Evaluation of antiinflammatory activity of fatty acids of *Ocimum sanctum* fixed oil

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*Ocimum sanctum* fixed oil and linolenic acid were found to possess significant antiinflammatory activity against PGE, leukotriene and arachidonic acid-induced paw edema. Plant lipids like linseed oil and soyabean oil containing linolenic acid when tested along with *O. sanctum* fixed oil, also showed significant inhibition of carrageenan-induced paw edema. The results suggest that linolenic acid present in *O. sanctum* fixed oil has the capacity to block both the cyclooxygenase and lipoxygenase pathways of arachidonate metabolism and could be responsible for the the antiinflammatory activity of the oil.

*Ocimum sanctum* L. (labiatae), popularly known as holy basil, is a well known sacred plant of the Hindus, to which several medicinal properties have been attributed in traditional system of medicine. *O. sanctum* leaves have been reported to have antiinflammatory and analgesic activity. Volatile oil distilled from leaves has been claimed to have antiinflammatory, antihistaminic, antibacterial and antifungal activity. Similarly *O. sanctum* fixed oil obtained from seeds, possesses significant antiinflammatory, antipyretic, analgesic, antiarthritic and antiulcer activities without any noticeable toxicity. In a clinical trial fixed oil has also been found to be effective in bovine mastitis. The fixed oil contains five fatty acids viz. palmitic (11.69%), stearic (3.19%), oleic (13.82%), linoleic (52.23%) and linolenic (16.63%) and the triglyceride fraction of oil possesses higher antiinflammatory and analgesic activities compared to the oil. In the present study the antiinflammatory activity of different unsaturated fatty acids of the oil has been tested, to evaluate the contribution of fatty acids towards antiinflammatory activity of oil. Antiinflammatory activity of some plant vegetable oils like linseed oil, soyabean oil and sunflower oil which contains varying proportions of oleic, linoleic and linolenic acids has also been studied.

**Materials and Methods**

Dried seeds of *O. sanctum* (collected from Maidan Garhi, New Delhi, India and authenticated by a resident botanist, Department of Genetics, Indian Council of Agricultural Research, New Delhi) were crushed and cold macerated in petroleum ether (40°-60°C) (S.D.Fine Chemicals Ltd., India) for 3 days. The petroleum ether was evaporated from the extract and oil was filtered to clarity. The fixed oil thus obtained was subjected to antiinflammatory studies. The unsaturated fatty acid composition (%) of the fixed oils used in the study was as follows linseed oil (oleic acid 12-30, linoleic acid 8-29, linolenic acid 35-67), soyabean oil (oleic acid 22-34, linoleic acid 50-60, linolenic acid 2-10), sunflower oil (oleic acid 49.41, linoleic acid 40-48).

**Pharmacological studies**

Different pharmacological activities of *O. sanctum* fixed oil by us so far have been evaluated following administration of oil by intraperitoneal route. It has been found that in PGE₂-induced rat paw edema model, ip administration of *O. sanctum* fixed oil produces much higher inhibition of edema compared to that obtained after oral administration of identical dose and the effect appears to be due to faster absorption of oil by ip route. In addition absorption from ip route is free from effects of gastric emptying or presystemic gastro-intestinal and gut wall metabolism which affects oral absorption. Keeping the same in view, in the antiinflammatory studies fixed oil and different fatty acids were administered by ip route.

**Carrageenan-induced inflammation**—Wistar albino rats weighing between 120-170 g (M/s Lucky Zoological House, New Delhi) were fasted for 24 hr
before experimentation but water was allowed ad libitum. They were divided into 5 groups of 6 animals each. Group I served as control and received distilled water (3ml/kg), group II received fixed oil of *O. sanctum* (3ml/kg), group III received linseed oil (3ml/kg)(M/s Arora Pharmaceuticals Ltd., New Delhi), group IV received soyabean oil (3ml/kg) (M/s SM Dychem Ltd. (Food Division), Bombay) and group V received sunflower oil (3ml/kg) (M/s Tina Oils and Chemicals Ltd., Bombay) ip. After 30 min inflammation was induced by injecting 0.1ml freshly prepared 1% carrageenan (Central Drug House, New Delhi) in normal saline into the planter aponeurosis of the right hind paw. The paw volume was measured plethysmographically immediately and 3 hr after carrageenan injection as prescribed by Winter et al.\(^9\). Statistical calculations were done by Student’s t test.

