

Anti-ulcer and Gastro Protective Effects of Honey in Combination with *Trigonella foenum graecum* Seeds Extract on Experimental Gastric Ulcer in Rats

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Abstract: The gastric protective effect of honey in combination with extract of fenugreek seeds was investigated in rats against ethanol-induced gastric damage. Macroscopically, oral administration of honey alone or honey in combination with seed extracts or with cimetidine induced a significantly decrease ($p < 0.05$) in gastric lesions. Histological studies revealed that honey in combination with aqueous seeds extract was significantly more effective than honey alone or honey in combination with alcoholic extract in preventing gastric lesions formation. These observations showed that fenugreek aqueous seed extracts possess anti-ulcer potential.

Key words: Gastro-protection, honey, *Trigonella foenum graecum* seed extract, cimetidine, ulcer inhibition

INTRODUCTION

Honey has been used topically for centuries to accelerate wound healing. It has been reported to be helpful in treating burns, decubitus ulcers and infected wounds^[1]. *In vitro* it has been shown to have antibacterial and antifungal activity to organisms commonly infecting surgical wounds. The wound-healing properties of honey are thought to result from the debriding properties of the enzyme catalase, absorption of edema because of honey's hygroscopic properties, its ability to promote granulation and reepithelialization from the wound edges and its antimicrobial properties.

Trigonella foenum graecum L. (Leguminosae) or fenugreek is a herbal medicine used in many parts of world. Its leaves are used for their cooling properties and its seeds for their carminative, tonic and aphrodisiac effects^[1]. The seeds of fenugreek (*T. foenum graecum*) are commonly used as a condiment in homes. The seeds are reported to have nutritive properties and stimulate digestive processes. The seeds have been used to treat a number of gastrointestinal disorders^[2-5]. Much work has been done on the beneficial effects of fenugreek seeds in diabetic^[6-9] stimulatory effect on immune function^[10] anti-inflammatory^[11,12] and hypercholesterolemia states^[13-16]. *T. foenum-graecum* seed are commonly used for a variety of kidney disorders and as diuretics^[7,18].

There are some reports concerning the anti-nociceptive effects of the plant *Trigonella foenum-graecum* (fenugreek), from the family Fabaceae, in Iranian folk medicine^[19-20]. On the other hand, this plant is known

to contain flavonoids^[21], alkaloids^[22], nicotinic acid^[23] and salicylate^[24].

The objective of the present study was to evaluate the effectiveness of honey alone or in combination with fenugreek seed extract in preventing the formation of gastric ulcer experimentally by ethanol-induced gastric damage in rats. Cimetidine, which is a commonly prescribed drug for increase gastric acid secretion and gastric ulcer, was used as a reference drug for comparison.

MATERIALS AND METHODS

Plant materials: *T. foenum-graecum* L. (Papilionaceae) dry seeds were collected from the local market and identified by the Plant Taxonomy Unit of our faculty. The shade-dried plant material were cleansed of extraneous matter and then ground to fine powder by using a grinder.

Preparation of aqueous and alcoholic extracts: *T. foenum-graecum* dry seeds were subject to both aqueous extraction and 95% ethanol extraction methods. Weighing 40 g of *T. foenum-graecum* seeds and mixing it with 800 mL of sterile distilled water in a conical flask using a ratio of 1:20. It was then heated and stirred on a hotplate for 3 h. After being left to cool, the residue was removed by filtration using a mesh and filter funnel. Rotary evaporator then extracted the filtered material.

Weighing 30 g of *T. foenum-graecum* seeds and mixing it with 600 mL of 95% ethanol in a conical flask using a ratio of 1:20 carried out the ethanol extraction

method. It was then covered with aluminium foil and left at room temperature (27°C) for 7 days. The residue was removed by filtration using a filter funnel and rotatory evaporator extracted the filtered material.

Both extracts were then submitted to lyophilization by a freeze-dryer, to produce powdered forms of the extracts. Lyophilization removes the solvents from the solutes and stabilizes the formulation so that it can retain satisfactory pharmacological activity during long-term storage. The freeze-dried products were stored in sterile universal bottles and refrigerated (4°C) until time of use.

