GASTRIC ACID ANTISECRETORY AND ACID NEUTRALIZATION EFFECT OF AQUEOUS *TRIGONELLA FEONUM-GRACUM* AND *LINUM USSITATISSIMUM* SEED EXTRACTS ON EXPERIMENTAL MODELS

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Summary

The majority of the Ethiopian population rely on traditional remedies such as barley and fenugreek infusion, and linseed mucilage to manage peptic ulcer. Previous studies showed that both *Trigonella feonum-gracum* and *Linum ussitatissimum* seeds have anti-ulcer effect. How anti-ulcer effect is mediated, however, has not well been established. The aim of this study is, therefore, to screen the aqueous extract of *T. foenum-gracum* and *L. usitatissimum* seeds for their anti-secretory and antacid action in an attempt to come up with the possible mechanisms. The present study revealed that *T. foenum-gracum* extract significantly reduced the basal total acid ($P< 0.05$) while *L. usitatissimum* extract slightly reduced it. Both aqueous extract of *T. foenum-gracum* and *L. usitatissimum* seeds neutralized the acid *in vitro*. *L. usitatissimum* extract was observed to have a higher acid neutralizing capacity than *T. foenum-gracum* extract. From this study it can be concluded that the aqueous extract of *T. foenumgracum* seeds may produce anti-ulcer effect through acid antisecretory and acid neutralizing action while that of *L usitatissimum* lonely through acid neutralizing action. To establish this, further investigation at molecular mechanism should be pursed.

Key words: *T. foenum-gracum, L. usitatissimum,* linseed, fenugreek, anti-secretory, antacid.

Peptic ulcer is the most common gastrointestinal disorder in clinical practice. Considering the several side effects of modern medicine (1), indigenous drugs with fewer side effects should be looked for as a better alternative for the treatment of peptic ulcer.

African ethnomedical systems employed numerous plant extracts for the treatment of diseases, including gastrointestinal disorders, in order to give a therapeutic choice to the population (2). *Linum ussitatissimum* (linseed) and *Trigonella foenum-gracum* (fenugreek) are traditionally used in the management of peptic ulcer in Ethiopia (3).

L. usitatissimum, member of Linaceae family, seed contains fixed oil, mucilage, protein and small quantities of cyanogenic glycosides. *L. usitatissimum* seed has been used as a poultice for local inflammation and employed to make demulcent preparation (4). In experimental studies, *L. usitatissimum* seed inhibits breast tumor development (5) and the growth of prostate cancer (6) in animal models. *L. usitatissimum* seed oil in men shows total cholesterol concentration reduction by 12.5 % (7).

T. foenum-gracum, member of the Leguminosae family, has been used to promote lactation and as a medicinal agent to treat diabetes, constipation, and hyperlipidemia. It has been used topically to treat inflammation (8). The seed contains alkaloid trigonelline, a number of steroidal sapogenins, fixed oil and mucilage (4). Study on animal model shows that *T. foenum-gracum* seed reduced total acid and pepsin activity in ethanol induced ulcer (9). Other studies reveal the possible hypoglycemic and antihyperlipidemic properties of oral *T. foenum-gracum* seed (10).

The majority of the Ethiopian population rely on traditional remedies such as *T. foenum-gracum* seed infusion and *L. ussitatissimum* seed mucilage to manage peptic ulcer (3). Previous study showed that both *T. feonum-gracum* and *L. ussitatissimum* seed extracts have gastroprotective action and wide safety margin (11). The mechanism for the anti-ulcer effect has not yet been well established. The aim of this study is, therefore, to screen the aqueous extract of *T. foenum-gracum* and *L. usitatissimum* seeds for their anti-secretory and antacid action in an attempt to suggest how their anti-ulcer effect is mediated.

Materials and Methods

Chemicals and drugs:

The following drugs and chemicals were used: Aluminum hydroxide and magnesium hydroxide (Demo S.A Pharmaceutical Industry, Greece); Atropine hydrochloride (Sigma Chemical Company, U.S.A.); Carbamoylcholine chloride (BDH Chemicals Ltd Poole, England); Cimetidine (Kwang Myung Pharm Co.Ltd, Korea); Ether (Nice Chemicals Pvt Ltd, India); Histamine dihydrochloride (Aldrich Chemical Company, U.S.A.); Hydrochloric acid (BDH Laboratory Supplies, England); Phenolphthalein (E.Merck Darmstadt, Germany); Phthalate pH buffer (Beckman instruments, Inc., U.S.A); Phosphate pH buffer (Beckman instruments, Inc., U.S.A.); and Sodium hydroxide (Loba Chemie, India).

