Evaluation of anti-inflammatory activity of plant lipids containing α-linolenic acid

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Two groups of fatty acids are essential to the body, the ω6 (n6) series derived from linoleic acid (18:2, n-6) and the ω3 (n3) series derived from α-linolenic acid (18:3, n-3). Fatty acids provide energy, are an integral part of the cell membranes and are precursors of prostaglandins, thromboxanes and leukotrienes collectively known as eicosanoids. Eicosanoids participate in development and synthesis of immunological and inflammatory responses. The fixed oils (1, 2, 3 ml/kg) containing α-linolenic acid, obtained from the seeds of Linseed (Linum usitatissimum), Soyabean (Glycine max) and Holy basil (Ocimum sanctum) were screened for their antiinflammatory activity using carrageenan, leukotriene and arachidonic acid induced paw edema models in rats and the antiinflammatory effects were compared with the standard drug indomethacin. Significant inhibition of paw edema was produced by all the oils in the highest dose (3ml/kg) in all the models. While O. sanctum oil produced the maximum percentage inhibition in leukotriene induced paw edema, L. usitatissimum oil produced maximum percentage inhibition in carrageenan and arachidonic acid induced paw edema models. The results show that oils with higher α-linolenic acid content (L. usitatissimum and O. sanctum) produced a greater inhibition of paw edema suggesting that modulation of the course of inflammatory disorders may be achieved by altering the eicosanoid precursor (i.e. poly unsaturated fatty acids; PUFA) availability through dietary manipulation.

Keywords: α–Linolenic acid, Eicosanoids, Glycine max, Linum usitatissimum, Ocimum sanctum, Paw edema

Research on lipids conducted in the last 20 years has upgraded our knowledge about essential fatty acids. Two groups of fatty acids are essential to the body. The ω6 (n6) series derived from linoleic acid (18:2, n-6) and the ω3 (n3) series derived from α-linolenic acid (18:3, n-3). Fatty acids provide energy, are an integral part of the cell membranes and are precursors of prostaglandins, thromboxanes and leukotrienes collectively known as eicosanoids. A number of experimental evidence supports that eicosanoids participate in development of immunological and inflammatory responses. Some novel fatty acids may be safe and effective anti-inflammatory and immunomodulatory agents. Certain plant lipids, notably those extracted from the seeds of evening primrose and borage plants contain relatively large amount of gammalinolenic acid (GLA). This fatty acid is rapidly converted to dihomogammalinolenic acid (DGLA) (20:3, n-6; a precursor of prostaglandin E1) which has anti-inflammatory and immunomodulatory properties. In humans, the 5-desaturase which converts DGLA to arachidonic acid is less active. Thus, the concentration of arachidonate for oxidative enzymes reduces, and this in turn reduces the production of cyclooxygenase products derived from arachidonate. In addition, DGLA cannot be converted to inflammatory leukotrienes by 5-lipoxygenase. GLA enrichment of diet has been found to suppress acute and chronic inflammation as well as joint tissue injury in several experimental animal models.

Several independent lines of evidence strongly indicate that changes in the course of hypertension, atherothrombotic and inflammatory disorders may be achieved by altering the eicosanoid precursor (poly unsaturated fatty acids; PUFA) availability through dietary manipulation

α-linolenic acid is an essential fatty acid but its occurrence is not so common. The main commercial sources for the pure compound are plants, where it is found in reasonable quantities in the seed oil. Evening primrose oil is the main source of linolenic acid, which contains 9% linolenic acid in its fixed oil. India has a rich heritage of medicinal plants. Systematic studies have been carried out on quite a number of plants but many aspects of these still remain unexplored. One of the widely grown Indian plants, O. sanctum contains 16.63% linolenic acid in the fixed oil of seeds. The fixed oil possesses significant

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anti-inflammatory, analgesic, antiarthritic, antipyretic, antiulcer, antimastitic and antimicrobial properties without any noticeable toxicity. Fixed oil of a number of plants contains large amounts of $\alpha$-linolenic acid. The fixed oils obtained from linseed and soyabean also contain reasonable amount of linolenic acid. The anti-inflammatory potential of these oils is yet to be explored.

With this background, the present study has been undertaken to screen fixed oils (containing $\alpha$-linolenic acid) from linseed (obtained from the seeds of *Linum usitatissimum* Linn.), soyabean (obtained from the seeds of *Glycine max* (L.) Merr) and Holy basil (obtained from the seeds of *Ocimum sanctum* Linn.) for their antiinflammatory activity using different animal models.

