Antiinflammatory, analgesic and antipyretic activities of *Linum usitatissimum* L. (flaxseed/linseed) fixed oil

Gaurav Kaithwas\(^a\), Alok Mukherjee\(^a\), A K Chaurasia\(^b\) & Dipak K Majumdar\(^c\)*

\(^a\)Department of Pharmaceutical Sciences, Faculty of Health & Medical Sciences
\(^b\)Department of Genetics and Plant Breeding (Seed Technology), Allahabad Agricultural Institute-Deemed University, Allahabad 211 007, India
\(^c\)Delhi Institute of Pharmaceutical Sciences and Research, (formerly College of Pharmacy), University of Delhi, Pushp Vihar, Sector III, New Delhi 110 017, India

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The fixed oil of *L. usitatissimum* (flaxseed/linseed) inhibited PGE\(_2\), leukotriene-, histamine- and bradykinin-induced inflammation. The oil also inhibited arachidonic acid-induced inflammation, suggesting its capacity to inhibit both cyclooxygenase and lipooxygenase pathways of arachidonic metabolism. In tail immersion model, the oil raised the pain threshold to a lesser extent than morphine but showed excellent peripherally acting, analgesic activity comparable to aspirin, against acetic acid-induced writhing in mouse. In typhoid paratyphoid A/B vaccine-induced pyrexia, the oil showed antipyretic activity comparable to aspirin. The oil contains 57.38\% \(\alpha\)-linolenic acid. Dual inhibition of arachidonic acid metabolism, antihistaminic and antibradykinin activities of the oil could account for the biological activity and the active principle could be \(\alpha\)-linolenic acid an omega-3 (18:3, n-3) fatty acid.

**Keywords:** Antibradykinin, Antihistaminic, Arachidonic acid, Dual-inhibitor, \(\alpha\)-Linolenic acid

*Linum usitatissimum* L, (also known as common flax or linseed) an annual herb believed to have originated in Egypt, is a member of the genus Linum in the family *Linaceae*. The seeds produce a fixed oil known as linseed oil or flaxseed oil. It is one of the oldest commercial oil and solvent processed flaxseed oil has been used for centuries as a drying oil in painting and varnishing. Raw oil is used as an astringent in fungicidal lotion, insecticide and has moderate insect repellent properties\(^1\). The oil contains unsaturated fatty acids like oleic acid (12-30\%), linoleic acid (8-29\%) and linolenic acid (35-67\%)\(^2\). These fatty acids appear to render drying property to the oil. *L. usitatissimum* fixed oil has been found to inhibit inflammation induced by carrageenan\(^3\). Therapeutic effect of *L. usitatissimum* fixed oil on acute and chronic arthritic models in albino rats has been reported\(^4\). The antiulcer activity of *L. usitatissimum* oil in animal models has also been demonstrated\(^5\). The antimicrobial activity of *L. usitatissimum* oil and its therapeutic efficacy in bovine mastitis, an inflammatory disorder caused by microbial infection, has been reported recently\(^6\). The present study has been undertaken to evaluate the antiinflammatory, analgesic and antipyretic potential of *L. usitatissimum* fixed oil.

**Materials and Methods**

**Plant material**—Flaxseed/Linseed, (Variety: JL-59) was obtained from Division of Seed Science, Department of Agronomy, Allahabad Agricultural Institute-Deemed University (AAI-DU), Allahabad, India. The seeds were authenticated at National Botanical Research Institute (NBRI, CSIR), Lucknow, India and voucher sample was deposited at NBRI.

**Extraction of oil**—Seeds were crushed and cold macerated in petroleum ether (40°-60°C) for 7 days. Petroleum ether was evaporated from the extract and the oil was filtered to clarity. The oil was stored at room temperature in amber colored airtight bottle. To avoid oxidation, the oil was purged with nitrogen and was filled to the brim of the bottle so that there was no head space. The yield of fixed oil was 17.50\% v/w with reference to dried seeds. The density of the oil was 0.952 g/ml. Gas chromatographic...
analysis [Agilent GC make 6890; column: DB-FFAP, dimension (30 M × 0.53 mm ×1.0 µm) using flame ionization detector; carrier gas: nitrogen; volume of injection 1 µL; internal standard: cetyl alcohol] of the methyl ester of oil revealed the presence of palmitic acid (5.53%), stearic acid (4.67%), oleic acid (19.05%), linoleic acid (13.67%) and linolenic acid (57.38%). The oil thus obtained was subjected to further studies.

