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RESEARCH ARTICLE

Evaluation of antiulcer and antisecretory potential of *Linum usitatissimum* fixed oil and possible mechanism of action

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Abstract The aim of the study was to evaluate the antiulcer activity of *Linum usitatissimum* fixed oil against aspirin-, indomethacin-, ethanol-, reserpine-, serotonin- and stress-induced gastric ulceration in rats and histamine-induced gastric ulceration in guinea pigs. Attempts were also made to evaluate the in vitro anticholinergic and antihistaminic activity and in vivo antisecretary and antiulcer activity of oil following pylorus ligation in rats. *L. usitatissimum* fixed oil exhibited significant antiulcer activity against different ulcerogens in experimental animal models. The fixed oil significantly inhibited acetylcholine- and histamine-induced contraction of guinea pig and rat ileums, respectively, suggesting its anticholinergic and antihistaminic activity. The oil also exhibited significant inhibitory effect on gastric secretion/total acidity and aspirin-induced gastric ulceration in pylorus-ligated rats. The lipoxygenase inhibitory, histamine antagonistic and antisecretory (anticholinergic) effects of the oil could probably have contributed towards antiulcer activity. *L. usitatissimum* fixed oil may be considered to be a drug of natural origin which possesses significant antiulcer activity. The present observation is the first experimental data showing antiulcer activity of *L. usitatissimum* fixed oil.

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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 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 (The Wealth of India 2006). The oil contains unsaturated fatty acids like oleic acid (12–30%), linoleic acid (8–29%), and linolenic acid (35–67%) (The Wealth of India 1976). These fatty acids appear to render drying property to the oil.

The most common side effect associated with the nonsteroidal anti-inflammatory drugs (NSAIDs, except selective COX-2 inhibitors) is gastric ulcer. Gastric damage caused by these agents (Non selective COX inhibitors) is mediated by two mechanisms: first by local irritation, allowing back diffusion of acid into gastric mucosa leading to tissue damage and second by inhibiting the biosynthesis of cytoprotective prostaglandins (PGE_2 and PGI_2). In a preliminary study, *L. usitatissimum* fixed oil has been found to inhibit inflammation induced by carrageenan (Singh and Majumdar 1997).Therapeutic effect of *L. usitatissimum* fixed oil on acute and chronic arthritic models in albino rats has been reported recently (Kaithwas and Majumdar 2010). The present study was, therefore, designed to evaluate the antiulcer activity of *L. usitatissimum* fixed oil using different in vivo models in rats and guinea pigs.

Materials and methods

Materials

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, Lucknow, India, and voucher sample was deposited at National Botanical Research Institute. Serotonin was purchased from Sigma–Aldrich, USA, and histamine acid phosphate was obtained from Acros chemicals, USA. Aspirin, indomethacin, and reserpine were received as gift sample from Arbro Pharmaceuticals Limited, New Delhi, India. Misoprostol (Misoprost), cimetidine (Lock-2) and promethazine hydrochloride (Phenargan) were procured from Cipla Pvt. Ltd., Zydus Cadila Pvt. Ltd., and Nicholas Piramal Pvt. Ltd., India, respectively. All other chemicals were of analytical grade.

Animals

Wistar strain of albino rats (150–200 g) and guinea pigs (400–500 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 12 h light/12 h 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 experiments the animals were fasted for a specific period and allowed water freely. The fasting time and the dose of ulcerogens used in the study were same as described by Singh and Majumdar (1999). The study was approved by the Institutional Animal Ethics Committee, and all experiments were 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 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. The oil thus obtained was subjected to 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] by methyl esterification which 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%) in the oil.

Aspirin-induced gastric lesions

Groups of rats, fasted for 36 h received control vehicle or *L. usitatissimum* fixed oil (1.0, 2.0, 3.0 ml/kg, i.p.) or misoprostol (100 μg/kg/0.5 ml, p.o.). After 30 min, ulcers were induced by administering aspirin 500 mg/kg, p.o. (suspended in 1% carboxymethyl cellulose in water). Animals were killed after 4 h (Parmar and Desai 1993).The stomach was removed and opened along the greater curvature to determine the ulcer index as given below:

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 ulcer index (Main and Whittle 1975). The volume of gastric juice was measured as described subsequently under "*Gastric secretion in pylorus-ligated rats*" (Singh and Majumdar 1999). The pH measurement of gastric juice was done using a pH meter (Milwaukee pH-600).