Honey: The honey used in this study was pure, unprocessed, unboiled commercial honey, obtained from the Faculty of Agriculture, University Putra Malaysia, Serdang, Selangor, Malaysia..

Preparation of oral administration: Oral pre-treatment of *T. foenum-graecum* in combination with honey was prepared by mixing *T. foenum-graecum* seed extracts with honey that had been filtered, at a concentration of (10% w/v) 500 mg kg⁻¹ body weight, (100 mg mL⁻¹ honey/animal). Mixtures were left to mix homogeneously at room temperature (27°C) overnight.

Cimetidine was used as a reference drug in this experiment and was mixed with filtered honey at a concentration dose of 50- mg kg⁻¹ body weight (10 mg mL⁻¹ honey/animal).

Experimental animals: Thirty male albino *Sprague-Dawley* rats, 6-8 weeks old (180-200 g) were used in this study. The animals were obtained from the animal house of the Faculty of Medicine, University of Malaya, Malaysia. The animals were left for 48 h to acclimatize to the animal room conditions and were maintained on a standard pellet diet and tap water. The rats were randomly divided into 5 groups of 6 animals each.

Induction of gastric lesions and treatment protocol: The animals were placed individually in cages (Bollman cages) with wide-mesh wire bottoms stainless-steel cages, to limit their movements and thus prevent coprophagy. The rats were deprived of food 48 h prior to the start of the experiment, but were given access to drinking water.

Group 1 animals were orally administered each with 1 mL of distilled water (5 mL kg⁻¹) by gastric intubations as control group. Group 2 rats were orally administered each with 1 mL of honey (5 mL kg⁻¹); Group 3 rats were orally administered each with 1 mL of honey in combination with aqueous extract of *T. foenum-graecum* (5 mL kg⁻¹) and Group 4 rats were received orally each 1 mL of honey in

combination with alcoholic extract (5 mL kg⁻¹) Group 5 rats were orally administered with 1 mL honey in combination with cimetidine (5 mL kg⁻¹). After 30 minutes, 1 mL of absolute ethanol (5 mL kg⁻¹) was orally administered to all rats by gastric intubations. 15 minutes later, all rats were sacrificed by an overdose of diethyl ether and prepared for dissection.

Each rat stomach was removed and inflated with 10 mL of 10% buffered formalin solution to fix the outer layer of the stomach. Each stomach was cut open along the greater curvature, rinsed with tap water to remove stomach contents and the mucosa were examined under the dissecting microscope grossly (10 x) with a square-grid eyepiece to access the formation of ulcers (hemorrhagic lesions). The maximum length of each lesion was determined and the sum of the lengths of all lesions, in mm, for each stomach was expressed as the ulcer index, as recommended by^[25-26]. Percentage inhibition (%I) was determined by following formula.

$$(\%I) = [(UI_{\text{control}} - UI_{\text{treated}}) \div UI_{\text{control}}] \times 100^{[27]}$$

Histological examinations: The stomach tissue samples were fixed in 10% buffered formalin overnight and then processed in an automated tissue processor. Stomach tissues were embedded, sectioned by a microtome and stained with Haematoxylin and Eosin (H&E) stain. Each section was examined by light microscopy with magnification of x10, x40 and x100 (oil immersion).

Statistical Analysis: The results were expressed as mean ± standard error mean. Statistical analysis of comparison between treatments was conducted by using Student's *t*-test (SPSS version 11.5 for Windows).

RESULTS

The results of the present study are summarized in (Table 1). In control animals, oral administration of absolute ethanol solution produced characteristic lesions in the glandular portion of rat stomachs. These necrotizing agents produced severe gastric damage

Table 1: Effect of honey alone or in combination with seed extract and in combination with cimetidine on absolute ethanol-induced gastric lesions/ulcer in rats

| Treatment | Dose (mL kg ⁻¹) | No. of animals | Mean ulcer index ± S.E.M. | Inhibition % |
|-------------------------|-----------------------------|----------------|---------------------------|--------------|
| Distilled water | 5 | 6 | 65.33 + 1.45 ^a | – |
| Honey alone | 5 | 6 | 31.33 + 1.23 ^b | 52.04 |
| Aqueous extract + honey | 5 | 6 | 17 + 1.45 ^c | 73.98 |
| Ethanol extract +honey | 5 | 6 | 23.33 + 1.12 ^d | 64.29 |
| Honey +Cimetidine | 5 | 6 | 20.5 + 1.41 ^d | 68.62 |