Animals—Wistar strain albino rats (100-150 g) and Swiss albino mice (20-25 g) were obtained from Central Animal House, Department of Animal Husbandry, Allahabad Agricultural Institute-Deemed University. Animals were housed under standard conditions of temperature (25° ± 1°C) with natural light dark cycle and had free access to commercial pellet diet and water. The animals were given week’s time to get acclimatized with the laboratory condition, before experimentation. The study was approved by the Institutional Animal Ethics Committee.

Drugs and chemicals—Carrageenan was purchased from CDH, New Delhi. Prostaglandin E₂, leukotriene (LTB₄, methyl ester), bradykinin and arachidonic acid were supplied by Sigma Aldrich. Histamine acid phosphate was obtained from Acros Chemicals, USA. Typhoid Paratyphoid A/B vaccine (Tyvax-Vi Plus) was procured from VHB Pharmaceuticals Ltd., Mumbai. Acetic acid was procured from S.D. Fine Chemicals Ltd., Mumbai and morphine (Rilimorfin) was purchased from BDH Industries, Mumbai, India. Aspirin, chlorpheniramine maleate, cyproheptadine hydrochloride and ketoconazole were received as gift sample from Arbro Pharmaceuticals Limited, New Delhi, India. All other chemicals were of analytical grade.

Antiinflammatory activity

Carrageenan and prostaglandin induced paw edema (po/im/ip route)—To ascertain whether the anti-inflammatory activity of L. usitatissimum fixed oil is dependent upon the route of administration, anti-inflammatory activity was evaluated following administration of oil (3 ml/kg) by oral, intramuscular and intraperitoneal routes in rats. Overnight fasted rats were divided into 6 groups of 6 animals each. Three groups of animals served as control and received distilled water (3 ml/kg) by three different routes whereas other three groups received fixed oil (3 ml/kg) by oral, intramuscular and intraperitoneal routes respectively. After 30 min of drug administration, inflammation was induced by injecting 0.1 ml of freshly prepared 1% carrageenan in normal saline into the planter aponeurosis of the right hind paw. The paw volume was measured immediately and 3 h after the carrageenan administration plethysmographically. In another set of experiment with similar groups, inflammation was induced by injecting prostaglandin E₂ (10⁻⁸ g/ml, 0.1 ml) and paw volume was measured after 30 min. One additional group of animals received a standard drug aspirin, dissolved in phosphate buffer pH 7.4 (100 mg/kg, ip), 30 min prior to prostaglandin E₂ administration.

Inflammatory mediator-induced paw edema—In another set of experiment, different inflammatory mediator/phlogistic agents were used to induce paw edema. The respective strength of oedemogens, the volume injected and the time for determination of edema are shown in the parenthesis; arachidonic acid [0.5% in 0.2 M carbonate buffer (pH 8.43-8.56), 0.1 ml, 30 min], leukotriene (1×10⁻⁶ g/ml, 0.1ml, 30 min), histamine (1 × 10⁻³ g/ml, 0.1ml, 60 min) and bradykinin (2 × 10⁻⁵ g/ml, 0.1ml, 60 min). The oedemogens were injected in the hind paw of rat after 30 min of administration of L. usitatissimum fixed oil (3 ml/kg) or standard drug or control vehicle (distilled water) ip, to groups of fasted rats. The standard drugs used in different edema model were as follows: arachidonic acid-induced edema, aspirin (100 mg/kg), chlorpheniramine maleate (25 mg/kg), cyproheptadine hydrochloride (25 mg/kg), ketoconazole (14 mg/kg); leukotriene-induced edema, aspirin (100 mg/kg), ketoconazole (14 mg/kg); bradykinin-induced edema, aspirin (100 mg/kg), chlorpheniramine maleate (25 mg/kg); histamine-induced edema, chlorpheniramine maleate (25 mg/kg). Chlorpheniramine maleate and cyproheptadine hydrochloride were administered as solution in distilled water while ketoconazole was administered as a solution in 0.01M HCl. Edema volume was measured plethysmographically as described earlier.