Indomethacin-induced gastric lesions

Groups of rats, fasted for 24 h were treated with control vehicle or *L. usitatissimum* fixed oil (1.0, 2.0, 3.0 ml/kg, i.p.) or misoprostol (100 μg/kg/0.5 ml, p.o.). Indomethacin (20 mg/kg, p.o.) was given after 30 min. The rats were killed 6 h after the administration of indomethacin (West 1982) and stomach was removed and examined for ulcer index as described previously. The volume of gastric juice and its pH were also measured as described earlier.

Ethanol-induced gastric lesions

Groups of rats fasted for 24 h received control vehicle or *L. usitatissimum* fixed oil (1.0, 2.0, 3.0 ml/kg, i.p.) or misoprostol (100 μg/kg/0.5 ml, p.o.). After 30 min, ulceration was induced by oral administration of 50% ethanol (5 ml/kg). The animals were killed after 1 h following administration of ethanol (Robert et al. 1979). The stomach was removed, opened along the greater curvature and sum

of length of lesions (mm) was calculated and expressed as lesion index. The volume of gastric juice and its pH were also measured as described earlier.

Serotonin-induced gastric ulcers

Groups of rats fasted for 24 h were treated with control vehicle or *L. usitatissimum* fixed oil (1.0, 2.0, 3.0 ml/kg, i.p.). After 30 min, serotonin creatinine sulphate (20 mg/kg, s.c.) was given to induce ulcers. Animals were killed after 18 h and the ulcer index was determined (Main and Whittle 1975).

Reserpine-induced gastric ulcers

Reserpine (5 mg/kg, i.m.) was administered to groups of rats (fasted for 24 h) 30 min after treatment with control vehicle or *L. usitatissimum* fixed oil (1.0, 2.0, 3.0 ml/kg, i.p.). All the animals were killed after 18 h and degree of ulceration was expressed as lesion index as described above.

Histamine-induced gastric ulcers in guinea pigs

Guinea pigs fasted for 36 h were divided into five groups of four animals each. Gastric ulceration was induced by intraperitoneal administration of 1 ml of histamine acid phosphate (100 mg/kg, base). To protect the animals against histamine toxicity, promethazine hydrochloride (10 mg/kg) was injected intraperitoneally to each animal, 15 min prior and 15 min after histamine administration. The control vehicle or *L. usitatissimum* fixed oil (1.0, 2.0, 3.0 ml/kg, i.p.) or cimetidine (32 mg/kg/1 ml, p.o.) was given 45 min prior to histamine administration (Parmar and Desai 1993). The animals were killed after 4 h following histamine administration, and the stomach was dissected out to determine the ulcer index as described earlier.

Stress-induced gastric ulcer

Groups of rats fasted for 12 h were treated with control vehicle or *L. usitatissimum* fixed oil (1.0, 2.0, 3.0 ml/kg, i.p.) or cimetidine (32 mg/kg/1 ml, p.o.). After 30 min, rats were immobilized in a stress cage and forced to remain in a cold chamber (4–6°C) for 3 h (Parmar and Desai 1993). The animals were then killed and sum of length of erosions was expressed as lesion index.

Aspirin-induced gastric ulcerations in pylorus-ligated rats

Six groups of rats fasted for 24 h were subjected to the operative procedure used for pylorus ligation as described by Shay et al. (1945). The animals were treated with control vehicle or *L. usitatissimum* fixed oil (1.0, 2.0, 3.0 ml/kg, i.p.) or cimetidine (32 mg/kg/1 ml, p.o.), or misoprostol (100 μg/kg/0.5 ml, p.o.), 30 min prior to pylorus ligation. The animals were anaesthetized with ether and the abdomen was opened by a small mid-line incision below the xiphoid process. The pylorus was secured and ligated with silk sutures, after which the abdominal wall was closed by sutures, and the animals were allowed to recover from the anaesthesia. Aspirin (100 mg/kg) suspended in 1% carboxymethyl cellulose in water was given orally to all the rats, 15 min after pylorus ligation. The animals were killed after 7 h, the stomach removed, and the degree of ulceration was expressed as lesion index (Okabe et al. 1974).

Gastric secretion in pylorus-ligated rats

Six groups of rats, fasted for 24 h were subjected to pylorus ligation as described earlier. Control vehicle or *L. usitatissimum* fixed oil (1.0, 2.0, 3.0 ml/kg, i.p.) or cimetidine (32 mg/kg/1 ml, p.o.), or misoprostol (100 μg/kg/0.5 ml, p.o.) was administered 30 min prior to pylorus ligation. Four hours later the animals were killed, abdomen was opened, and cardiac end of the stomach was ligated. The stomach was removed and its content drained into a graduated centrifuge tube and centrifuged at 3,000 rpm for 10 min. The supernatant volume was measured. The total acid content of gastric juice was determined by titrating with 0.01 N NaOH using phenolphthalein as an indicator and expressed as mEq/l (Singh and Majumdar 1999). Ulcer index was also determined as described earlier.