Animal preparation:

Swiss Albino mice (25-30g) of both sex and female Wistar rats were used for the study. These were purchased from the Ethiopian Health and Nutrition Research Institute and the animals were acclimatized for a week in the Faculty of Medicine prior to experimentation. Animals were fed on pellet and water *ad libitum*. They were deprived from food for 24 hrs before the experiment but were allowed free access to water. Animals were kept in cages with grating floors to prevent coprophagy (12).

Plant materials and extract preparations:
The *T. foenum-gracum* and *L. usitatissimum* seeds used in this study were purchased from a supermarket in Addis Ababa. The plants were identified and authenticated by Mr. Melaku Wondafrash of the National Herbarium, Department of Biology, Faculty of Science, Addis Ababa University. A voucher specimen of the plant was deposited in the National Herbarium. Unpowdered whole seeds of *L. usitatissimum* were macerated in distilled water in a ratio of 1 g: 6 ml for 48 hrs and the aqueous viscous material was separated from the mixture by straining using muslin. Powdered *T. foenum-gracum* seeds were soaked in distilled water in a ratio of 1 g: 100 ml for 12 hrs; and the supernatant (aqueous layer) was separated from the mixture by decantation. The aqueous extracts of both seeds were then lyophilized using a freeze-drier. The extracts were subjected for the pharmacological investigation. The extracts and standard drugs used for the pharmacological investigations were dissolved in distilled water freshly.

Extract and vehicle administration:
The mice were given a volume of 0.8ml per 30g body weight of the dissolved extract which is equivalent to each dose or vehicle *per os*. Different doses-500mg, 1000mg and 1500mg/kg were found effective for their antiulcer effect from previous study (11). A 1000mg/kg dose was used for antisecretory and antacid *in vivo* activity studies.

Anti-secretory studies:
a. Effect of extracts on Secretagogue induced gastric secretion
One hour after extracts 1000mg/kg, distilled water 0.8ml/30g, atropine sulphate 10mg/kg, and cimetidine 100mg/kg had been given, the mice were anesthesized with ether; and the pylorus was ligated (13). After closure, acid secretion was evoked by either histamine dihydrochloride (4mg/kg) or carbamoylcholine chloride (0.5mg/kg). The animals were killed 3hrs later using ether, and ligature was placed on esophago-cardiac junction. Then their stomach content was drained in to the test tube, which was centrifuged at a speed of 2000rpm for 10 minutes. After washing the mucosal side of the stomach with 2ml of distilled water, the gastric secretion volume and the pH were determined (14). The total acid of the gastric juice (meq/lt) was determined by titrating against 0.01N NaOH using phenolphthalein as indicator (15). Grouping of animals were carried out as follows:

Histamine induced:
- Distilled water +Histamine dihydrochloride
- *T. foenum-gracum* extract +Histamine dihydrochloride
- *L.usitatissimum* extract + Histamine dihydrochloride
- Cimetidine + Histamine dihydrochloride

Carbachol induced
- Distilled water + carbamoylcholine chloride
- *T.foenum-gracum* + carbamoylcholine chloride
- *L.usitatissimum* + carbamoylcholine chloride
- Atropine + carbamoylcholine chloride

b. Effect of the extracts on basal gastric acid secretion

Albino mice weighing 25-30g were housed in cages and fasted for 24hrs prior to pylorus ligation. Care was taken to avoid coprophagy by using grating floor. The abdomen was opened by a small medline incision below the xiphoid process under light ether anesthesia. Pyloric portion of the stomach was slightly lifted out and ligated avoiding traction to the pylorus or damage to its blood supply (16). The extracts (1000mg/kg), cimetidine(100mg/kg) and vehicle (i.e. distilled water in volume of 10ml/kg) were administered in to the duodenal lumen(15). After the drug or vehicle administration, the stomach was placed back carefully and the abdominal wall closed by interrupted sutures. The animals were deprived of both food and water during the postoperative period and are sacrificed at the end of 6hrs after operation. A ligature was placed at the end of esophago-cardiac junction, and the stomachs were removed carefully, and dissected out; contents were drained in to tubes. The volume of gastric secretion was measured after centrifugation, and was subjected to analysis for pH and total acid (meq/lt) as described by Hosseinzadeh (13).

