Effects of the Oil and Mucilage from Flaxseed (Linum Usitatissimum) on Gastric Lesions Induced by Ethanol in Rats

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Abstract: The anti-ulcer activity of the oil and mucilage obtained from flaxseed (Linum usitatissimum) was evaluated in a rat model of ethanol-induced gastric ulcer. Our results show that pretreatment of rats with flaxseed oil and flaxseed mucilage significantly reduced the number and length of gastric ulcers induced by ethanol. Flaxseed oil was more effective than flaxseed mucilage in reducing the number of ulcers. The reduction in ulcer severity (cumulative length in mm) provided by an oral dose of flaxseed oil (5 ml/kg) was more prominent than that obtained by ranitidine (50 mg/kg). This study indicates that both flaxseed oil and flaxseed mucilage can provide a cytoprotective effect against ethanol-induced gastric ulcers in rats.

Key words: gastric ulcer, flaxseed, ethanol.

Introduction

Ethanol is a well known cause of gastric mucosal damage, but its pathogenetic mechanisms are not well understood. The oxygen-derived free radicals generated by ethanol may be responsible for the induction of gastric damage [1,2]. Free radicals are extremely reactive products leading to oxidative damage through lipid peroxidation [3,4].

Plant extracts and herbs are recognized as sources of natural antioxidants that can protect against oxidative stress and thus can play an important role in the chemoprevention of diseases resulting from lipid peroxidation [5]. Several plant extracts that have traditionally been used to treat gastric ulcers have also been tested for their cytoprotective effects in experimental animals. These include *Zingiber officinalis* [6], *Glycyrrhiza globra* [7], *Curcuma lunga* [8], *Brassica oleracea* [9], *Matricaria chamomilla, Matrcaria recutita* [10], *Althaea officinalis* [11], *Punica garanatum* and *Trigonella foecum* [12].

Flaxseed, also known as linseed, is derived from the flax plant (*Linum usitatissimum*), of the family Linaceae, which is cultivated worldwide for its fiber and oil. Flaxseed contains 6% mucilage or soluble fibers, insoluble fibers 18%, 25% proteins, and 30-40% oil, with alpha-linolenic acid (ALA) making up about 50-60% of the total fatty acids [13]. ALA is a precursor of omega-3 fatty acids, which makes flaxseed the leading source of plant-derived omega-3 [14]. Several experimental and clinical studies have demonstrated that ALA reduces total cholesterol [15], coronary heart diseases [16] and colon cancer [17]. The lignan constituents of flaxseed (but not its oil) possesses *in vitro* anti-oxidant and possible estrogen receptor agonist/antagonist properties, prompting hypotheses on its utility in the treatment of breast cancer [18], prostate cancer [19], inflammatory bowel disease [20], lupus nephritis [21], and type 2 diabetes [22]. There are only few studies on the health benefits of flaxseed mucilage, such as reduction of total cholesterol [14] and blood glucose levels [23].

Natural compounds rich in omega-3 (a known antioxidant) are being tested in animal models of gastric ulcers. Al Harbi *et al* [24] demonstrated that fish oil produced a significant inhibition of gastric mucosal damage induced by pyloric ligation, non-steroidal anti-inflammatory drugs (NSAIDs), reserpine, ethanol and hypothermic restraint in rats.

From these observations we hypothesized that the flaxseed plant may provide protection against experimentally-induced gastric ulcers in rats. To achieve this objective, we studied the effects of both flaxseed oil and mucilage on ethanol-induced gastric ulcers in rats.

Materials and methods

Plant material: Flaxseed (*Linum usitatissimum*) was obtained from a farm in the region of Misrata, east of Tripoli.

Preparation of flaxseed extracts

Preparation of flaxseed oil: One kilogram of powdered flaxseeds was macerated in a 1:1 mixture of petroleum ether and ethyl acetate for 24 h. After filtration, the filtrate was concentrated in a rotary evaporator under reduced pressure. About 450 ml of oil was obtained.

Preparation of flaxseed mucilage: One kilogram of flaxseed was milled and placed in 1 L of distilled water for 24 h. The mucilage was obtained by filtration of the supernatant followed by evaporation of excess water in a rotary evaporator.

Preparation of drug solutions: Ranitidine hydrochloride (Sigma chemicals Co, Germany) was dissolved in distilled water at 5.0%, ethanol (Farmitalia, Carlo Erba, Italy) was diluted with distilled water to obtain 75% solution.

Animals: Male Wistar rats (bred at the Animal Care Unit, Department of Pharmacology and Clinical Pharmacy, Al-Fateh University for Medical Sciences) weighing 165-250 g were used in the study. They were housed in an ambient temperature of 23°C with

a 12-h light-dark cycle. Animals were fed a balanced diet and given free access to water. All animals were fasted 36 h before starting the experiments. The study was approved by the faculty and the experiments were done according to the ethics guidelines of Al-Fateh University for Medical Sciences.