Indomethacin-induced gastric lesions following oral administration of oil

Groups of rats containing five animals each were fasted for 24 h and treated with control vehicle (3 ml/kg, p.o.) or *L. usitatissimum* fixed oil (3 ml/kg, p.o.) or *L. usitatissimum* fixed oil (3 ml/kg, i.p.) or misoprostol (100 μg/kg/0.5 ml, p.o.). Indomethacin (20 mg/kg, p.o.) was given after 30 min. The rats were killed 6 h after the administration of indomethacin (West 1982) and their stomach was removed and examined for pH, volume and total acidity of gastric juice, and ulcer index as described previously.

In-vitro anticholinergic activity

Cumulative dose response curve of acetylcholine (0.1, 0.2, 0.4, 0.8, 1.6 ml of 100 μg/ml) was plotted in the presence and absence of *L. usitatissimum* fixed oil (0.1, 0.2, 0.3 ml) and atropine (1 ml of 100 μg/ml). The experiment was done (in triplicate) using isolated guinea pig ileum preparation (2 cm) from an overnight-fasted animal. Log dose

versus % response curve of acetylcholine was plotted to calculate the dose ratio of acetylcholine with *L. usitatissimum* fixed oil by the method of Kulkarni (2005).

In-vitro antihistaminic activity

Cumulative dose response curve of histamine (0.1, 0.2, 0.4, 0.8, 1.6 ml of 10^{-4} M) was plotted using isolated rat ileum preparation (2 cm) in the presence and absence of *L. usitatissimum* fixed oil (0.3 ml) and promethazine (1 ml of 0.5 μg/ml) to ascertain the antihistaminic potential. Log dose versus % response curve was plotted to calculate the dose ratio. The experiment was performed in triplicate (Nkeh et al. 1996).

Statistical analysis

All the data are presented as Mean ± SEM and analyzed by One-way ANOVA followed by Dunnett 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, USA).

Results

Aspirin-induced gastric ulcers

Aspirin produced gastric lesions in the glandular region of the stomach and treatment with *L. usitatissimum* fixed oil (1.0, 2.0, 3.0 ml/kg) inhibited the formation of gastric lesions in a dose-dependent manner. Significant antiulcer effect was observed at the dose of 1 ml/kg (i.e. 29.42%) with maximization of response at 3 ml/kg (81.08%), which was slightly better than the standard, misoprostol (78.98%).The oil at 3 ml/kg dose significantly raised the gastric pH to 3.37 and showed 32.63% inhibition of gastric juice secretion compared with control (Table 1).

Indomethacin-induced gastric ulcers

Like aspirin, indomethacin also produced haemorrhagic lesions in the glandular region of the stomach. Intraperitoneal treatment with *L. usitatissimum* fixed oil (1.0, 2.0, 3.0 ml/kg) reduced the ulcer scores compared with vehicle control. *L. usitatissimum* fixed oil at 3 ml/kg dose showed significant protective response of 68.61%, which was comparable to the standard, misoprostol (70.56%). The oil at 3 ml/kg dose significantly raised the gastric pH to 4.42 and inhibited gastric juice secretion by 33.81% compared with control (Table 1).

Table 1 Effect of *L. usitatissimum* fixed oil on aspirin-, indomethacin- and ethanol-induced gastric ulcer/lesions in rats