Acid neutralizing effect of the extracts

a. Incubation of gastric juice

Twenty-four hours fasted female rats (200-250g) were used. The abdomens of six rats per group were opened under ether anesthesia, and the pylorus of each animal was ligated. The stomachs were removed 6 hrs later. The gastric juice, which was obtained from each one after centrifugation at a speed of 2000rpm for 10 minutes, was incubated with the extracts (1000mg/kg) or vehicle (distilled water, 20ml/kg in volume) at 37 ^{0}C for 4hrs *in vitro* and the pH was determined (17).

b. Determination of acid neutralizing capacity

Acid neutralizing capacities (ANC), which is the number of milliequivalents of hydrochloric acid required to maintain 1ml of antacid suspension at pH 3 for 2 hours *in vitro* (18), of the extracts were determined by dissolving the 100mg of the extracts with 1ml of water. Antacid suspension, containing mixture of Aluminum hydroxide 310mg/5ml and Magnesium trisilicate 620mg/5ml, was used as a standard drug while distilled water, in which the extracts dissolved, was used as a control. The test was carried out three times with each preparation, and then the arithmetic mean was calculated.

Statistical analysis

The Data, expressed as mean $\pm$ standard error mean, obtained from groups consisting of 6-8 animals, were analyzed using one-way analysis of variance (ANOVA). *Post hoc* comparisons between two groups were made with Dunnett's test using SPSS 10 statistical software package. A P value of less than 0.05 was considered statistically significant.

Results

Effect of the extracts on secretagogue induced gastric secretion:

Administration of both *L. usitatissimum* and *T. foenum-gracum* extract slightly decreased the carbachol induced total acid secretion and did not significantly alter the pH. *L. usitatissimum* extract increased the volume of gastric juice, while *T. foenum-gracum* decreased it.

The reduction in the total acid and the raise in pH by both extracts was less than the standard drug, atropine (Table 1).

Table 2 depicts the effect of *L. usitatissimum* and *T. foenum-gracum* extract on histamine induced gastric secretion. The vehicle treated animals had a volume of secretion, pH, and total acid of 0.52± 0.05ml, 3.27 ± 0.32 and 172.98 ± 0.65meq/lt, respectively. *L. usitatissimum* and *T. feonum-gracum* extracts did not alter the pH of gastric secretion but slightly decreased the total acidity of the secretion. *L. usitatissimum* extract increased the volume of secretion, while *T. feonum-gracum* did not change it. The change in the volume, pH and total acidity of gastric secretion by both plant extracts were not statistically significant as compared to the control. The acid anti-secretary action of both extracts was lower than the standard drug, cimetidine.

Table 1. Effect of intragastric administration of *L. usitatissimum and T. feonum-gracum* seed extracts on carbachol induced gastric secretion in pylorus-ligated mice

Treatment	**Dose (Mg/kg)**	N	**Volume of gastric juice**	**pH**	**Total acid Meq/lt**
Control	--	7	0.75 ± 0.11	2.95 ± 0.26	140.34 ± 18.5
L. usitatissimum	1000	7	0.92 ± 0.1	2.92 ± 0.09	105.31± 4.02
T. feonum-gracum	1000	6	0.57 ± 0.08	3.3 ± 0.24	109.57 ±16.88
Atropine	10	6	0.53 ± 0.15	6.02 ± 0.46**	74.1 ±11.78*

N-number of mice. * P<0.01 and **P<0.001, statistically significant relative to control

Table 2. Effect of intragastric administration of *L. usitatissimum and T. feonum-gracum* seed extracts on histamine induced gastric secretion in pylorus-ligated mice

Treatment	**Dose (Mg/kg)**	**N**	**Volume of gastric juice**	**pH**	**Total acid Meq/lt**
Control	------	6	0.52 ± 0.05	3.27 ± 0.32	172.98 ± 0.65
L. usitatissimum	1000	6	0.79 ± 0.05	3.27 ± 0.35	129.75 ± 12.14
T. feonum-gracum	1000	6	0.54 ± 0.06	3.00 ± 0.27	130.95 ± 10.95
Cimetidine	100	7	0.43± 0.1	5.58 ± 0.6*	91.97 ± 16.57**

N- Number of animal. *P<0.01 and ** P<0.001, statistically significant relative to control

Effect of the extracts on basal gastric acid secretion:

The effects of the extracts on basal gastric acid secretion are shown in table 3. *T. foenum-gracum* extract significantly reduced (P<0.05) the total acidity of the gastric juice, raised the pH of the gastric content and decreased the volume of the gastric content, though statistically insignificant. *L. usitatissimum* extract, although significantly diminished the volume of gastric secretion (P<0.01), did not show significant change in gastric pH and total acidity. The potency of the extracts is much less than that of the standard, cimetidine.

