Mast cell stabilizing and lipoxygenase inhibitory activity of
*Cedrus deodara* (Roxb.) Loud. wood oil


Department of Pharmacology, Bombay College of Pharmacy, Kalina, Mumbai 400 098, India

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Volatile oil of *C. deodara*, administered orally at the doses of 50, 100 and 200 mg/kg body weight, significantly inhibited the pedal edema induced by compound 48/80 in rats. The oil significantly inhibited compound 48/80 induced degranulation of isolated rat peritoneal mast cells at concentrations ranging from 25-200 µg/ml. *C. deodara* wood oil also significantly inhibited the enzyme lipoxygenase at a concentration of 200 µg/ml. Thus, the anti-inflammatory activity of *C. deodara* wood oil could be attributed to its mast cell stabilizing activity and the inhibition of leukotriene synthesis.

*Cedrus deodara* (Roxb.) Loud. (Family-Pinaceae) is commonly known as Devadaru in Sanskrit, Deodar in Hindi and Marathi and Cedar in English. It is an ornamental and evergreen tree growing extensively on the slopes of Himalayas. Deodar forests are common from Kashmir to Garhwal. *Cedrus deodara* has been used since ancient days in the Ayurvedic Medical Practice for the treatment of inflammatory conditions such as rheumatoid arthritis and bronchitis.

Airway inflammation as seen in bronchitis is a complex interaction of various cells and mediators that result in the bronchial hyper-responsiveness and airway obstruction as well as ongoing inflammation. Mast cells and leukotrienes have been implicated in the pathogenesis of allergic and inflammatory disorders like bronchitis and rheumatoid arthritis.

In earlier studies the anti-inflammatory activity of the volatile oil of *C. deodara* was evaluated using various acute and chronic models of inflammation. Considering the significance of mast cells and leukotrienes in bronchitis and rheumatoid arthritis, in the present study the oil was further evaluated for anti-allergic and anti-inflammatory activity using compound 48/80 induced pedal edema in rats. To characterize the involvement of mast cells and leukotrienes, *in vitro* studies have also been carried out to evaluate the effect of the oil on compound 48/80 induced degranulation of isolated rat peritoneal mast cells and lipoxygenase enzyme.

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**Materials and Methods**

**Preparation of sample**—The oil obtained by steam distillation of the wood of *C. deodara*, was emulsified by using a mixture of acacia and tragacanth as emulsifying agents and administered orally at the doses of 50-200 mg/kg body weight for the *in vivo* study.

Solutions of oil prepared in alcohol (25-200 µg/ml) were used for *in vitro* studies.

**Drugs**—Ketotifen fumarate and phenidone were obtained as generous gift samples from FDC Ltd., Mumbai and Hoechst Marion Roussel Ltd., Mumbai respectively.

**Animals**—Male Wistar rats (150-200 g) were used. Animals were housed under standard conditions of temperature (23° ± 1°C), relative humidity (55 ± 10%), 12/12 hr light/dark cycles and fed with standard pellet diet (Gold Mohur brand, Lipton India Ltd.) and water *ad libitum*.

**Effect on compound 48/80 induced pedal edema in rats**—Rats (6/group) were injected with 0.1 ml solution of compound 48/80 in saline (100µg/ml) into the subplantar region of right hind paw. The paw volume was measured before the injection and 10, 20, 30, 45, 60, 120 and 180 min after the injection of compound 48/80 by volume displacement method using a mercury column connected to pressure transducer. The output from transducer was led to 4 channel polygraph (Polyrite, Recorders and Medicare Systems, Ambala), amplified and recorded by a pen recorder (Omnscribe, Digital Electronics, Mumbai). The edema volume was determined and...
expressed as percentage swelling, compared with the initial hind paw volume of each rat.
The control group received normal saline solution. The oil (50, 100 and 200 mg/kg) and ketotifen (1 mg/kg) as the reference standard were administered orally 1 hr before the induction of inflammation.

Effect on compound 48/80 induced degranulation of isolated rat peritoneal mast cells—Isolation of rat peritoneal mast cells:

Tyrode solution (10 ml) containing 5 units/ml heparin was injected in rats intraperitoneally. After a gentle massage for about 30-45 sec, the mast cell rich peritoneal fluid was collected over ice and centrifuged at 2000 r.p.m. for 5 min. The cells were washed twice with chilled Tyrode and resuspended in 1 ml Tyrode solution. These isolated peritoneal mast cells were used to study the effect of compound 48/80 induced mast cell degranulation in vitro.