Treatment	Aspirin (500 mg/kg, p.o.)				Indomethacin (20 mg/kg, p.o.)			Ethanol (50%, 5 ml/kg, p.o.)	
	pH (units)	Volume of gastric juices (ml/100 g)	Ulcer index	pH (units)	Volume of gastric juices (ml/100 g)	Ulcer index	pH (units)	Volume of gastric juices (ml/100 g)	Lesion index (mm)
Control (distilled water, 3.0 ml/kg, i.p.)	2.43 ± 0.13	6.21 ± 0.21	3.33 ± 0.12	3.30 ± 0.18	3.28 ± 0.33	3.60 ± 0.10	2.25 ± 0.09	12.21 ± 0.98	4.80 ± 0.21
L. usitatissimum fixed oil (1.0 ml/kg, i.p.)	2.74 ± 0.09	5.21 ± 0.31 (5.18)	2.35 ± 0.06 (29.42)*	3.71 ± 0.11	3.11 ± 0.32 (16.10)	3.16 ± 0.10 (12.22)	3.37 ± 0.07	11.62 ± 0.91 (4.83)	3.06 ± 0.19 (36.25)*
L. usitatissimum fixed oil (2.0 ml/kg, i.p.)	2.80 ± 0.07	4.61 ± 0.29 (12.19)	1.82 ± 0.18 (45.34)*	4.10 ± 0.14*	2.88 ± 0.24 (25.76)	2.73 ± 0.08 (24.17)*	3.85 ± 0.08	9.12 ± 0.43 (25.31)	2.18 ± 0.12 (54.58)*
L. usitatissimum fixed oil (3.0 ml/kg, i.p.)	3.37 ± 0.04*	4.11 ± 0.17 (32.62)	0.63 ± 0.15 (81.08)*	4.42 ± 0.05*	2.21 ± 0.20 (33.81)	1.13 ± 0.06 (68.61)*	4.47 ± 0.07*	8.56 ± 0.13 (29.81)	0.86 ± 0.03 (82.08)*
Misoprostol (100 μg/kg/ 0.5 ml, p.o.)	3.32 ± 0.05*	4.17 ± 0.14 (33.84)	0.70 ± 0.09 (78.98)*	4.04 ± 0.08*	2.17 ± 0.19 (32.85)	1.06 ± 0.11 (70.56)*	4.51 ± 0.12*	5.34 ± 0.21 (56.26)	0.82 ± 0.09 (82.91)*

Values in *parenthesis* represent percentage inhibition

(Values are Mean ± SEM), each group contains six animals

All groups were compared with group 1 (control) by Dunnett test (*$P < 0.05$)

Ethanol-induced gastric lesions

Striated lesions, characteristic of oral ethanol treatment were observed in the glandular portion of the rat stomach and *L. usitatissimum* fixed oil pretreatment provided a significant dose-dependent protection against the ulceration caused by ethanol. Mean ulcer score was reduced from 4.80 (control) to 0.86 with 3 ml/kg dose of *L. usitatissimum* fixed oil (i.e. 82.08% inhibition). Misoprostol, which was taken as a positive control, produced results equipotent to *L. usitatissimum* fixed oil (3 ml/kg). The oil at 3 ml/kg dose significantly raised the gastric pH to 4.47 and inhibited gastric juice secretion by 29.81% compared with control (Table 1).

Serotonin-induced ulcers

Subcutaneous administration of serotonin produced characteristic mucosal lesions. Pretreatment with *L. usitatissimum* fixed oil (1.0, 2.0, 3.0 ml/kg) provided significant dose-dependent protective effect. The oil at 3 ml/kg dose exhibited maximum protection of 83.38%, in comparison with control (Fig. 1).

Reserpine-induced ulcers

L. usitatissimum fixed oil exhibited a dose-dependent protection against the gastric mucosal damage induced by reserpine. The oil exhibited 34.45% (1 ml/kg) to 87.08% (3 ml/kg) protection in comparison with control (Fig. 2).

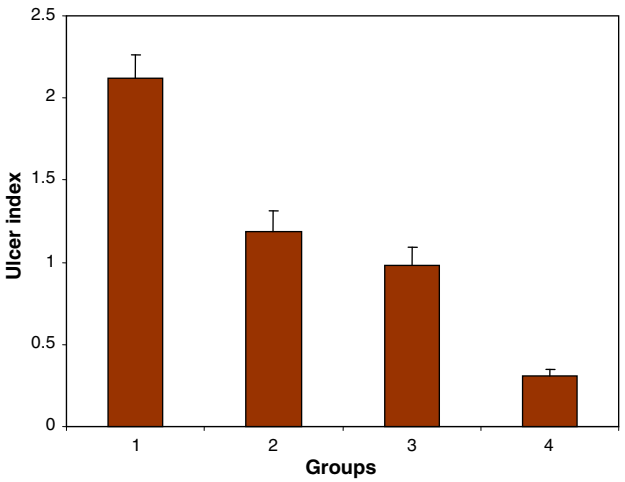

Fig. 1 Effect of *L. usitatissimum* fixed oil on serotonin-induced gastric ulcers in albino rats. Values are Mean ± SEM, each group contains six animals. All groups were compared to group 1 by Dunnett test. *Group 1* control; *Group 2 L. usitatissimum* fixed oil (1 ml/kg); *Group 3 L. usitatissimum* fixed oil (2 ml/kg); *Group 4 L. usitatissimum* fixed oil (3 ml/kg)