Table 3. Effect of intraduodenal administration of *L. usitatissimum and T. feonum-gracum* extracts on basal gastric secretion in pylorus-ligated mice

Treatment	Dose (Mg/kg)	N	Volume of gastric Juice	pH	Total acid Meq/lt
Control	----	6	1.08 ± 0.05	2.78 ± 0.46	91.22± 5.85
T. feonum-gracum	1000	6	0.77 ± 0.11	4.04 ± 0.49	64.73± 5.23*
L. usitatissimum	1000	6	0.56 ± 0.08**	3.58 ± 0.40	72 ± 3.50
Cimetidine	100	6	0.86 ± 0.14	5.25 ± 0.73*	41.7 ± 7.84**

N-number of mice. *P< 0.05 and **P<0.01, statistically significant compared with control

Acid neutralizing capacity of extracts

As shown in Table 4, the ANC of *L. usitatissimum* (100mg/ml) and *T.foenum-gracum* (100mg/ml) extract were found to be significantly higher than that of the vehicle though less than that of the standard antacid suspension. The ANC of *L.usitatissimum* extract was significantly higher than that of *T .foenum-gracum* extract.

Table 4. Acid neutralizing capacity of *L ussitatissimum* and *T. foenum-gracum* seed aqueous extract

Preparations	Acid neutralizing capacity
Vehicle (control)	$0.002 \pm 1.7 \times 10^{-4}$
T. foenum-gracum extract (100mg/ml)	$0.05 \pm 2.888 \times 10^{-3}$ [a]
L. ussitatissimum extract (100mg/ml)	$0.148 \pm 1.01 \times 10^{-2}$ [a, b]
Antacid suspension	2.033 ± 0.117 [a]

The test was carried out three times.

a- P<0.001, statistically significant relative to control

b- P<0.001, statistically significant relative to *T.foenum-gracum* extract

Effect on Gastric Juice *in vitro*

Figure 1 demonstrates the acid neutralizing effect of the extracts on the gastric juice. A dose 1000mg/kg *T. foenum-gracum* extract increased the pH (30.4%), though not statistically significant. The same dose of *L. usitatissimum* extract reduced the acidity of the gastric juice after incubation. *L. usitatissimum* extract increased the pH by 62.4 % (P<0.001).

Discussions

Independent of the inciting or injurious agent, peptic ulcers develop as a result of an imbalance between mucosal protection/ repair and aggressive factors (19). To regain the balance, different therapeutic agents including herbal preparations are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanism by increasing mucus production.

