Research Article

Effect of *L. usitatissimum* (Flaxseed/Linseed) Fixed Oil against Distinct Phases of Inflammation

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Received 18 December 2012; Accepted 10 January 2013

Academic Editors: A. Jalili, B. Kim, and D. Szukiewicz

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The present investigation summarizes the effect of *Linum usitatissimum* fixed oil against different phases of acute inflammatory reaction, namely, protein exudation, peritoneal capillary permeability, and leukocyte migration. The fixed oil exhibited dose-dependent inhibition of protein exudation vascular permeability, comparable to standard aspirin. The oil also inhibited the leukocyte migration in pleural exudates in a dose-dependent manner. Production of less vasodilatory (PGE₃) and chemotactic (LTB₅) eicosanoids through EPA (derived from linolenic acid) metabolism could account for the above observations.

1. Introduction

*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 oils, 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 and as an 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. In earlier studies, the *L. usitatissimum* fixed oil has been reported to exhibit significant anti-inflammatory [3], antiarthritic [4], antiulcer [5], and antidiabetic [6] properties along with the efficacy against experimental esophagitis in experimental animals [7]. 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 [8]. Considering the significant anti-inflammatory and antiarthritic activity, the present study has been undertaken to evaluate the possible effect of fixed oil against distinct phases of inflammation using suitable animal models.

2. Materials and Methods

2.1. Materials

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

2.1.2. 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
2.2. Methods

2.2.1. Inflammatory Exudation (Protein Exudation). Group of albino mice were treated either with \textit{L. usitatissimum} fixed oil (1–3 mL/kg) or control vehicle (distilled water, 3 mL/kg) or standard drug (aspirin, 100 mg/kg) intraperitoneally. After 30 min, 0.25 mL of 1.2% acetic acid was administered i.p. to each mouse. After 3 h, visceras were exposed by abdominal incision; abdomen was irrigated with physiological saline containing 0.1 mM disodium EDTA. The pooled washing was made to 6 mL with physiological saline and then centrifuged. Total amount of protein in the supernatant was estimated with Folin’s reagent [9].

2.2.2. Peritoneal Capillary Permeability (Dye Leakage Method). \textit{L. usitatissimum} fixed oil (1–3 mL/kg) or control vehicle (distilled water, 3 mL/kg) or standard drug (aspirin, 100 mg/kg) was administered intraperitoneally to group of albino mice (n = 6), and after 3 h, each mouse received 4 mL of 0.05 N acetic acid in 0.9% saline i.p. followed by immediate i.v. administration of 0.1 mL of 4% pontamine sky blue (dye). One hour after the administration of the dye, animals were sacrificed by cervical dislocation and the peritoneal cavity was drained. The collected exudate was centrifuged for 10 min, at 2000 rpm, and 0.5 mL of the supernatant was diluted with 4.5 mL of physiological saline. Absorbance of the diluted supernatant was measured in a colorimeter at 625 nm using normal saline as blank. The mean percentage of the light absorption was then calculated for both control and drug-treated groups [10].

2.2.3. Leucocyte Migration. Carrageenan (0.1 mL of 1% aqueous suspension) was injected into the pleural cavity of anesthetized rats as described previously [11], pretreated i.p. 30 min earlier with \textit{L. usitatissimum} fixed oil (1–3 mL/kg) or control vehicle (distilled water, 3 mL/kg) or standard drug (phenylbutazone, 100 mg/kg). After 5 h, the animals were sacrificed and 2 mL of Hank’s solution [12] was injected into the pleural cavity. The animals were then rocked to allow the mixing of the solution with the exudates, which was then collected with a pipette. The total number of leucocytes present in the exudate was counted with a haemocytometer [13].

2.2.4. Statistical Analysis. All the data are presented as mean ± SEM and analyzed by one-way ANOVA followed by Dunnett’s test for the possible significance identification between the various groups. P < 0.05 was considered statistically significant. Statistical analysis was carried out using Graphpad prism 3.0 (Graphpad software, San Diego, CA).

3. Results and Discussion

\textit{L. usitatissimum} fixed oil demonstrated a significant (P < 0.05) dose-dependent inhibition of protein exudation (i.e., rise in protein concentration in peritoneal fluid) and inhibited the vascular permeability evidenced by inhibition to dye leakage. Degree of inhibition of protein exudation and vascular permeability was comparable to standard aspirin (Table 1). Similarly, in carrageenan-induced pleurisy in rats, \textit{L. usitatissimum} fixed oil demonstrated a significant (P < 0.05) dose-dependent inhibition in leucocyte migration in pleural exudates and slightly better results were observed at a dose level of 3 mL/kg (i.p.), in comparison to phenylbutazone (Table 2).

The present investigation revealed the effect of \textit{L. usitatissimum} fixed oil on distinct phases of inflammatory process: firstly an acute phase of local vasodilatation and increased capillary permeability leading to exudation, followed by leucocytes migration. The momentous characteristic of the inflammatory reaction is the transient increase in permeability of small blood vessels to proteins, initially involving the venules, whereas the delayed phase of increased permeability involves the capillaries as well, and is more relevant to chronic inflammation [14]. The effect of \textit{L. usitatissimum} fixed oil on the intensity of peritoneal inflammation was evaluated by direct measurement of exuded plasma protein using Folin’s reagent [9] and leakage of protein bound dye into the peritoneal cavity [10]. \textit{L. usitatissimum} fixed oil significantly reduced the peritoneal vascular permeability, indicating the suppression of the vascular response in the process of acute inflammation. Anti-inflammatory potential was further confirmed by inhibition of carrageenan-induced pleurisy in rats, as \textit{L. usitatissimum} fixed oil significantly inhibited the migration of leucocytes in pleural exudates. The key inflammatory mediators are the n-6 eicosanoids, prostaglandin E\textsubscript{2} (PGE\textsubscript{2}), and leukotriene B\textsubscript{4} (LTB\textsubscript{4}) which...
Table 1: Effect of *L. usitatissimum* fixed oil and aspirin on acetic acid induced peritoneal inflammation and peritoneal capillary permeability in mice.