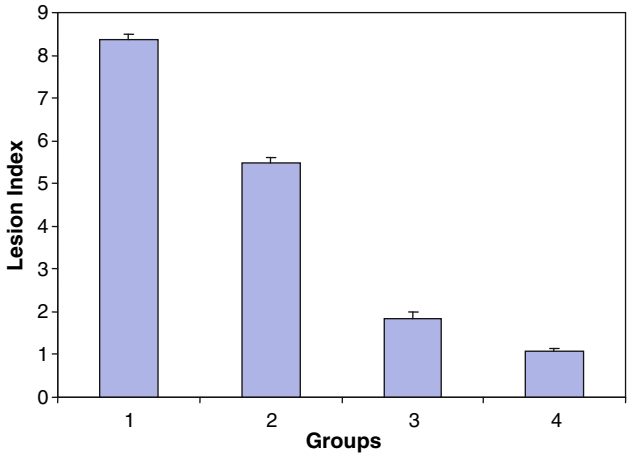

Fig. 2 Effect of *L. usitatissimum* fixed oil on reserpine-induced gastric lesions in albino rats. Values are Mean ± SEM, each group contains six animals. All groups were compared to group 1 by Dunnett test. *Group 1* control; *Group 2 L. usitatissimum* fixed oil (1 ml/kg); *Group 3 L. usitatissimum* fixed oil (2 ml/kg); *Group 4 L. usitatissimum* fixed oil (3 ml/kg)

Histamine-induced ulcers

Intraperitoneal administration of histamine produced gastric mucosal damage in the glandular region of the stomach. *L. usitatissimum* fixed oil (1.0, 2.0, 3.0 ml/kg) treatment provided 31.33, 46.99, and 68.67% protection, respectively (Table 2).

Stress-induced ulcers

Restraining the animal at a cold temperature induced considerable haemorrhagic mucosal lesions in stomach. *L. usitatissimum* fixed oil showed significant gastroprotective effect in a dose-dependent manner. Maximum protective effect with 65.52% inhibition (Table 2) was observed at 3 ml/kg dose.

Pylorus ligation-induced gastric ulceration

Ligation of pyloric end induced haemorrhagic mucosal lesions in stomach, increase in volume of gastric juices, and total acidity. *L. usitatissimum* fixed oil (3 ml/kg) significantly reduced the ulcer index (87.07%), gastric volume (61.46%), and total acidity (36.11%) in pylorus-ligated rats in comparison with control (Table 3). Treatment with the oil at 3 ml/kg dose provided 87.07% protection in the ulcer index in comparison with control. Cimetidine and misoprostol produced 72.74 and 84.24% inhibition, respectively.

Table 2 Effect of *L. usitatissimum* fixed oil on histamine- and stress-induced gastric ulcer/lesions

Treatment	Histamine-induced Ulcer index ($n = 4$)	Stress-induced Lesion index (mm) ($n = 6$)
Control (distilled water, 2.0 ml/kg, i.p.)	4.15 ± 0.09	14.01 ± 0.63
L. usitatissimum fixed oil (1.0 ml/kg, i.p.)	2.85 ± 0.10 (31.33)*	9.67 ± 0.33 (30.98)*
L. usitatissimum fixed oil (2.0 ml/kg, i.p.)	2.20 ± 0.16 (46.99)*	8.83 ± 0.74 (36.97)*
L. usitatissimum fixed oil (3.0 ml/kg, i.p.)	1.30 ± 0.07 (68.67)*	4.83 ± 0.16 (65.52)*
Cimetidine (32 mg/kg/1 ml, p.o.)	1.51 ± 0.08 (63.61)*	3.25 ± 0.11 (76.80)*

Values in *parenthesis* represent percentage inhibition

(Values are Mean ± SEM), *n* signifies number of animals

All groups were compared with group 1 (control) by Dunnett test (*$P < 0.05$)

Table 3 Effect of *L. usitatissimum* fixed oil on gastric secretion and aspirin-induced ulceration in pylorus-ligated rats