Experimental Design

Effects of flaxseed oil on ethanol-induced gastric ulcer

Animals were randomly divided into 5 groups of 6 rats. Three treatment groups received 2.5, 5.0 and 10.0 ml/kg of flaxseed oil (FSO) by gastric gavage, respectively. The negative control group was given 5.0 ml/kg of corn oil, and the positive control group received 50 mg/kg of ranitidine hydrochloride. Thirty minutes later, all animals were given 1.0 ml of 75% (v/v) ethanol orally to induce gastric ulcers.

One hour following ethanol administration, all rats were killed by an overdose of ether and the stomachs were rapidly removed, opened along their greater curvature, rinsed with warm distilled water, pinned flat on a paraffin plate, and fixed in 10% formalin for 24 h. The stomachs were then examined for the number of ulcers and the length of ulcers (in mm) was measured with a ruler under an illuminated magnifier (3X).

Effects of flaxseed mucilage on ethanol-induced gastric ulcer

Animals were randomly divided into 4 groups of 5 rats. Two treatment groups received 10.0 and 20.0 ml/kg of flaxseed mucilage (FSM) by gastric gavage, respectively. The negative control group was given 5.0 ml/kg distilled water and the positive control group was given 50 mg/kg ranitidine hydrochloride. After 30 min, all animals were given 1.0 ml of 75% (v/v) ethanol orally to induce gastric ulcers.

One hour following ethanol administration, all rats were killed by an overdose of ether and the numbers and lengths of ulcers were determined as described above.

Statistical analysis:

Data were analyzed with the unpaired student's t-test (two sample equal variance) by using SPSS 11.0. P values <0.05 were considered significant. Results are presented as mean ± SEM.

Results

Effects of flaxseed oil on ethanol-induced gastric ulcer

Pre-treatment with FSO significantly reduced in a dose-dependent manner the number of ulcers and the cumulative length (severity) of the ulcers induced by ethanol (Fig. 1). FSO at a dose of 5.0 ml/kg was the most effective in reducing the number ($p < 0.001$) and length of ulcers ($p < 0.001$) relative to control animals pre-treated with an equivalent amount of corn oil. Noteworthy, FSO at 10.0 ml/kg was less protective than at 5.0 ml/kg. At 5.0 ml/kg, it was more potent than ranitidine (positive control) in reducing the length of gastric ulcers. Representative stomachs of rats pretreated with corn oil, ranitidine and different doses of FSO are shown in figure 3.

Effect of flaxseed mucilage on ethanol-induced gastric ulcers

Flaxseed mucilage at the lower dose of 10 ml/kg significantly reduced the number and length of gastric ulcers induced by ethanol ($p<0.05$) (Fig. 2). The reduction of ulcer length by 10.0 ml/kg FSM was about half the reduction obtained by 50 mg/kg ranitidine. At a higher dose of 20.0 ml/kg, FSM significantly reduced the length but not the number of ethanol-induced ulcers ($p<0.001$). Representative stomachs of rats treated with distilled water, ranitidine and different doses of FSM are shown in figure 4.

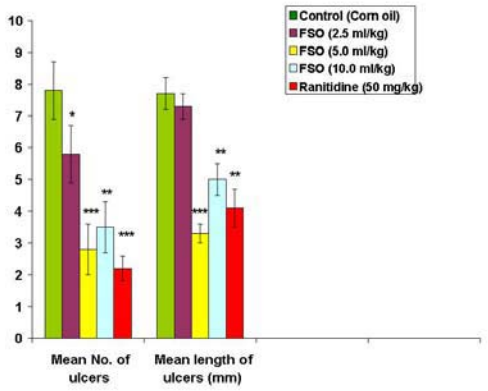

Figure 1: Effect of Linum usitatissimum seed oil on ethanol-induced gastric ulcer. Data are expressed as means + SEM (n=5-6). *P<0.05; **p<0.01; ***p<0.001 versus control (corn oil +ethanol). *FSO = flaxseed oil; EtOH = ethanol.*

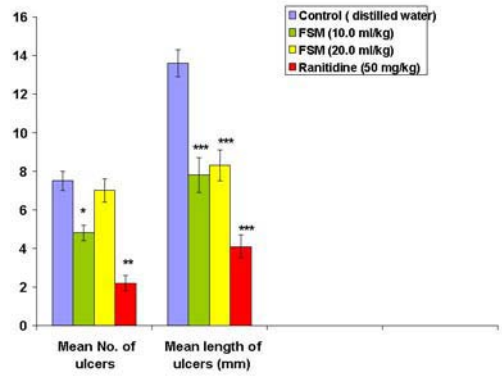

Figure 2: Effect of linum usitatissimum seed mucilage on ethanol-induced gastric ulcer. Data are expressed as means + SEM (n=5-6). *P<0.05; ***p<0.001 versus control (corn oil +ethanol). *FSM = flaxseed mucilage. EtOH = ethanol.*