Analgesic activity

Tail immersion test—Swiss albino mice were divided into 5 groups of 6 animals each. Each mouse was inserted in a conoid paper receptacle with its tail protruding. The protruding tail was entirely immersed in a pot of water maintained at 58°C. The time in seconds for withdrawal of the tail clearly out of water was taken as the reaction time and measured by a stopwatch. The cut off time for the experiment was 15 sec. The reaction time was determined before and
periodically (1.0, 2.0 and 3.0 h) after administration of *L. usitatissimum* oil (1.0, 2.0 and 3.0 ml/kg, ip). Morphine, 10 mg/kg, ip was used as positive control.

**Acetic acid induced writhing**—Swiss albino mice of either sex were divided into 5 groups of 6 animals each. Group I served as control and received distilled water (3 ml/kg, ip); group II to IV received *L. usitatissimum* fixed oil 1.0, 2.0 and 3.0 ml/kg (ip), respectively. Group V received aspirin, dissolved in phosphate buffer pH 7.4, at a dose of 100 mg/kg (ip). After 60 min, 0.6% v/v acetic acid solution in normal saline (10 ml/kg) was administered ip to each animal. Immediately after the acetic acid administration, numbers of writhings or stretches (a syndrome characterized by the wave of contraction of the abdominal muscle followed by the extension of the hind limb) were counted for 15 min. Reduction in writhing number as compared to control was considered as the evidence for the presence of analgesia.

**Antipyretic activity**—Wistar albino rats were fasted overnight but the animals had free access to water. The animals were divided into 5 groups of 6 animals each. Group I served as control and received only typhoid paratyphoid A/B vaccine, at dose of 1 ml/kg, sc. Group II to IV received *L. usitatissimum* oil in dose level of 1, 2, 3 ml/kg, ip along with the vaccine while group V received a standard drug, aspirin (dissolved in phosphate buffer pH 7.4, 100 mg/kg) along with the vaccine. Rectal temperature was measured 1 min before the administration of vaccine and drugs, followed by hourly measurement for 4 h.

**Toxicity studies**

**Acute toxicity study**—Acute toxicity was evaluated after administration of *L. usitatissimum* oil through intraperitoneal route. Swiss albino mice of either sex were fasted overnight but the animals had free access to water. No mortality was observed in the animals treated with fixed oil up to 20 ml/kg. As per the OECD guidelines the liquid is considered to be safe if no mortality is observed up to 20 ml/kg.

**Sub-acute toxicity study**—Albino rats (20) of either sex were divided into two groups. The first group received control vehicle (normal saline) and other group received fixed oil in a dose of 3.0 ml/kg, i.p. daily. Both groups of rats were kept in a similar laboratory conditions and were allowed to take usual food and water. The animals were observed for their general condition, gross behavior, body weight etc. All the animals were sacrificed by cervical dislocation under light ether anesthesia on the 15th day; the viscera were removed and examined for the histopathological changes including gastric erosion in stomach.

**Statistical analysis**—The data are presented as mean±SE and analyzed by One way ANOVA followed by Student’s Newman Keul’s multiple comparison tests for the possible significance identification between the various groups. *P*<0.05 was considered statistically significant. Statistical analysis was carried out using Graph pad prism 3.0 (Graph pad software, San Diego, CA).

**Results and Discussion**

The physicochemical characterization of *L. usitatissimum* fixed oil was done as per Indian Pharmacopoeia. The pale yellow colored viscous oil (viscosity 33.57cps) showed acid value, saponification value and ester value of 3.197, 196.15 and 192.95 respectively. The high iodine value (198.38) of the oil corresponds to its higher content of unsaturated fatty acids namely oleic (19.05%), linoleic (13.67%) and linolenic acids (57.38%).

In order to explore the effect of route of administration on anti-inflammatory activity, the antiinflammatory activity of *L. usitatissimum* fixed oil was evaluated against carrageenan- and PGE2-induced paw edema in rats following administration of oil by oral, intramuscular and intraperitoneal routes. In carrageenan-induced paw edema model significant inhibition (34.38%) of paw edema was observed after oral administration of oil but the extent of edema inhibition was inferior to that observed with intramuscular (62.34%) and intraperitoneal (69.35%) administration. Similarly in prostaglandin-induced paw edema model oral administration of the oil produced 14.29% inhibition which was much smaller than the edema inhibition obtained with intramuscular (55.55%) and intraperitoneal (80.88%) administration. Intraperitoneal administration of aspirin produced 77.94% edema inhibition (Table 1). The lower edema inhibition obtained with oral administration of oil could be due to fasting/fatty meal-induced delayed gastric emptying resulting in reduced absorption.