All values are expressed as mean ± S.E.M.; means with different superscripts are significantly differences (p<0.05).

visible from the outside of the stomach as dark lines. Upon opening the stomach, elongated bands of thick, black and dark red lesions were found in the mucosa. Animals pre-treated with honey-significantly ($p < 0.05$) produced inhibition of gastric ulceration. Rats pre-treated with seed extract in combination with honey significantly ($p < 0.5$) prevent or reduced gastric ulceration. Also animals pre-treated with cimetidine in combination with honey significantly ($p < 0.05$) reduced the ulcer index.

DISCUSSION

Absolute ethanol method of inducing gastric lesions is rapid and convenient way of screening plant extracts for anti-ulcer potency and cytoprotection in macroscopically and microscopically visible lesions. Animals pre-treated with honey produced significantly ($p < 0.05$) lower ulcer index in rats compared with the control. These results are in agreement with those reported by Ali *et al.*,^[28] and Gharzouli *et al.*,^[29] about the almost total protection afforded by natural honey.

Our results also show that the aqueous seed extract of *T. foenum-graecum* is significantly ($p < 0.05$) a more potent protector of the gastric mucosa against absolute ethanol solution compared with honey alone or honey in combination with alcoholic seed extract or in combination with cimetidine. The cytoprotective effect of the seeds seemd to be not only due to the anti-secretory action but also to the effects on mucosal glycoproteins. The fenugreek seeds also prevented the rise in lipid peroxidation induced by ethanol presumably by enhancing antioxidant potential of gastric mucosa thereby lowering mucosal injury^[5].

Ethanol-induced gastric ulcer has been widely used for the experimental evaluation of anti-ulcer activity. Disturbances in gastric secretion, damage to gastric mucosa, alterations in permeability, gastric mucus depletion and free-radical production are reported to be the pathogenic effects of ethanol^[30].

The pathogenesis of ethanol-induced gastric lesions is complex. Depletion of non-protein sulfhydryls concentration^[31], modulation of nitric oxide system^[32], reduction of gastric mucosal blood flow^[33] and other factors such as the autonomic nervous system Ko *et al.*,^[34] have been suggested to be involved in the development of gastric lesions. Some evidence has been given about the combined beneficial effects of nitric oxide and non-protein sulfhydryl in gastric protection induced by honey against ethanol-induced damage^[35,36].

Absolute ethanol-induced gastric lesion formation may be due to stasis in gastric blood flow, which contributes to the development of the hemorrhagic and necrotic aspects of tissue injury^[37]. Absolute ethanol is highly corrosive to the gastric mucosa. Its pathogenic

mode of action on rat gastric mucosa involves, in addition to superficial aggressive cellular necrosis, the release of tissue-derived mediators such as histamine and leucotrine C₄. These mediators act on gastric microvasculature, triggering a series of events that result in mucosal and possibly submucosal tissue destruction^[38]. The results suggest that the seed extracts prevent the generation or necrotic action of these mediators on the gastric microvasculature.

The present study found that oral administration of honey in combination with cimetidine markedly reduced ethanol-induced gastric mucosal necrosis. Cimetidine has a direct cytoprotective effect on the gastric mucosa, cimetidine can reduce the back diffusion through gastric acid inhibition or neutralizing action, via chemical interaction, cimetidine may reduce the corrosive effect of ethanol. In the clinic, cimetidine is effective in preventing and curing gastric lesions in patients suffering from gastrointestinal ulcer^[39,40]. The inhibition of gastric secretion may play an important role in cimetidine induced prevention and healing of gastric ulcer

In the present study, pretreatment with seed extract protect the gastric mucosa against the ulcerogenic actions of ethanol. The anti-secretory activity of the seeds might be important in protecting the gastric mucosa. The cytoprotective effect was confirmed by histological examination showing prevention of mucosal hyperemia and edema^[5]. Fenugreek seeds exert their anti-ulcer activity through the flavonoids since flavonoids are reported to protect the mucosa by preventing the formation of lesions by various necrotic agents^[41].

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