Treatment	Pylorus ligated rats			Aspirin induced gastric lesions in pylorus ligated rats
	Volume of gastric juices (ml/100 g)	Total acidity (mEq/l)	Ulcer index (mean ± SEM)	Lesion index (mm)
Control (distilled water, 3.0 ml/kg, i.p.)	5.89 ± 0.35	144.00 ± 2.78	5.65 ± 0.04	12.67 ± 0.42
L. usitatissimum fixed oil (1.0 ml/kg, i.p.)	5.21 ± 0.21 (11.55)	132.00 ± 1.54 (8.3)	4.73 ± 0.03 (16.28)	9.89 ± 0.65 (21.94)
L. usitatissimum fixed oil (2.0 ml/kg, i.p.)	3.24 ± 0.14 (44.99)*	105.00 ± 1.59 (27.08)*	2.20 ± 0.06 (61.06)*	6.33 ± 0.49 (50.04)*
L. usitatissimum fixed oil (3.0 ml/kg, i.p.)	2.27 ± 0.21 (61.46)*	92.00 ± 2.21 (36.11)*	0.73 ± 0.02 (87.07)*	3.83 ± 0.31 (69.77)*
Cimetidine (32 mg/kg/0.2 ml, p.o.)	1.78 ± 0.11 (69.78)*	56.00 ± 2.31 (61.11)*	1.54 ± 0.05 (72.74)*	4.52 ± 0.15 (64.33)*
Misoprostol (100 μg/kg/0.5 ml, p.o.)	4.54 ± 0.17 (22.92)	87.00 ± 1.14 (39.58)*	0.89 ± 0.09 (84.24)*	4.15 ± 0.12 (67.24)*

Values in *parenthesis* represent percentage inhibition

(Values are Mean ± SEM), each group contains six animals

All groups were compared with group 1 (control) by Dunnett test (*$P < 0.05$)

Aspirin-induced gastric ulcerations in pylorus-ligated rats

Aspirin (100 mg/kg, p.o.) induced severe ulceration in the gastric mucosa in pylorus-ligated rats. Pre-treatment with *L. usitatissimum* fixed oil (3.0 ml/kg) markedly reduced the lesion index (3.83, i.e. 69.77%) in comparison with control (12.67). Treatment with cimetidine and misoprostol provided significant protection of 64.33 and 67.24%, respectively, in comparison with control (Table 3).

Indomethacin-induced gastric lesions following oral administration of oil

Effect of oral administration of *L. usitatissimum* fixed oil on indomethacin-induced gastric ulcers in rats is shown in Table 4. Oral administration of oil at 3.0 ml/kg dose significantly reduced the ulcer index, gastric volume, and total acidity and raised the gastric pH in comparison with control.

In-vitro anticholinergic activity

Dose ratio of acetylcholine was found to be 1.93 ± 0.17, 2.75 ± 0.21 and 3.15 ± 0.14 in the presence of *L. usitatissimum* fixed oil (0.1, 0.2, 0.3 ml), respectively (Fig. 3), depicting dose-dependent anticholinergic activity slightly less than atropine (4.07 ± 0.24).

In-vitro antihistaminic activity

Dose–response curve of histamine in the presence of *L. usitatissimum* fixed oil and promethazine shows more right shift in the curve depicting better histamine antagonistic action of promethazine. Dose ratio of histamine was calculated to be 2.19 and 3.01 in the presence of *L. usitatissimum* fixed oil and promethazine, respectively, from the dose response curve (Fig. 4).

Table 4 Effect of *L. usitatissimum* fixed oil (p.o./i.p.) on indomethacin-induced gastric ulcers in rats

Treatment	pH (units)	Volume of gastric juices (ml/100 g)	Total acidity (mEq/l)	Ulcer index
Control (distilled water, 3.0 ml/kg, p.o)	3.01 ± 0.07	3.12 ± 0.19	156.11 ± 6.95	2.98 ± 0.13
L. usitatissimum fixed oil (3.0 ml/kg, p.o.)	4.21 ± 0.12*	2.33 ± 0.09* (25.32)	106.85 ± 2.99* (31.55)	1.66 ± 0.08* (44.29)
L. usitatissimum fixed oil (3.0 ml/kg, i.p.)	4.46 ± 0.18*	1.91 ± 0.20* (38.78)	98.23 ± 4.66* (37.07)	1.18 ± 0.07* (60.40)
Misoprostol (100 μg/kg/0.5 ml, p.o.)	4.51 ± 0.15*	1.88 ± 0.09* (39.74)	80.03 ± 5.85* (48.73)	1.06 ± 0.05* (64.42)

Values in *parenthesis* represent percentage inhibition

(Values are Mean ± SEM), each group contains five animals

All groups were compared with group 1 (control) by Dunnett test (*$P < 0.05$)