**Materials and Methods**

*Plant material* — Fixed oil of the dried seeds of *O. sanctum*, *L. usitatissimum* and *G. max* were extracted by cold maceration using petroleum ether (40°-60°C) for 3 days. The dried seeds, procured from Unani pharmacies in Delhi were authenticated by resident botanist at Jamia Hamdard. A voucher specimen of each has been retained at the Institute for further reference (AIIMS/Pharma/SS/2007-009-011). The fixed oils thus obtained were subjected to following pharmacological studies.

*Animals* — Wistar albino rats of either sex weighing between 120-200 g from Institute breeding stock were used for the study. Ethical clearance was obtained from the Institutional Animal Ethics Committee, AIIMS, New Delhi, India, before conducting the study. The rats were maintained at 25°C±1°C with a 12:12 hr light-dark cycle for 5 days with free access to food and water. They were deprived of food 24 hr before the experiment. Inflammation was induced by subcutaneous administration of carrageenan, leukotriene or arachidonic acid into the hind paw of the animal. The anti-inflammatory efficacies of the fixed oils were evaluated in all the models.

*Induction of paw edema in rats* — Overnight fasted rats were divided into 5 groups of 6 each for evaluating fixed oil. Group I received 2 ml/kg distilled water and served as the control, groups II-IV received 1, 2 and 3 ml/kg of fixed oil respectively and group V received indomethacin (10 mg/kg, ip). Carrageenan (0.1 ml, 1% freshly prepared in normal saline), or 0.1 ml leukotriene (0.1mg LTB$_4$ methyl ester) or arachidonic acid (in 0.2M carbonate buffer; pH 8.43-8.56) was injected into the right hind paw of rats 30 min after treatment with either distilled water, fixed oil or standard drug. Paw volume was measured immediately and 3 hr after the administration of carrageenan, leukotriene or arachidonic acid.

**Statistical analysis** — Statistical analysis was done using One-way ANOVA, followed by Dunnett’s Multiple Comparison. *P*<0.05 was considered to be significant.

**Results**

*Effect of various plant lipids on carrageenan induced paw edema* (Table 1) — All the test compounds, viz. *O. sanctum* oil (OS oil), *L. usitatissimum* oil and *G. max* oil showed a dose dependent inhibition of paw swelling. The reduction in paw swelling was highly significant (*P*<0.01) as compared to the control in all doses of *O. sanctum* and *L. usitatissimum* oil, but significant reduction was seen only with the higher dose groups (2 and 3 ml/kg) of *G. max* oil. Maximum inhibition was seen with 3ml/kg of linseed oil (75.80%) as compared to the standard drug indomethacin (64.51%).

*Effect of various plant lipids on leukotriene induced paw edema* (Table 1) — All the test compounds, viz. *O. sanctum* oil, *L. usitatissimum* oil and *G. max* oil showed a dose dependent inhibition of paw swelling. The reduction in paw swelling was highly significant (*P*<0.01) as compared to the control in all doses of *O. sanctum* and the higher two doses (2 and 3 ml/kg) of *L. usitatissimum* oil and *G. max* oil. Maximum inhibition was seen with 3ml/kg of *Ocimum sanctum* oil (58.06%) as compared to the standard drug indomethacin (51.61%).

*Effect of various plant lipids on arachidonic acid induced paw edema* (Table 1) — The test compounds, viz. *O. sanctum*, *L. usitatissimum* and *G. max* oil showed a dose dependent inhibition of paw swelling. The reduction in paw swelling was highly significant (*P*<0.01) as compared to the control in all doses of *L. usitatissimum* and *G. max* oil. In the case of *O. sanctum* oil, the reduction was significant only in the higher doses (2 and 3 ml/kg). Maximum inhibition was seen with 3ml/kg of *L. usitatissimum* oil (74.19%) as compared to the standard drug indomethacin (61.29%).