*L. usitatissimum* fixed oil significantly inhibited carrageenan-induced inflammation which involves three distinct phases of mediator release including histamine and serotonin in first phase, kinins in second phase and prostaglandin in third phase.
The oil also inhibited prostaglandin E₂ (PGE₂)-induced inflammation. Hence, subsequently, the anti-inflammatory effect of *L. usitatissimum* oil was evaluated against other inflammatory mediators like leukotriene, histamine, and bradykinin. In leukotriene-induced edema the oil significantly inhibited the edema (72.6%) comparable to ketoconazole (a leukotriene antagonist) (79.45%). In histamine-induced edema, the oil significantly inhibited the edema (62.31%); the inhibitory effect was however less than that observed with chlorpheniramine (an antihistaminic) (74.46%). Similarly the oil also inhibited bradykinin-induced edema (84.28%) but the effect was much greater than chlorpheniramine (48.57%). Chlorpheniramine was used in the study as antihistamines inhibit bradykinin-induced increase in vascular permeability. Thus the oil significantly inhibited the leukotriene-, histamine- and bradykinin-induced inflammation whereas aspirin, a cyclooxygenase inhibitor, failed to inhibit leukotriene and bradykinin-induced inflammation (Table 1). The results suggest that the oil possesses anti-prostaglandin, antileukotriene, antihistaminic and antibradykinin effects. In body tissue inflammation starts from arachidonic acid which could be metabolized by cyclooxygenase and lipoxygenase pathways. Activation of cyclooxygenase pathway produces prostaglandins while lipoxygenase pathway yields leukotrienes, and both prostaglandin and leukotriene are proinflammatory. *L. usitatissimum* fixed oil inhibited inflammation caused by exogenous prostaglandin and leukotriene. Now the question arises whether the oil would inhibit the *in vivo* synthesis of prostaglandin and leukotrienes from the substrate arachidonic acid. To explore the same, the effect of oil was evaluated against inflammation induced by arachidonic acid. In arachidonic acid-induced edema, significant edema inhibition was observed with the oil (83.78%), chlorpheniramine (60.81%), cyproheptadine (70.27%) and ketoconazole (62.16%) while aspirin did not inhibit the edema formation and the inhibitory effect of oil was much greater than the rest. The edema inhibition by chlorpheniramine (antihistaminic) and cyproheptadine (antihistaminic/antiserotonin agent) appears to be due to inhibition of mast cell mediator release indicating that mast cell mediator release, may partly contribute towards arachidonic acid-induced paw edema. The results suggest that the oil has the capacity to inhibit the synthesis of prostaglandin and...

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<tr>
<th>Table 1—Effect of <em>L. usitatissimum</em> fixed oil on inflammatory mediator-induced paw edema.</th>
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<td>Values are mean ± SE from 6 animals in each group</td>
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<thead>
<tr>
<th>Group (–)</th>
<th>Control (Dist. water, 3 ml/kg, po)</th>
<th>Fixed oil (3 ml/kg, po)</th>
<th>Aspirin (100 mg/kg, ip)</th>
<th>Chlorpheniramine (25 mg/kg, ip)</th>
<th>Cyproheptadine Ketocanaole (25 mg/kg, ip)</th>
<th>Prostaglandin</th>
<th>Histamine</th>
<th>Leukotriene</th>
<th>Bradykinin</th>
<th>Arachidonic Acid</th>
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<td>Carrageenan</td>
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<td>Prostaglandin</td>
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<td>Leukotriene</td>
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<td>Bradykinin</td>
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<td>Arachidonic Acid</td>
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Statistical significance for intramuscular and intraperitoneal route was compared to oral route by Student-Newman-Keuls’ test (*P<0.05) Statistical significance compared to control as per Student-Newman-Keuls’ Test (**) (P<0.15)
leukotrienes by cyclooxygenase and lipoxygenase from arachidonic acid substrate or it is a dual inhibitor of arachidonic acid metabolism. It is known that arachidonic acid-induced paw edema is highly sensitive to inhibition by dual inhibitors of arachidonic acid metabolism, corticosteroids, and antihistaminic/antiserotonin agent. Hence the anti-inflammatory effects of *L. usitatissimum* oil could be contributed by dual inhibition of arachidonic acid metabolism. Further, antihistaminic and antibradykinin effects of the oil, also, could contribute towards anti-inflammatory effect.

