td>
<td>400</td>
<td>0.525±0.111</td>
</tr>
</tbody>
</table>

Values represent the mean ± S.E.M. (N=6) a p<0.001, b p<0.01, c p<0.05 significant level and were analyzed by one way analysis of variation (ANOVA) followed by Dunnet’s test.
Table-II: Effect of C. zedoaria 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 hours</th>
<th>3rd hours</th>
<th>4th hours</th>
<th>5th hours</th>
<th>6th hours</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>
</tr>
<tr>
<td>SD-I</td>
<td>10</td>
<td>0.416±0.016</td>
<td>0.550±0.341</td>
<td>0.683±0.047</td>
<td>0.700±0.036</td>
<td>0.683±0.065</td>
<td>0.667±0.066</td>
</tr>
<tr>
<td>SD-II</td>
<td>200</td>
<td>0.367±0.044</td>
<td>0.416±0.030</td>
<td>0.583±0.054</td>
<td>0.617±0.030</td>
<td>0.425±0.047</td>
<td>0.583±0.030</td>
</tr>
<tr>
<td>ETE</td>
<td>200</td>
<td>0.380±0.037</td>
<td>0.520±0.037</td>
<td>0.680±0.020</td>
<td>0.640±0.040</td>
<td>0.600±0.032</td>
<td>0.580±0.037</td>
</tr>
<tr>
<td>ETE</td>
<td>400</td>
<td>0.450±0.035</td>
<td>0.533±0.022</td>
<td>0.667±0.033</td>
<td>0.667±0.033</td>
<td>0.583±0.040</td>
<td>0.533±0.021</td>
</tr>
<tr>
<td>AE</td>
<td>200</td>
<td>0.516±0.477</td>
<td>0.783±0.031</td>
<td>1.033±0.088</td>
<td>0.800±0.082</td>
<td>0.775±0.086</td>
<td>0.750±0.057</td>
</tr>
<tr>
<td>AE</td>
<td>400</td>
<td>0.483±0.047</td>
<td>0.85±0.062</td>
<td>0.967±0.1174</td>
<td>0.916±0.101</td>
<td>0.883±0.185</td>
<td>0.867±0.067</td>
</tr>
</tbody>
</table>

Values represent the mean ± S.E.M. (N=6) a p<0.001, b p<0.01, c p<0.05 significant level and were analyzed by one way analysis of variation (ANOVA) followed by Dunnet’s test.

Figure no: 1 Root extracts of C. zedoaria percentage Protection against carrageenan and histamine

Percentage inhibition was calculated. C –C/ C × 100. C= Control group and C= drug treated groups

References: