Effect of *Linum usitatissimum* (Linseed/Flaxseed) Fixed oil on Experimental Esophagitis in Albino Rats

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Abstract

Introduction : **The present study was undertaken to elucidate the effect of *Linum usitatissimum* fixed oil on experimental esophagitis in albino rats.**

Methods : **Group of rats (n = 6), treated with vehicle control (0.9% NaCl, 3 mL/kg, i.p.) or *L. usitatissimum* fixed oil (1, 2, 3 mL/kg, i.p.) or omeprazole (30 mg/kg, i.p.). Rats were subjected to pylorus and forestomach ligation to induce esophagitis and were compared to a control sham group. Animals were sacrificed after 6 h and evaluated for the gastric pH, gastric volume, total acidity and esophagitis index. Esophageal tissues were further subjected to estimations of sialic acid, collagen, thiobarbituric acid reactive substances, tissue glutathione, catalase and superoxide dismutase.**

Results : **Treatment with fixed oil significantly inhibited the gastric secretion, total acidity and esophagitis index. The oil also altered the levels of sialic acid and collagen towards normal with significant antioxidant activity in esophageal tissues.**

Conclusion : **The lipoxygenase inhibitory, histamine antagonistic, antisecretory (anticholinergic) and antioxidant activity of the oil was attributed for its effect in reflux esophagitis.** (Acta gastroenterol. belg., **2012**, 75, **331-335**).

Key words : Esophagitis, Flaxseed, Linolenic acid, *Linum usitatissimum*, Omega three fatty acids.

Introduction

Gastrooesophageal reflux disease (GERD) is described as a condition that develops due to reflux of gastric contents into the esophagus leading to mucosal damage and oxidative stress ; the condition may be asymptomatic or result in symptoms. The incidence of GERD is estimated to be 10-20% in western countries, making it one of the most prevalent gastrointestinal disorders (1). Recent investigations have reported that the mucosal damage in GERD is due to several causative agents in the refluxate (2) that stimulate mucosal and sub-mucosal cells to release mediators, eliciting an inflammatory reaction and leading to visceral hypersensitivity and other symptoms of GERD (3,4). Inflammatory process seems to play a key role in the underlying mechanisms of the symptoms and pathogenesis of other gastrointestinal conditions as well, such as functional dyspepsia (FD) and irritable bowel syndrome (IBS) (5).

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. Gas chromatographic analysis of the oil has been shown to reveal the presence of fatty acids viz. palmitic acid (5.53%), stearic acid (4.67%), oleic acid (19.05%), linoleic acid (LA) (13.67%) and alpha linolenic acid (ALA) (57.38%) respectively (6). These fatty acids appear to render drying property to the oil. In earlier studies, the oil has been reported to exhibit antiinflammatory, analgesic, antipyretic and antiarthritic activity. The study also enumerated the possible effect of ip/po/im route of administration against carrageenan and prostaglandin induced inflammation, depicting better anti-inflammatory activity by intraperitoneal route (7,8). The antimicrobial activity of *L. usitatissimum* fixed oil and its therapeutic efficacy in bovine mastitis, an inflammatory disorder caused by microbial infection, has been reported (6). Recently, the oil (i.p.) has been found to exhibit significant antiulcer activity against different animal models (9). Considering the previous reports, it was considered worthwhile to evaluate the possible effects of *L. usitatissimum* fixed oil (i.p.) on experimentally induced esophageal lesions in animal model.

Materials and methods

Materials

Flaxseed/Linseed (Variety : JL-59) was obtained from Division of Seed Science, Department of Agronomy, Allahabad Agricultural Institute-Deemed University (Allahabad, India). The seeds were authenticated at National Botanical Research Institute, Lucknow, India, and voucher sample was deposited at National Botanical Research Institute. Omeprazole (Omez) was purchased from Dr. Reddy's Laboratories Pvt. Ltd. (India).

Animals

Wistar strain of albino rats (175-200 g) were obtained from Central Animal House, Department of Animal

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Husbandry, Allahabad Agricultural Institute-Deemed University. Animals were housed under standard conditions of temperature (25 ± 1°C) with 12 h light/dark cycle and had free access to commercial pellet diet and water. Animals were given week's time to get acclimatized with the laboratory condition, before experimentation. Prior to experiment the animals were fasted for 24 h and allowed water freely. The study was approved by the Institutional Animal Ethics Committee, and the experiment was performed according to the CPCSEA guidelines for the laboratory animals and ethics, Department of Animal Welfare, Govt. of India.

Methods

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 oil was filtered to clarity. The oil was stored at room temperature in amber-colored air-tight 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 oil was 17.50% v/w with reference to dried seeds. The density of the oil was 0.952 g/mL.

Induction of Esophagitis

Groups of rats (n = 6), fasted for 24 h received normal saline (3 ml/kg, i.p.) (Sham control), or vehicle (normal saline, 3 mL/kg, i.p.), *L. usitatissimum* fixed oil (1, 2, 3 mL/kg, i.p.), or omeprazole (30 mg/kg, i.p.) (operated groups). After 1 h, coeliotomy was performed under pentobarbitone sodium anesthesia (50 mg/kg, i.p.) and esophagitis was induced (except in sham control) by ligating the forestomach and corpus with 2-0 silk suture, and pylorus ligation as per the method described by Rao and Vijaykumar (10). After 6 h, the animals were sacrificed by cervical dislocation and the chest was opened with a midline incision and the tissue esophagus and stomach were removed. The stomach was opened along the greater curvature and the esophagus was dissected out by extending the dissection line along the major axis to determine the esophagitis index as described for "ulcer index" under the section "Gastric secretion in pylorus ligated rats" by Kaithwas and Majumdar (9). The severity of esophagitis was calculated using the following scores :

Erosion (mm)	**Score**
1 or less	1
1-2	2
> 2	3

The sum of scores was divided by a factor of ten which was designated as the esophagitis index (9,11). The volume of gastric juice was measured as described subsequently under "Gastric secretion in pylorus ligated rats" (9). The pH measurement of gastric juice was done using a pH meter (Milwaukee pH-600).

Estimation of sialic acid

Protein bound sialic acid was estimated by thiobarbituric acid assay (12). Samples of esophageal homogenate were treated with 90% ethanol and the precipitate, thus obtained was dissolved in 0.2 N sulphuric acid. It was oxidized with periodic acid and incubated at 37°C for 30 s. After terminating oxidation using sodium arsenate, 6% thiobarbituric acid and cyclohexane were added. The mixture was centrifuged to get a clear pink layer of cyclohexane and the intensity of the colour was measured at 550 nm. Results are expressed as μg/100 mg tissue of gastric mucosa.

Estimation of collagen content

The esophageal mucosal biopsies of known weight were hydrolyzed in 6 mol/L HCl at 110°C for 18 h in tightly capped polypropylene microcentrifuge tubes. The acid hydrolysates were evaporated in a heat block at 95°C. The dry residues were washed three times with 1.0 ml deionized water (Millipore, Billerica, MA, U.S.A.) with complete evaporation between each wash step. The acid free samples were reconstituted in 1.0 ml acetate citrate buffer and sonicated for 30 min. The collagen content was determined by the estimation of hydroxyproline and expressed as mg/100 mg tissue (13).

Estimation of free radical generation

Esophageal tissue was minced well, homogenized in ice-cold 0.01M Tris-HCl buffer, pH 7.4 and subjected to the estimations of thiobarbituric acid reactive substances (TBARS) (14), tissue glutathione (GSH) (15), catalase (16) and superoxide dismutase (SOD) (17).

Statistical Analysis

All data were presented as mean ± S D and analyzed by one way ANOVA followed by Dunnett test 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

Ligating the forestomach and pylorus developed reflux esophagitis in all the animals marked by macroscopically visible necrosis and significant ulceration in the esophagus. Intraperitoneal administration of *L. usitatissimum* fixed oil (1, 2, 3 mL/kg) significantly inhibited the esophagitis in a dose dependent manner. *L. usitatissimum* fixed oil (3 mL/kg) significantly inhibited the esophagitis index (79.37%), gastric volume (44.87%), and total acidity (68.83%) in comparison with vehicle control (Table 1). Omeprazole produced 81.95% inhibition of esophagitis index respectively.

Table 1. — **Effect of *L. usitatissimum* fixed oil on pH, gastric volume, total acidity and esophagitis index**

S. No.	Treatment	pH	Volume of gastric juices (ml/100 g)	Total acidity (mEq/l)	Esophagitis index (mean ± SD)
Group-I	Sham Control (Normal saline, 3 ml/kg, ip)	3.22 ± 0.11	3.86 ± 0.22	49.23 ± 3.22	0.18 ± 0.04
Group-II	Toxic Control (Normal saline, 3 ml/kg, ip)	1.91 ± 0.13	7.42 ± 0.76	154.00 ± 5.09	5.43 ± 1.23
Group-III	*L. usitatissimum* fixed oil (1 ml/kg, ip)	2.98 ± 0.09*	5.23 ± 0.16 (29.51) *	135.00 ± 7.79 (12.34)	5.21 ± 0.97 (4.05)
Group-IV	*L. usitatissimum* fixed oil (2 ml/kg, ip)	3.32 ± 0.14*	4.27 ± 0.65 (42.45) *	89.00 ± 5.45 (42.21) *	2.53 ± 0.67 (53.41)*
Group-V	*L. usitatissimum* fixed oil (3 ml/kg, ip)	3.45 ± 0.21*	4.09 ± 0.27 (44.87) *	48.00 ± 2.98 (68.83) *	1.12 ± 0.37 (79.37)*
Group-VI	Omeprazole (30 mg/kg, ip)	3.61 ± 0.16*	4.16 ± 0.35 (43.94) *	51.00 ± 4.31 (66.88) *	0.98 ± 0.51 (81.95)*

Each group contains 6 animals ; Values in parenthesis represent percentage inhibition in comparison to Group-II.
All data were presented as mean ± SD, analyzed by one way ANOVA followed by Dunnett test.
*p < 0.05 was considered statistically significant.

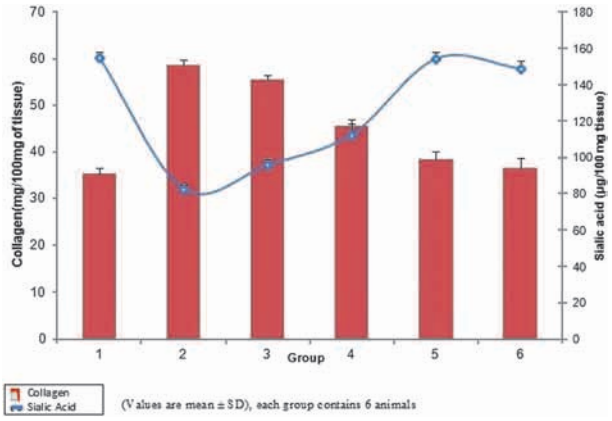

Fig. 1. — Effect of *L. usitatissimum* fixed oil on collagen and sialic acid in experimental esophagitis.
(Values are mean ± SD), each group contains 6 animals.

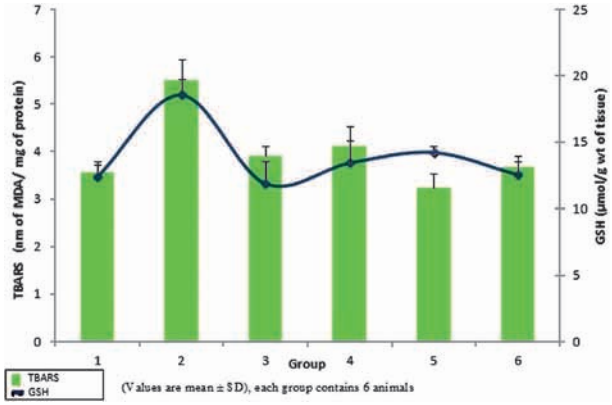

Fig. 2. — Effect of *L. usitatissimum* fixed oil on tissue TBARS and GSH in experimental esophagitis
(Values are mean ± SD), each group contains 6 animals.

Figure 1 enumerates the decrease in sialic acid and increase in collagen content after reflux esophagitis. Whereas, treatment with fixed oil altered the levels of sialic acid and collagen towards normal level.

As depicted in figure 2, levels of tissue TBARS and GSH were significantly increased in the toxic control animals (i.e. vehicle control) ; however intraperitoneal administration of *L. usitatissimum* fixed oil significantly reduced the levels of TBARS and GSH. Similar pattern of results were observed for the antioxidant defense of SOD and catalase in control and treatment groups (Fig. 3).

The present study reveals that *L. usitatissimum* fixed oil exhibit significant protection against the reflux esophagitis in experimental animals following intraperitoneal administration. Ligation of the forestomach and pyloric end produced the erosive and/or ulcerative type

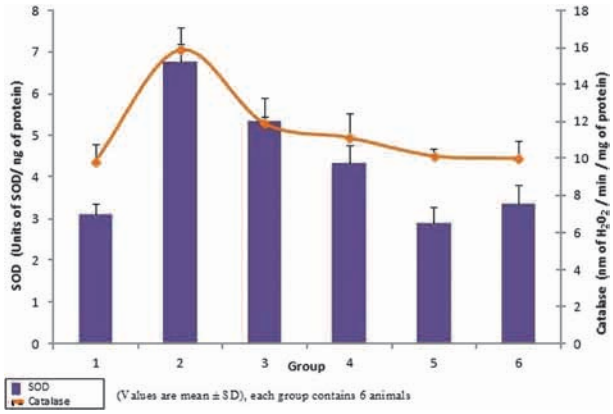

Fig. 3. — Effect on *L. usitatissimum* fixed oil on tissue SOD and catalase in experimental esophagitis.
(Values are mean ± SD), each group contains 6 animals.

of lesions. The damage produced during the process could be primarily attributed to the reflux of gastric content containing significant amount of pepsin. Presence of acid also aggravates the ulcer formation by its corrosive action thus keeping an optimum environment for pepsin activity (18,19). However, intraperitoneal administration of *L. usitatissimum* fixed oil, in the present experiment significantly decreased the gross volume of gastric juice secretion, total acidity, esophagitis index and raised the gastric pH in comparison to control.

Significant antiulcer activity of plant lipids i.e. *L. usitatissimum* and *O. sanctum* fixed oil was reported against NSAID's-, alcohol-, histamine-, reserpine-, serotonin-, stress- and pylorus ligation- induced ulcers in animal models (9,20). The antisecretory effect of the *L. usitatissimum* oil was observed in the experiments on NSAIDs- and ethanol-induced gastric ulcers in rats. However, a much more pronounced antisecretory effect was demonstrated in the experiment on pylorus-ligated rats where the oil inhibited both the gastric output and total acidity. Pylorus ligation-induced ulcers are particularly mediated due to increased accumulation of gastric acid and pepsin, leading to autodigestion of gastric mucosa. *L. usitatissimum* fixed oil decreased the gastric output and total acidity in pylorus ligated rats, which further confirms the antisecretory potential. The fixed oil also exhibited significant anticholinergic and antihistaminic activity, evaluated against isolated tissue preparations in a dose-dependent manner, which seems to account for the antisecretory effect (9).

The fixed oil obtained from *L. usitatissimum* and *O. sanctum* contains ALA (18:3, n-3), 57.38% and 16.63% respectively. Previous reports in the literature have reported ALA as a dual inhibitor of arachidonic acid metabolism i.e. inhibiting both the cyclooxygenase as well as lipoxygenase pathway (9,21). The antiulcer activity of oil was congregately attributed to the lipoxygenase inhibitory, histamine antagonistic, and antisecretory (anticholinergic) effects (9,20), which seems to accounts for decrease in gross volume of gastric juice secretion, total acidity and esophagitis index in the present experiment as well.

The severity of reflux esophagitis impaired strength and flexibility of collagen fibres, and collagen constitutes the major structural protein in the extracellular matrix, providing mechanical strength and structural integrity to the various connective tissues of the body (22). In the present experiment, reflux esophagitis in rats caused a rise in hydoxyproline content, a marker of collagen. The protective effect of fixed oil in reflux esophagitis normalizes the hydroxyproline concentration underlining the importance of antioxidants to prevent esophageal mucosal damage. Mucosal glycoprotein constitutes and serves as first line of defense against ulcerogens. Sialic acids usually occupy exposed terminal positions on oligosaccharide chain of the glycoproteins. This implies that the release of the sialic acid will expose the masked sugars, e.g. galactose and fructose making them susceptible to hydrolysis by acid and proteolytic enzymes both in solution and on cell surfaces. Thus, sialic acid, derived from neuraminic acid is one of the first molecules encountered by biochemical compounds and their determination during clinical investigation is well established (23). Our results showed that the sialic acid was significantly decreased in toxic control and the oil increased the same in dose dependent manner.

Previous studies have elaborated the role of free radicals in the pathogenesis of reflux esophagitis in experimental animals. Reflux esophagitis has been reported to increase malondialdehyde, a stable product of lipid peroxidation and a sensitive marker of membrane damage in esophageal tissues (17,24). Our results suggest that reflux esophagitis produces free radical species that attack lipid components leading to lipid peroxidation, and concomitant administration of the *L. usitatissimum* fixed oil significantly inhibited the lipid peroxidation evidenced by decreased TBARS levels. Free radical damage led to consumption of GSH in the first few hours of oxidative stress, directing decreased GSH level, a marker of short-term oxidative stress (25,26) and treatment with *L. usitatissimum* fixed oil significantly helped to restore the same.

The antioxidant enzymes, SOD and catalase constitute the major supportive team of defense against free radicals. SOD by scavenging the superoxide radical generates hydrogen peroxide and molecular oxygen. Catalase existing in the cells catalyses the dismutation of hydrogen peroxide (produced due to scavenging effect of SOD) to water and molecular oxygen. Previous studies have suggested the decreased levels of SOD in oxidative stress (27,28). However, in the present study, a significant rise in SOD level in animals subjected to reflux esophagitis (control) was observed, which could be the consequence of physiological compensatory mechanisms to combat oxidative stress in early phase. Simultaneous increase in the catalase activity is expected with increase in SOD activity to overcome the deleterious effects of esophagitis-induced free radicals generation, which was observed in our study. Previous studies on PUFAs (Polyunsaturated fatty acids) (LA and ALA) have demonstrated protection against lipid peroxidation by increasing the levels of several cellular antioxidants such as ascorbic acid, alpha-tocopherol and GSH (29). Baydas *et al.* (30) also suggested that the diet supplemented with antioxidants like LA exhibit significant antioxidant activity in rats. Thus antioxidant activity observed in the present study could be attributed to the presence of ALA (18:3, n-3) (57.38%) and LA (13.67%) in *L. usitatissimum* fixed oil.

Conclusion

From the above discussion, we would like to conclude that the protective effect of *L. usitatissimum* fixed oil against reflux esophagitis could be attributed to the antisecretory, antioxidant and lipoxygenase inhibitory activ-

ities. The present observation is the first experimental data demonstrating protective effect of plant derived lipids on experimental reflux esophagitis in animal model. Thus drugs that possess antiinflammatory property and activity against reflux esophagitis could be of great therapeutic importance as most of the antiinflammatory drugs used in modern day medicine are ulcerogenic. Further studies are required to establish the usefulness of *L. usitatissimum* in reflux esophagitis.

Competing interest

Authors declare that we have no competing interest.

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