**PGE\(_2\)-induced inflammation**—In another set of experiment, albino rats were selected and divided into 5 groups of 6 animals each. Group I served as control and received distilled water (3ml/kg), group II received oleic acid methyl ester (Sigma, USA) (0.41ml/kg, equivalent to 3ml/kg of fixed oil of *O. sanctum*) calculated according to percentage of oleic acid (i.e. 13.82%) in fixed oil of *O. sanctum*, group III received linoleic acid methyl ester (Sigma USA) (1.56 ml/kg, equivalent to 3ml/kg of fixed oil of *O. sanctum*), group IV received linolenic acid methyl ester (Sigma, USA) (0.49 ml/kg, equivalent to 3ml/kg of fixed oil of *O. sanctum*) and group V received fixed oil of *O. sanctum* (3ml/kg) ip. Prostaglandin E\(_2\) (Sigma, USA) (10\(^{-6}\) g/ml, 0.1ml) was injected into the right hind paw of the rats after 30 min of administration of either of the fatty acids or fixed oil of *O. sanctum* and the edema volume was measured after 30 min of PGE\(_2\) injection\(^9\) as done previously following Winter et al.\(^9\).

**Leukotriene-induced inflammation**—In order to ascertain whether the fatty acids had any inhibitory effect on lipoxygenase (the enzyme involved in the lipoxygenase pathway of inflammation), inflammation was induced by leukotriene, a selective lipoxygenase mediator for inflammation. In this experimental set up, groups of albino rats, received either fatty acids or fixed oil of *O. sanctum* ip, at the same dose level, as given in previous experiment. After 30 min, edema was produced by injecting leukotriene (LTB\(_4\) methyl ester, Sigma USA, 0.1ml (0.1 µg LTB\(_4\)) into subplanter aponeurosis in the right hind paw of rats and edema were measured after 30 min plethysmographically as described earlier.

**Arachidonic acid-induced inflammation**—To further explore the mechanism of antiinflammatory action, arachidonic acid-induced inflammation in rats was studied as described by Di Martino et al.\(^8\). Paw edema was induced by a single subplanter injection of 0.1ml (0.5%) of arachidonic acid (in 0.2 M carbonate buffer, pH 8.43-8.56) into the right hind paw of rats 30 min after ip administration of either of the fatty acids or *O. sanctum* fixed oil. The edema volume was measured after 30 min plethysmographically as described earlier.

### Results and Discussion

The results are presented in Tables 1 and 2. *O. sanctum* fixed oil contains a mixture of five fatty acids viz. palmitic (11.69%), stearic (3.19%), oleic (13.82%), linoleic (52.23%) and linolenic (16.63%) acid. To ascertain the antiinflammatory property of *O. sanctum* fixed oil, a number of plant fixed oils like linseed oil, soyabean oil and sunflower oil which possess varying proportions of unsaturated fatty acids were tested for antiinflammatory activity using carrageenan-induced paw edema model in rats. The results (Table 1) indicate that *O. sanctum* fixed oil, linseed oil and soyabean oil significantly reduced the paw edema. Linseed oil which contains highest percentage of linolenic acid (35-67%) offered maximum inhibition (75.41%). *O. sanctum* fixed oil containing 16.63% linolenic acid provided 70.50% inhibition, while soyabean oil which contains 2-10 % linolenic acid offered least inhibition (45.91%). Sunflower oil containing no linolenic acid failed to

| Table 1—The effect of various fixed oils on carrageenan-induced paw edema in rats |
|-------------------------------|-------------------------------|--------------------------|
| Treatment                     | Edema volume (ml)             |
|--------------------------------|-------------------------------|--------------------------|
| Control (dist. water)         | 0.61 ± 0.03                   |
| *O. sanctum* fixed oil        | 0.18 ± 0.01*                  |
| Linseed oil                   | 0.15 ± 0.01*                  |
| Soyabean oil                  | 0.33 ± 0.01**                 |
| Sunflower oil                 | 0.46 ± 0.07                   |
| P values * < 0.01; ** < 0.05 |

[Values are mean ± SE from 6 animals in each group. Figures in parentheses are % inhibition in edema volume]
produce any significant effect. The results indicate the likely involvement of linolenic acid towards antiinflammatory activity of *O. sanctum* fixed oil.

In order to ascertain the contribution of unsaturated fatty acids of *O. sanctum* towards antiinflammatory activity, inflammation was induced by PGE$_2$ in rats. The results indicate that linolenic acid provided maximum inhibition (52.84%) followed by *O. sanctum* fixed oil (45.29%) (Table 2) and other fatty acids namely oleic and linoleic acid did not produce any significant effect. Therefore at this stage, it can be suggested that antiinflammatory effect of fixed oil could be at least partially due to inhibition of prostaglandin (cyclooxygenage pathway of arachidonic acid metabolism) by linolenic acid.

Lipoxygenase inhibitors also possess significant antiinflammatory effect$^{19}$. To explore the same, inflammation was next induced by leukotriene (LTB$_4$, methyl ester), a selective lipoxygenase mediator for inflammation involving lipoxygenase pathway and the inhibitory effect of various fatty acids and fixed oil of *O. sanctum* were studied. The results show that *O. sanctum* fixed oil and linolenic acid significantly inhibited the edema formation. Fixed oil and linolenic acid afforded 49.19% and 57.38% inhibitions while oleic and linoleic acids had no significant inhibition (Table 2), which indicates that linolenic acid is responsible for the antiinflammatory activity and the same may also act by inhibition of lipoxygenase pathway of arachidonate metabolism.