Inflammation is characterized by redness, swelling and pain. These occur due to local vasodilatation, increased capillary permeability and infiltration of leukocytes. Inflammatory mediators are released predominantly from activated leukocytes. The key inflammatory mediators are the n-6 eicosanoids, prostaglandin E₂ (PGE₂) and leukotriene B₄ (LTB₄) derived from polyunsaturated fatty acid arachidonic acid (AA; 20:4, n-6) metabolism by the action of cyclooxygenase and lipoxygenase. Other mediators are the cytokines; tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), IL-6 and IL-8 which have been found in high concentrations in synovium of rheumatoid arthritis patients.

PGE₂, a powerful vasodilator, synergizes with other inflammatory vasodilators such as histamine and bradykinin and the combined dilator action on pre-capillary arterioles contributes to the redness and increased blood flow in areas of acute inflammation. PGE₂, on its own, does not increase the permeability of post-capillary venules but potentiates the effect of histamine and bradykinin to increase permeability. PGE₂ also potentiates the effect of bradykinin by sensitizing afferent C fibers to increase pain. LTB₄ is a powerful chemotactic agent for both neutrophils and macrophages. LTB₄ promotes adhesion of neutrophils to vascular endothelial cells and their transendothelial migration to the inflammatory site. PGE₂ and LTB₄ acting together can cause vascular leakage and extravasation of fluid. Thus, the lipid mediators are involved in the redness, swelling and pain that follow acute inflammation. The *L. usitatissimum* oil by inhibiting prostaglandin E₂ (PGE₂), leukotriene B₄ (LTB₄), histamine and bradykinin could render anti-inflammatory effect.

It has been proposed that eicosanoids mediate much of the early pathology of inflammatory joint disease such as swelling, pain and leukocyte infiltration and cytokines mediate the late destructive phase of the disease which involves cartilage loss, bone resorption and joint failure. Overexpression of COX-2 has been observed in synovium of rheumatoid arthritis patients and in the joint tissue of arthritic rats. Arachidonate metabolites such as PGE₂, LTB₄ and 5-hydroxyeicosatetraenoic acid are found in the synovium of patients with rheumatoid arthritis. Hence, dual inhibition of arachidonic acid metabolism and inhibition of histamine and bradykinin by *L. usitatissimum* oil could be responsible for its antiarthritic activity reported earlier. Inhibition of lipoxygenase/leukotriene and histamine by the oil appears to impart antiulcer activity to the lipid.

Recently Singh et al. reported anti-inflammatory effect of *L. usitatissimum* oil against leukotriene (LTB₄) and arachidonic acid-induced paw edema in rats using indomethacin as a standard drug. The oil significantly inhibited the edema caused by both the oedemogens, as observed in the present study. But the researchers observed inhibition of leukotriene and arachidonic acid-induced edema by indomethacin too, which is quite unexpected scientifically. According to DiMartino et al., arachidonic acid-induced paw edema in rats is highly sensitive to inhibition by corticosteroids (dexamethasone, prednisolone), dual inhibitors of arachidonate metabolism (phenidone, SK & F 86002), anti-histamine/serotonin agents (chlorpheniramine, cyproheptadine) but is insensitive to cyclooxygenase inhibitors (indomethacin, piroxicam, naproxen, ibuprofen, meclofenamic acid and tiflamizole). Thus indomethacin cannot inhibit arachidonic acid-induced paw edema as it is a cyclooxygenase/prostaglandin inhibitor. Similarly indomethacin cannot inhibit leukotriene (LTB₄)-induced edema, as LTB₄ is a lipoxygenase metabolite. LTB₄-induced edema is not inhibited by cyclooxygenase/prostaglandin inhibitor. Had it been so, oral administration of indomethacin would not have caused gastric ulceration, as leukotriene antagonists are antiulcer drugs. The aforesaid discussions clearly suggest the inability of indomethacin to inhibit leukotriene or arachidonic acid-induced paw edema and the findings contradict results of earlier studies.

The antipyretic activity of the *L. usitatissimum* oil was evaluated by testing against typhoid-paratyphoid A/B vaccine induced pyrexia in rats. It was observed that the oil had a definite antipyretic property, when given intraperitoneally at a dose of 1 ml/kg and
above. Appreciable reduction in the temperature was noted with in the 2nd and 4th h of oil administration (Fig. 1). Antipyretic activity of the fixed oil at 3 ml/kg dose was comparable to aspirin. Drugs having anti-inflammatory activity generally possess antipyretic activity e.g. non-steroidal anti-inflammatory drugs. The mode of action of non-steroidal anti-inflammatory drugs is not fully clear. It has been suggested that PGE mediates pyrogen fever; the ability of non-steroidal anti-inflammatory drugs to inhibit prostaglandin synthesis can help to explain the antipyretic activity.