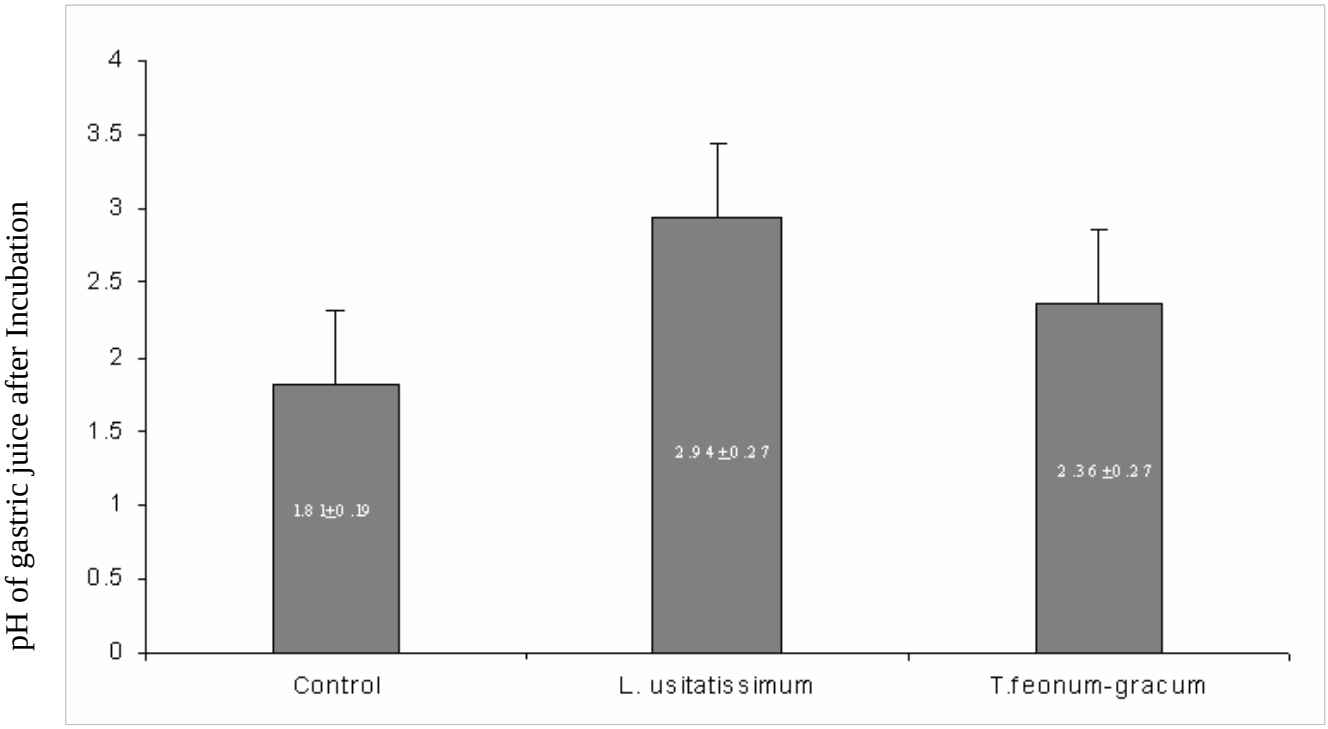

Figure 1. Effects of *L. usitatissimum* (1000mg/kg) and *T. feonum-gracum* (1000mg/kg) seed extracts on the pH of gastric juice from rats after incubation *in vitro*. Results are expressed as mean ± standard error of the mean (n=6). *P<0.001, compared with control.

The present study was undertaken to evaluate the anti-secretory and antacid action of aqueous extracts of *T. foenum-gracum* and *L. ussitatissimum* seeds. *T. foenum-gracum* extract reduced the total acid significantly and reduced the pH slightly. This action was observed by the systemic action of the plant extract as it was administered by intra-duodenal route. *T. foenum-gracum*, in ethanol-induced lesion followed by pylorus ligated rats, was also found to reduce the total acid (9). The anti-secretory effect of *T. foenum-gracum* extract, in addition to its antioxidant effect (9, 20), may be important in its gastroprotective action. The slight reduction of total acid and rise in pH of gastric content by *L. usitatissimum* extract showed that gastroprotective action of *L. ussitatissimum* is not mainly dependent on the anti-secretory effect.

The two extracts at the administered dose slightly reduced the total acidity induced by histamine and carbachol. *T. foenum-gracum* showed significant anti-secretory effect in basal gastric secretion but not in secretagogue induced gastric secretion. This depicts the weak anti-secretory property of *T. foenum-gracum* extract that could be influenced by acid secretogogue. The *L. ussitatissimum* mucilage is known to slow gastric emptying (21). This may be the reason why it increased the gastric content in pylorus-ligated mice when it was administered by gastric gavage.

Antacids have been effective in accelerating healing of duodenal and gastric ulcers. The ulcer healing action of antacids was thought to be due to the neutralization of gastric luminal acid (22). In the present study, *L. ussitatissimum* extract significantly raised the pH of the gastric content *in vitro* ($P<0.001$). The acid neutralizing capacity of the extract also supports this result. This suggests that the acid neutralizing action, in addition to the mechanical protection by mucilage, may contribute for its gastroprotective effect. Although *T. foenum-gracum* extract had higher acid neutralizing capacity than the control, the rise in the pH of the gastric content was insignificant.

From the present study, it can be concluded that antiulcer effect of the aqueous *T. foenum-gracum* seed extract may be through acid anti-secretory action while that of *L. usitatissimum* seed extract may be through antacid effect. Further investigations have to be carried out at molecular level.

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