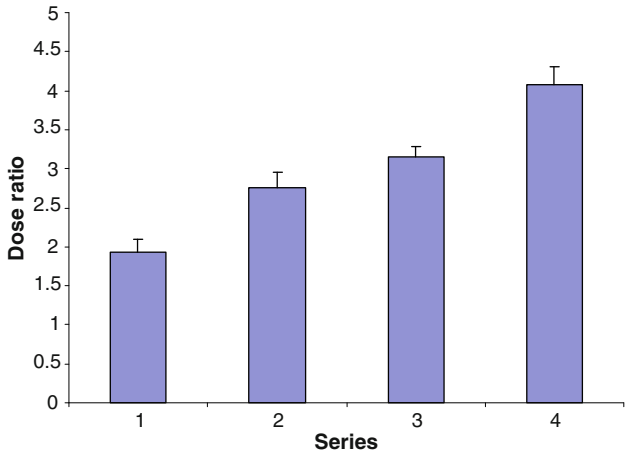

Fig. 3 In-vitro anticholinergic activity of *L. usitatissimum* fixed oil. *Series 1 L. usitatissimum* fixed oil (0.1 ml); *Series 2 L. usitatissimum* fixed oil (0.2 ml); *Series 3 L. usitatissimum* fixed oil (0.3 ml); *Series 4* atropine (1 ml of 100 μg/ml). Values are Mean ± SEM, $n = 3$

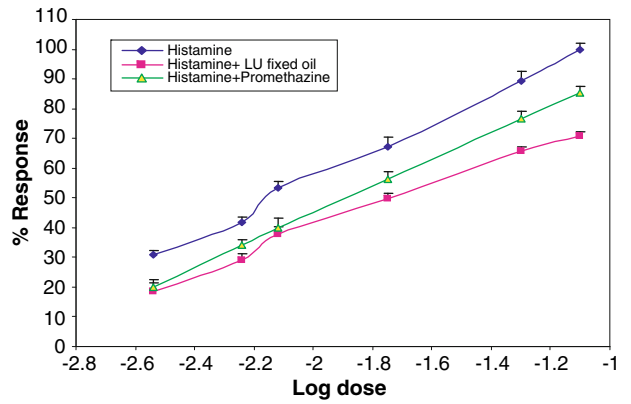

Fig. 4 Dose–response curves of the contractile effects of histamine alone and in presence of *L. usitatissimum* fixed oil and promethazine. Values are Mean ± SEM, $n = 3$

Discussion and conclusions

The results of present study indicate that *L. usitatissimum* fixed oil administered intraperitoneally exhibits potent antiulcer activity as evidenced by significant inhibition of gastric ulcers induced by various ulcerogens. Intraperitoneal administration provides faster absorption of oil compared with oral administration. In addition, absorption from intraperitoneal route is free from effects of gastric emptying or presystemic gastro-intestinal and gut wall metabolism which affects oral absorption. Considering the same the antiulcer activity of oil was evaluated following intraperitoneal administration.

The gastrointestinal irritant properties of nonsteroidal anti-inflammatory drugs are the major impediment to their use as antiinflammatory drugs. Aspirin and indomethacin inhibit the biosynthesis of cytoprotective prostaglandins (due to non specific COX inhibition) which results in overproduction of leukotrienes (products of arachidonic acid metabolism by lipoxygenase) (Rainsford 1987). Ethanol induces stasis in gastric blood flow which results in haemorrhage and necrotic gastric lesions, and prostaglandin [misoprostol, a synthetic prostaglandinE_1 (PGE_1) analog, used in the present study] offers protection against ethanol-induced ulceration by preventing stasis in gastric mucosal microcirculation (Guth et al. 1984). Researchers have reported that leukotriene antagonist and 5-lipoxygenase inhibitors are capable of inhibiting alcohol- and NSAID-induced gastric ulceration in rats (Parnham and Brune 1987). Plant lipids containing linolenic acid, e.g., *L. usitatissimum* fixed oil, possess lipoxygenase inhibitory activity (Singh and Majumdar 1997; Singh et al. 2008). Therefore, the protection offered by *L. usitatissimum* fixed oil against NSAIDs and ethanol-induced gastric ulcers could be due to lipoxygenase inhibitory activity. In NSAIDs- and ethanol-induced gastric ulcer models, *L. usitatissimum* fixed oil significantly raised the pH of gastric juice by inhibiting the gastric secretion suggesting antisecretory effect of the oil. Thus, the antisecretory effect of the oil could supplement its lipoxygenase inhibitory activity, in providing protection against NSAIDs- and ethanol-induced gastric ulceration.