The analgesic effect of L. usitatissimum fixed oil was evaluated using tail immersion method. The oil in 3 ml/kg dose after 2 h and 2-3 ml/kg dose after 3 h showed some analgesic activity, but the analgesic activity was significantly lower than morphine. It is known that centrally acting analgesic drugs elevate the pain threshold of mice towards heat. The results suggest that the oil raised the pain threshold to a lesser extent compared to morphine, and it may not be centrally acting. In order to distinguish between central and peripheral analgesic action of L. usitatissimum fixed oil, the oil was tested against acetic acid-induced writhing response in mouse. The oil significantly inhibited the acetic acid-induced writhing response in a dose dependent manner. The percentage inhibition of writhing produced by 3 ml/kg of oil was comparable to that of aspirin (100 mg/kg), a known standard analgesic drug (Table 2). It appears that the analgesic activity of the oil is peripherally mediated. It has been reported that in addition to non-steroidal anti-inflammatory drugs, antihistaminic and anticholinergics can inhibit writhing response. L. usitatissimum fixed oil possesses antihistaminic and anticholinergic activity. The oil also possesses significant anti-inflammatory activity against histamine, bradykinin and PGE2-induced inflammation. Prostaglandins themselves do not produce pain but potentiate the effect of bradykinin by sensitizing afferent C fibers to cause pain. Thus the analgesic activity of L. usitatissimum fixed oil may be due to combined inhibitory effects of prostaglandin, histamine, bradykinin and acetylcholine.

L. usitatissimum fixed oil was well tolerated up to 20 ml/kg in acute toxicity study and administration of fixed oil for a longer duration of time in sub-acute toxicity studies did not reveal any untoward effect of the oil on behavioral, body weight, normal reflexes and visceral appearance in rats. The oil did neither produce any ulcerogenic effect nor any histopathological changes.

L. usitatissimum fixed oil at 3.0 ml/kg or 2.85 g/kg (wt/ml of oil is 0.95) dose possesses antiinflammatory, analgesic and antipyretic activity. The oil contains 57.38% α-linolenic acid. α-linolenic acid, an ω-3 fatty acid (18:3, n-3), is metabolized in the body to eicosapentaenoic acid (EPA) (20:5, n-3) which can competitively inhibit arachidonate metabolism by cyclooxygenase and lipoxygenase pathways. Thus, α-linolenic acid, being the precursor of EPA, has the capacity to block both cyclooxygenase and lipoxygenase pathways of arachidonic metabolism, and could be responsible for the biological activity of the oil.

Thus it can be concluded that L. usitatissimum fixed oil possesses antinflammatorv, analgesic and antipyretic activities. Dual inhibition of arachidonic acid metabolism, antihistaminic and antibradykinin activities of the oil could account for the biological activity and the active principle could be α-linolenic acid. However, further studies are needed to comment more in this respect.

<table>
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<th>Groups (dose)</th>
<th>Writhing count</th>
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<tr>
<td>Control (3 ml/kg, ip)</td>
<td>33.16±1.57</td>
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<tr>
<td>Fixed oil (1 ml/kg, ip)</td>
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<tr>
<td>Fixed oil (2 ml/kg, ip)</td>
<td>18.30±0.66</td>
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<tr>
<td>Fixed oil (3 ml/kg, ip)</td>
<td>8.17±0.40</td>
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<tr>
<td>Aspirin (100mg/kg, ip)</td>
<td>5.83±0.95</td>
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Table 2—Effect of L. usitatissimum fixed oil on acetic acid-induced writhing in mice

*Values are mean ± SE from 6 animals in each group*

Statistical significance compared to control as per Student-Newman-Keul’s Test (*P<0.05*)

Fig. 1—Effect of L. usitatissimum fixed oil and aspirin on typhoid-paratyphoid A/B vaccine-induced pyrexia in rats. [Values are mean ± SE from 6 animals in each group. All groups were compared to the group 1 by Student Newman Keul’s Test]
Acknowledgement

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References