Similarly in arachidonic acid-induced edema model, the results (Table 2) show that percentage inhibition of edema with linolenic acid (66.13%) was more than that produced by *O. sanctum* fixed oil (58.07%), while other fatty acids failed to produce significant inhibition of paw edema. Arachidonic acid-induced paw edema in rats is highly sensitive to inhibition by dual inhibitors of arachidonic acid metabolism, corticosteroids and antihistamine/antiserotonin agents but is insensitive to cyclooxygenase inhibitors$^{18}$.

Hence linolenic acid in fixed oil of *O. sanctum* could possibly account for dual inhibition of arachidonate metabolism. Lipids extracted from the seeds of evening primrose and borage plants have been reported to be antiinflammatory due to the presence of relatively large amounts of gammalinolenic acid (GLA), an omega-6(18:3, n-6) fatty acid (All cis-6,9,12 octadecatrienoic acid) which contains the first double bond at 6$^\text{th}$ carbon atom from the methyl (omega) end of the fatty acid chain. GLA is rapidly converted to dihomgammalinolenic acid (DGLA)(20:3,n-6)(a precursor of antiinflammatory prostaglandin E$_1$) which competes with arachidonate for oxidative enzymes thereby reducing production of cyclooxygenase products derived from arachidonate. In addition DGLA is converted by 5-lipoxygenase to 15-hydroxy DGLA which possesses 5-lipoxygenase inhibitory activity$^{20,21}$. The results of the present study show that linolenic acid could inhibit both cyclooxygenase and lipoxygenase pathways of inflammation. Linolenic acid an omega-3 (18:3,n-3) fatty acid (All cis-9,12,15 octadecatrienoic acid) is progressively metabolised in the body to 6,9,12,15 octadecatetraenoic acid (18:4,n-3), stearadonic acid (20:4,n-3) and eicosapentaenoic acid (20:5,n-3)$^{21}$. The end product eicosapentaenoic acid has the capacity to competitively inhibit the formation of

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose(ip)</th>
<th>Edema volume (ml)</th>
<th>a</th>
<th>b</th>
<th>c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (dist. water)</td>
<td>3.0 ml/kg</td>
<td>0.50 ± 0.03</td>
<td>0.61 ± 0.03</td>
<td>0.62 ± 0.02</td>
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<tr>
<td>Oleic acid methyl ester</td>
<td>0.41 ml/kg</td>
<td>0.48 ± 0.03 (9.44)</td>
<td>0.55 ± 0.03 (9.84)</td>
<td>0.56 ± 0.04 (9.68)</td>
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<tr>
<td>Linoleic acid methyl ester</td>
<td>1.56 ml/kg</td>
<td>0.46 ± 0.03 (13.21)</td>
<td>0.50 ± 0.05 (18.04)</td>
<td>0.51 ± 0.06 (17.75)</td>
<td></td>
</tr>
<tr>
<td>Linolenic acid methyl ester</td>
<td>0.49 ml/kg</td>
<td>0.25 ± 0.02* (52.84)</td>
<td>0.26 ± 0.01* (57.38)</td>
<td>0.21 ± 0.01* (66.13)</td>
<td></td>
</tr>
<tr>
<td>*P values &lt; 0.01</td>
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Table 2—Effect of various fatty acids and *O. sanctum* fixed oil on (a) PGE$_2$ (b) leukotriene (LTB$_4$, methyl ester), and (c) arachidonic acid induced paw edema in rats

Values are mean ± SE from 6 animals in each group. Figures in parentheses are % inhibition in edema volume.
prostaglandins and leukotrienes derived from arachidonate while serving as a substrate for prostaglandins with three double bonds and leukotrienes with five double bonds \(^{22-24}\) which could be one possible mechanism of dual inhibition and antiinflammatory activity of linolenic acid. Octadecatetraenoic acid and stearadonic acids possessing four double bonds could also act in the same way. In addition linolenic acid containing three double bonds could compete with arachidonic acid and offer itself as a substrate for cyclooxygenase and reduce the formation of prostaglandins. Polyunsaturated fatty acid having two cis double bonds separated by a methylene group is a substrate for lipoxygenase \(^{25}\). Linolenic acid since contains three double bonds in this configuration, could therefore, also act as a substrate for lipoxygenase and inhibit formation of leukotrienes. Thus linolenic acid or its metabolites could competitively inhibit the formation of prostaglandins and leukotrienes from arachidonic acid and thereby produce antiinflammatory effect. But further studies are needed to comment on exact mechanism of action.

Thus it may be concluded that linolenic acid present in the fixed oil of *O. sanctum* could be responsible for its antiinflammatory activity and the same has the potential to inhibit both the cyclooxygenase and lipoxygenase pathways of arachidonate metabolism (dual inhibition property).

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**References**