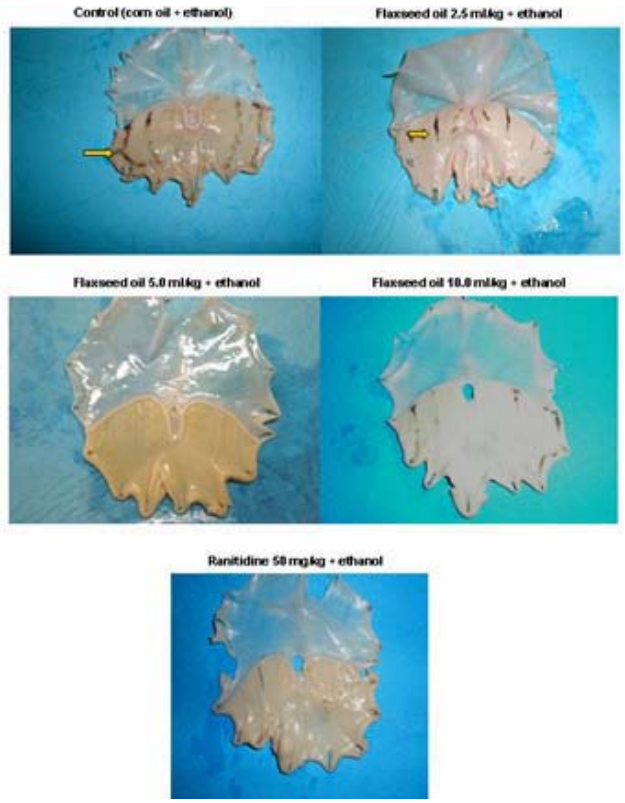

Figure 3. Representative stomachs showing the effects of pretreatment with flaxseed oil on ethanol-induced gastric lesions in rats. The top part in each stomach represents the f while the bottom is the corpus, (yellow arrows indicate gastric lesions).

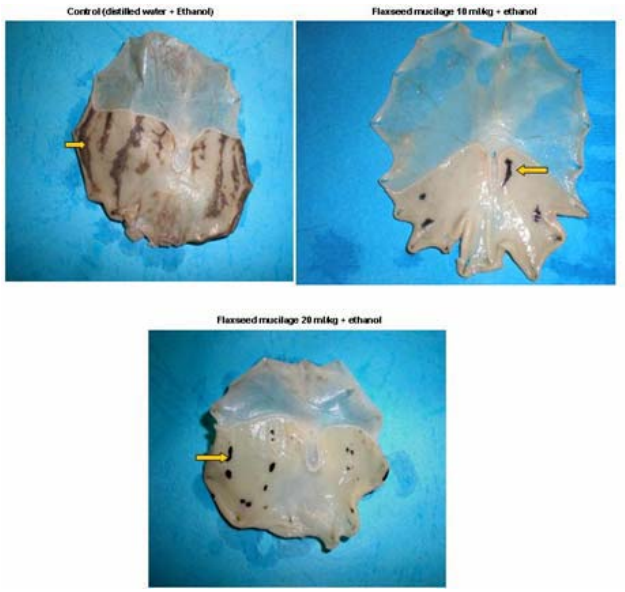

Figure 4. Representative stomachs showing the effects of pretreatment with flaxseed mucilage on ethanol-induced gastric lesions in rats. The top part in each stomach represents the forestomach while the bottom is the corpus, (yellow arrows indicate gastric lesions).

Discussion

The results of this preliminary study clearly demonstrate that pretreatment of rats with flaxseed oil and mucilage protects the gastric mucosa against ethanol-induced gastric ulcer. The ethanol-induced gastric ulcer model is mainly used to study the protective effects of substances with possible antioxidant and free radical scavenging properties [10].

Despite the extensive research carried on the effects of FSO in the prevention and treatment of several pathological conditions, including reduction of cholesterol, and prevention of cancer and diabetes, it has not been studied in the context of gastrointestinal diseases, particularly peptic ulcer.

Studies have shown that free-radical scavengers, such as vitamin E, vitamin A, and plant extracts from Falcaria vulgaris, Shankha bhasma, Kielmeyera coriacea, Ginkgo biloba, and Nigella sativa L, provide protective effects against experimentally induced gastric lesions [25-29]. Administration of fish oil, which is known to contain half the quantity of omega-3 compared with flaxseed oil, was found to significantly protect against ethanol-induced gastric ulcers in rats at doses of 5 and 10 ml/kg body weight [24].

Our results show that flaxseed oil provided protection against ethanol-induced gastric ulcers in rats. The reduction in ulcer length by 5.0 ml/kg FSO was significantly greater than that obtained with 50 mg/kg of the standard drug ranitidine.

The mucilage obtained from flaxseed also produced a significant reduction in the severity of ulcer pathology (ulcer length) and to a lesser extent in its incidence (ulcer number).

In conclusion, this study provides clear evidence that the products of flaxseed (oil and mucilage) have gastroprotective effect against ethanol-induced gastric ulcers. Further studies are needed to explore the effects of the chronic administration of FSO and FSM in the ethanol model of gastric ulcer and in other models, such as nonsteroidal anti-inflammatory drug-induced ulcers, pyloric ligation, and stress-induced gastric ulcers. Moreover, the effects of flaxseed oil and mucilage on mucus production and gastric acid secretion will be tested in an attempt to explore the possible mechanisms of their antiulcer effects.

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