| Serial number | Treatment                  | Dose   | Total amount of protein (mg/mL) exudation 3 h after acetic acid administration (0.25 mL of 1.2%, i.p.) | Absorbance at 625 nm |
|---------------|----------------------------|--------|-------------------------------------------------------------------------------------------------|----------------------|
| 1             | Control (distilled water)  | 3 mL/kg| 5.01 ± 0.21                                                                                     | 0.49 ± 0.02          |
| 2             | *L. usitatissimum* fixed oil | 1 mL/kg| 4.15 ± 0.24* (17.17)                                                                           | 0.39 ± 0.05* (20.41) |
| 3             | *L. usitatissimum* fixed oil | 2 mL/kg| 2.82 ± 0.21* (43.71)                                                                            | 0.31 ± 0.03* (36.73) |
| 4             | *L. usitatissimum* fixed oil | 3 mL/kg| 1.59 ± 0.18* (68.26)                                                                            | 0.19 ± 0.05* (61.22) |
| 5             | Aspirin                    | 100 mg/kg| 1.57 ± 0.16* (68.66)                                                                          | 0.20 ± 0.03* (59.18) |

Values in parenthesis represent percentage inhibition (values are mean ± SEM). Each group contains six animals; all groups were compared to the group 1 by Dunnett’s test (*P* < 0.05).

Table 2: Effect of *L. usitatissimum* fixed oil and phenylbutazone on carrageenan-induced pleurisy (leucocytes migration) in albino rats.

| Serial number | Treatment                  | Dose   | Number of leucocytes (x10³/mm³) |
|---------------|----------------------------|--------|---------------------------------|
| 1             | Control (distilled water)  | 3 mL/kg| 8854.25 ± 112.21                |
| 2             | *L. usitatissimum* fixed oil | 1 mL/kg| 6524.24 ± 302.14* (26.31)       |
| 3             | *L. usitatissimum* fixed oil | 2 mL/kg| 3954.25 ± 124.25* (55.34)       |
| 4             | *L. usitatissimum* fixed oil | 3 mL/kg| 3548.45 ± 258.26* (59.92)       |
| 5             | Aspirin                    | 100 mg/kg| 3654.88 ± 222.45* (58.72)       |

Values in parenthesis represent percentage inhibition (values are mean ± SEM); each group contains six animals; all groups were compared to the control by Dunnett’s test (*P* < 0.05).

are derived from polyunsaturated fatty acid arachidonic acid (AA; 20:4, n-6) [15] by the action of cyclooxygenase and lipoxigenase. PGE₂ is a potent vasodilator, whereas LTB₄ is a powerful chemotactic agent. PGE₂ does not increase the permeability of postcapillary venules but potentiates the effect of histamine and bradykinin to increase permeability. PGE₂ also potentiates the effect of bradykinin by sensitising afferent C fibres to increase pain [16]. On the other side, leukotriene (LTB₄) is a potent chemotactic agent for both neutrophils and macrophages. LTB₄ binds to its specific surface cell receptors (BLT-1 and BLT-2) and stimulates a number of leukocyte functions, that is, adhesion of neutrophils to vascular endothelium, transendothelial migration, and chemotaxis [17, 18]. Vasodilation and increased capillary permeability permits fluids and large molecules to leave the blood stream and increased movement of leucocytes into the surrounding tissues by diapedesis. Thus, PGE₂ and LTB₄ together can cause/promote vascular leakage and extravagation of fluids and are significantly involved in the physiology of acute inflammation [15].

The anti-inflammatory activity of various plant lipids has been reported, and the results revealed that the lipids containing α-linolenic acid (ALA, 18:3, n-3), for example, linseed oil, *O. sanctum* fixed oil, and soybean oil had a significant anti-inflammatory activity. Subsequently, methyl esters of various fatty acids, namely, oleic acid, linoleic acid, and ALA, were tested against PGE₂, LTB₄, and arachidonic acid-induced paw edema in rats, where only ALA showed a significant edema inhibition against all the inflammatory mediators suggesting the ALA as a dual inhibitor of arachidonate metabolism [19]. Previously, we also observed antiarthritic activity of the *L. usitatissimum* fixed oil against turpentine oil-induced joint oedema, formaldehyde-induced and adjuvant-induced arthritis in albino rats [4]. ALA (18:3, n-3) is a precursor for eicosapentaenoic acid (EPA, 20:5, n-3), which competes with AA (20:4, n-6, precursor for lipid mediators of inflammation) for cyclooxygenase and lipoxigenase pathway. EPA by acting as a substrate for cyclooxygenase and lipoxigenase pathway produces PGE₃ (less potent vasodilator than PGE₂) [20] and LTB₃ (100 times less potent chemotactic than LTB₄) [21–23]. Thus, less vasodilatory (PGE₃) and chemotactic (LTB₃) response of lipid mediators derived from EPA (metabolic product of ALA metabolism) accounts for the inhibition of fluid and protein exudation along with diminished leucocytes migration observed in the present experiment.

**Conflict of Interests**

The authors declare that they have no conflict of interests.

**Acknowledgment**

The authors are thankful to Arbro Pharmaceuticals Limited (New Delhi, India) for providing the drugs mentioned in the paper as a generous gift sample.

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