To 0.1 ml peritoneal mast cell suspension, 0.1 ml alcoholic solution of C. deodara wood oil (25-200 μg/ml) was added and incubated in a constant temperature bath (37°C) for 15 min. In control, 0.1 ml mast cell suspension was incubated with 0.1 ml alcohol. Then 0.1 ml degranulating agent (compound 48/80, 10 μg/ml) was added and further incubated for 10 min. The cells were then stained with neutral red and % protection of degranulation of mast cells in the control and treated groups were calculated by counting the mast cells under high power (45X) of light microscope.

Effect on lipoxygenase enzyme activity—The effect of C. deodara wood oil on lipoxygenase enzyme activity was determined by an in vitro assay method using soyabean lipoxygenase as enzyme and linoleic acid as substrate. Soyabean lipoxygenase was procured from Sigma Chemical Company, U.S.A. and the lipoxygenase inhibitory activity was determined according to the procedure recommended by the manufacturer as follows.

Reagent A: (2M Borate buffer, pH 9)
Boric acid (6.18 g) was suspended in 300 ml of water. pH was adjusted to 9 using NaOH solution and the volume was made upto 500 ml with water.

Reagent B: (Substrate solution)
To 0.05 ml linoleic acid in a volumetric flask 0.05 ml of 95% undenatured alcohol was added and mixed gently till an emulsion was formed. Then volume was made upto 50 ml.

For assay : Diluted 5 ml of solution to 30 ml with Reagent A.

Reagent C: (Enzyme solution)
Prepared a solution of approximately 10,000 Units/ml of enzyme in ice cold Reagent A.

For assay: Approximately 500 U/3ml reaction volume was used.

Test agent i.e. C. deodara wood oil predissolved in alcohol (0.05 ml) was preincubated for 5 min at 25°C with 0.05 ml buffered enzyme before lipoxygenation was started by addition of linoleic acid (2ml). Appropriate blanks (heat denatured enzyme) and controls (buffer containing equivalent quantity of alcohol instead of test drug) were run through the same procedure. Reagent B was quickly added to the cuvet, mixed well and the absorbance was measured spectrophotometrically for 4 min at 234nm using Shimadzu UV-160A spectrophotometer. The rate of increase of absorbance was highest between 1-3 min after which it falls off. The maximal ΔA234 nm/min between 1-3 min interval was recorded.

Phenidone (100 μg/ml) was used as a reference standard. The inhibitory activity was determined using the formula:

\[
\text{Enzyme activity is expressed as} \frac{\Delta A_{234} \text{ / min}}{0.001 \times \text{mg enzyme / 3.0 reaction volume}}
\]

\[
\text{Units / mg solid} = \frac{\Delta A_{234} \text{ / min}}{0.001 \times \text{mg enzyme / 3.0 reaction volume}}
\]

Statistical analysis—Data were statistically analyzed by Student's t test. P<0.05 was considered to be statistically significant.

Results

Effect on compound 48/80 induced pedal edema in rats—Injection of compound 48/80 induced edema formation which reached its peak at 10 mins after the administration. C. deodara wood oil (50, 100 and 200 mg/kg, po) and ketotifen (1 mg/kg, po) significantly inhibited the paw edema induced by compound 48/80. The inhibition of edema by C. deodara wood oil at 50, 100 and 200 mg/kg, p o was to the extent of 34.58, 48.83 and 72.59% respectively in 10 min.
Ketotifen inhibited the edema to the extent of 59.16%. (Fig. 1)

Table 1—Effect of C. deodara wood oil on compound 48/80 induced degranulation of isolated rat peritoneal mast cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (µg/ml)</th>
<th>No. of mast cells (%)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>6 ± 2</td>
<td>--</td>
</tr>
<tr>
<td>Ketotifen</td>
<td>25</td>
<td>82 ± 5 *</td>
<td>80.85</td>
</tr>
<tr>
<td>C.d.</td>
<td>25</td>
<td>39 ± 2 *</td>
<td>35.11</td>
</tr>
<tr>
<td>C.d.</td>
<td>50</td>
<td>46 ± 6 *</td>
<td>42.55</td>
</tr>
<tr>
<td>C.d.</td>
<td>100</td>
<td>54 ± 5 *</td>
<td>51.06</td>
</tr>
<tr>
<td>C.d.</td>
<td>200</td>
<td>62 ± 5 *</td>
<td>59.58</td>
</tr>
<tr>
<td>C.d.: C. deodara wood oil</td>
<td>*: P&lt;0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Effect on compound 48/80 induced degranulation of isolated rat peritoneal mast cells—C. deodara wood oil at 25, 50, 100 and 200 µg/ml concentrations produced significant reduction of degranulation of mast cells induced by compound 48/80. Inhibition of degranulation was to the extent of 80.85% by ketotifen (25 µg/ml) and 35.11, 42.44, 51.09, 59.58% by C. deodara wood oil at the doses of 25, 50, 100 and 200 µg/ml respectively. (Table 1)