Histamine-induced gastric ulceration is mediated through both enhanced gastric acid secretion and

vasospastic effects of histamine (Cho and Pfeiffer 1981). *L. usitatissimum* fixed oil appears to suppress the histamine-induced vasospastic effects and gastric secretion. The oil significantly inhibited histamine-induced contraction of rat ileum suggesting its histamine-antagonistic activity. The antisecretory effect of the oil has been observed in the experiments on NSAIDs- and ethanol-induced gastric ulcers in rats. However, a much more pronounced antisecretory effect of the oil is demonstrated in the experiment on pylorus-ligated rats where the oil inhibited both the gastric output and total acidity.

Reserpine-induced gastric ulceration has been attributed to degranulation of the gastric mast cells providing endogenous release of histamine, provoking gastric secretion and mucosal defects (Kirsner 1957). Reserpine also depresses the adrenergic activity with simultaneous increase in the cholinergic tone and reserpine-induced ulceration is believed to be both histamine- and cholinergically mediated (Sandor and Cuparencu 1977; Cho et al. 1985). Consequently, the antiulcer effect of *L. usitatissimum* fixed oil could be due to its histamine antagonistic and anticholinergic effects.

Serotonin-induced ulceration is believed to arise from a disturbance of gastric mucosal microcirculation, and the *L. usitatissimum* fixed oil appears to improve such a disturbance. Since the development of ulcers by serotonin and reserpine usually takes about 18 h, it may also be inferred that the oil has a sustained effect.

Stress plays important role in the pathogenesis of gastric ulcers. Stress-induced ulcers are mediated by histamine release (due to mast cell degranulation), increased acid production, enhanced gastric motility, vagal over activity (Cho et al. 1976), and decreased gastric mucosal blood flow (Hase and Moss 1973). Stress-induced ulcers can be prevented partially or entirely by vagotomy (Brodie and Hanson 1960). Accordingly, protective effect of *L. usitatissimum* fixed oil in stress-induced ulcers could be due to its histamine antagonistic, anticholinergic and antisecretory activity.

Pylorus ligation-induced ulcers are particularly mediated due to increased accumulation of gastric acid and pepsin, leading to autodigestion of gastric mucosa (Goel and Bhattacharya 1991). *L. usitatissimum* fixed oil in the present study decreased the gastric output and total acidity in pylorus-ligated rats, which suggests its antisecretory potential. *L. usitatissimum* fixed oil exhibited significant anticholinergic activity on isolated guinea pig ileum in a dose-dependent manner which compliments the antisecretory potential of *L. usitatissimum* oil.

The antiulcer activity of *L. usitatissimum* fixed oil described so far has been conducted following intraperitoneal administration of oil. However, intraperitoneal administration is not a normal, pharmaceutically accepted route of administration. Thus, antiulcer activity of oil was evaluated following oral administration of oil, and the oil significantly reduced the ulcer index, gastric volume, and total acidity and raised the gastric pH in comparison with control.

Antiulcer activity of plant lipid, e.g., *Ocimum sanctum* fixed oil is available. *O. sanctum* fixed oil possesses significant antiulcer activity against NSAID-, alcohol-, histamine- and reserpine-, serotonin- and stress-induced ulceration in experimental animals. The fixed oil also possesses antisecretory activity (Singh and Majumdar 1999). The oil contains linolenic acid (16.63%) which can inhibit both cyclooxygenase and lipoxygenase (Singh and Majumdar 1997).The antiulcer activity of the oil has been attributed to lipoxygenase inhibitory, histamine antagonistic, and antisecretory effects of the oil (Singh and Majumdar 1999). Since *L. usitatissimum* fixed oil, used in the present study, also contains higher quantity of linolenic acid (57.38%), one would expect similar biological activity from *L. usitatissimum* fixed oil too. As per expectation, *L. usitatissimum* fixed oil has been found to possess significant antiulcer activity.

In conclusion, one would like to say that *L. usitatissimum* fixed oil possesses significant antiulcer activity which may be due to the lipoxygenase inhibitory, histamine antagonistic, and antisecretory (anticholinergic) effects of the oil. The present observation is the first experimental data showing antiulcer activity of *L. usitatissimum* fixed oil in various animal models. The fixed oil also possesses anti-inflammatory activity. The drug that possesses both anti-inflammatory and antiulcer activity could be of great therapeutic importance as most of the antiinflammatory drugs used in modern day medicine are ulcerogenic. However, further studies are required to comment more in this respect.

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