**Discussion**

In the present study, a dose dependent reduction was seen in carrageenan, leukotriene and arachidonic
acid induced paw edema in all the plant lipids tested, viz. *O. sanctum* oil, *L. usitatissimum* oil, and *G. max* oil. Results demonstrate that oils containing higher amount of linolenic acid inhibited phlogistic agent induced paw edema to a greater extent. Maximum inhibition was obtained with linseed oil (containing the highest amount of linolenic acid), followed by *O. sanctum* oil. The results also indicate that linolenic acid in all fixed oils could possibly account for the anti-inflammatory activity of the oil by dual inhibition (eicosapentaenoic acid has the capacity to competitively inhibit the formation of prostaglandins and leukotrienes and serves as a substrate for synthesis of prostaglandins with three double bonds and leukotrienes with five double bonds) of arachidonate metabolism\(^{14-17}\), as it significantly inhibited the paw edema in both leukotriene and arachidonic acid induced paw edema. These plant lipids contain relatively large amount of gammalinolenic acid (GLA), an omega-6 (18:3, n-6) fatty acid (all cis-6,9,12 octadecatetraenoic acid) which contains the first double bond at 6\(^{th}\) carbon atom from the methyl (\(\omega\)) end of the fatty acid chain. GLA is rapidly converted to dihomogammalinolenic acid (DGLA) (20:3, n-6) (a precursor of anti-inflammatory prostaglandin E\(_1\)) which competes with arachidonic 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\(^2\). The results of the different studies shows that linolenic acid could inhibit both cyclooxygenase and lipoxygenase pathways of inflammation\(^18\). Linolenic acid, an \(\omega-3\) (18:3, n-3) fatty acid (all cis-9, 12, 15 octadecatetraenoic acid), is progressively metabolized 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). The end product eicosapentaenoic acid has the capacity to competitively inhibit the formation of prostaglandins and leukotrienes derived from arachidone while serving as a substrate for synthesis of prostaglandins with three double bonds and leukotrienes with five double bonds, which are antiinflammatory. This could

<table>
<thead>
<tr>
<th>Fixed oil</th>
<th>Groups and Drug Dose</th>
<th>Carrageenan induced paw edema</th>
<th>Leukotriene induced paw edema</th>
<th>Arachidonic acid induced paw edema</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>a</td>
<td>b</td>
<td>a</td>
</tr>
<tr>
<td><em>O. sanctum</em> oil</td>
<td>Group I (Control-2ml/kg normal saline)</td>
<td>0.62±0.02</td>
<td>0.62±0.02</td>
<td>0.61±0.03</td>
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<td>Group II (1ml/kg)</td>
<td>0.50±0.02**</td>
<td>19.35</td>
<td>0.49±0.03**</td>
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<td>Group III (2ml/kg)</td>
<td>0.39±0.02**</td>
<td>37.09</td>
<td>0.40±0.02**</td>
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<td>Group IV (3ml/kg)</td>
<td>0.26±0.01**</td>
<td>58.06</td>
<td>0.26±0.01**</td>
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<tr>
<td></td>
<td>Group V (Indomethacin–10mg/kg)</td>
<td>0.22±0.02**</td>
<td>64.51</td>
<td>0.30±0.02**</td>
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<tr>
<td><em>L. usitatissimum</em> oil</td>
<td>Group I (Control-2ml/kg normal saline)</td>
<td>0.62±0.03</td>
<td>0.62±0.01</td>
<td>0.62±0.03</td>
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<tr>
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<td>Group II (1ml/kg)</td>
<td>0.46±0.01**</td>
<td>25.80</td>
<td>0.53±0.01*</td>
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<td>Group III (2ml/kg)</td>
<td>0.35±0.02**</td>
<td>43.54</td>
<td>0.43±0.01**</td>
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<tr>
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<td>Group IV (3ml/kg)</td>
<td>0.15±0.01**</td>
<td>75.80</td>
<td>0.30±0.01**</td>
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<td>Group V (Indomethacin–10mg/kg)</td>
<td>0.22±0.01**</td>
<td>64.51</td>
<td>0.30±0.02**</td>
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<tr>
<td><em>G. max</em> oil</td>
<td>Group I (Control-2ml/kg normal saline)</td>
<td>0.62±0.02</td>
<td>0.56±0.03</td>
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<td>Group II (1ml/kg)</td>
<td>0.57±0.02</td>
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<td>61.29</td>
<td>0.22±0.01**</td>
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</tbody>
</table>

\(a = \) Increase in paw volume (ml) at 3hr

\(b = \) Inhibition (%) of paw edema at 3hr

Statistical analysis by One-way ANOVA followed by Dunnett’s Multiple Comparison. \(P\) values: *<0.05; **<0.01
be a possible mechanism for the anti-inflammatory activity of linolenic acid15.

The present findings suggest that incorporation of these agents in the diet could help in modulating the course of inflammatory disorders by altering the eicosanoid precursor (i.e. PUFA) availability.

References