Effect on lipoxygenase enzyme activity—C. deodara wood oil at 25, 50, 100 µg/ml concentrations, was found to have no significant inhibitory activity on enzyme lipoxygenase. However, at 200 µg/ml it showed significant (P<0.001)

Table 2—Effect of C. deodara wood oil on lipoxygenase enzyme activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (µg/ml)</th>
<th>Mean change in absorbance per min</th>
<th>Enzyme activity (units/mg solid)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0.01 ± 0.001</td>
<td>2222.5 ± 315.85</td>
<td>--</td>
</tr>
<tr>
<td>Phenindone</td>
<td>100</td>
<td>0.005 ± 0.001</td>
<td>1109.67 ± 220.50</td>
<td>50.07</td>
</tr>
<tr>
<td>C.d.</td>
<td>25</td>
<td>0.016 ± 0.005</td>
<td>3445.00 ± 1695.02</td>
<td>--</td>
</tr>
<tr>
<td>C.d.</td>
<td>50</td>
<td>0.01 ± 0.004</td>
<td>2296.67 ± 921.54</td>
<td>--</td>
</tr>
<tr>
<td>C.d.</td>
<td>100</td>
<td>0.009 ± 0.003</td>
<td>2076.67 ± 712.91 **</td>
<td>6.56</td>
</tr>
<tr>
<td>C.d.</td>
<td>200</td>
<td>0.005 ± 0.001</td>
<td>1183.33 ± 127.02 *</td>
<td>46.76</td>
</tr>
</tbody>
</table>

C.d.: C. deodara wood oil
*: P<0.001
**: Non-significant

Fig. 1—Effect of C. deodara on time course of compound 48/80 induced pedal edema in rats (Values are mean ± S.D. of 6 observations)
lipoxygenase inhibitory activity which was comparable to that of the reference standard i.e. phenidone at 100 μg/ml (Table 2).

Discussion

*C. deodara* wood oil at the dose range of 50-200 mg/kg, po significantly inhibited compound 48/80 induced rat paw edema *in vivo*. The oil at the concentrations of 50-200 μg/ml also inhibited compound 48/80 induced degranulation of isolated peritoneal mast cells *in vitro*.

Compound 48/80 is one of the most potent mast cell degranulators which causes liberation of mediators of inflammation such as histamine, leukotrienes, platelet activating factors, chemotactic factors for eosinophils and neutrophils etc from mast cells\(^\text{11}\). These mediators are responsible for immediate as well as delayed inflammatory reactions. Inhibition of compound 48/80 induced rat paw edema by *C. deodara* wood oil could be due to its ability to interfere in the release and/or synthesis of mediators of inflammation. Further, the inhibition of compound 48/80 induced degranulation of isolated mast cells by *C. deodara* wood oil is indicative of its mast cell stabilizing activity. Thus the anti-inflammatory activity of *C. deodara* wood oil could be attributed to its mast cell stabilizing activity leading to inhibition of the release of inflammatory mediators from mast cells.

In the present study effect of *C. deodara* on leukotriene synthesis i.e. on enzyme 5-lipoxygenase was studied in an *in vitro* model. The study was carried out using soyabean lipoxygenase as enzyme and linoleic acid as substrate, since it has been shown by Bailey and Chalkein that lipoxygenase reaction cascade occurs both in mammalian and plant tissue\(^\text{12}\). It was found that at 200μg/ml concentration, *C.deodara* wood oil showed significant inhibition of enzyme lipoxygenase indicating its ability to inhibit the synthesis of leukotrienes. Leukotrienes are one of the important inflammatory mediators that are released from mast cells and other variety of cells along with the other mediators. They have direct toxic effects or recruit other cells which phagocytosize the damaged tissue and in the process lead to further release of inflammatory mediators, thus resulting in perpetuation of this vicious cycle. Inhibition of lipoxygenase enzyme by *C. deodara* wood oil leading to inhibition of synthesis of leukotrienes and in turn the vicious cycle of inflammation could be one of the mechanisms of its anti-inflammatory activity.

Although effect of *C. deodara* wood oil on synthesis of other mediators of inflammation remains to be studied, the mast cell stabilizing action and lipoxygenase inhibitory activity of the oil can partly explain the mechanism of its anti-inflammatory activity. Thus the present study justifies the rationale behind the use of the wood of *C. deodara* in the treatment of rheumatoid arthritis and bronchitis as claimed in ancient Ayurvedic System of Medicine.

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References