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Investigation of *Trigonella Foenum Graceum* In Combination with Honey for their Antiulcer Activity

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ABSTRACT

Ulcers are the sores occurring on the lining of the digestive tract. The anti-ulcer activity of aqueous extract of seeds of *Trigonella foenum graceum* in combination with honey in rat ulcer models was performed. This was investigated in experimental rats by ethanol induced ulcer models and stress induced models. In both models the common parameter determined was ulcer index. Aqueous extract of *Trigonella foenum graceum* in combination with honey at doses of 250 and 500 mg/kg produced significant inhibition of the gastric lesions in ethanol induced gastric ulcer and stress induced ulcer. The extract (250 mg/kg & 500 mg/kg) showed significant reduction in gastric volume and ulcer index as compared to control. This present study indicates that aqueous extract of *Trigonella foenum graceum* have potential antiulcer activity in the both the models. These results may further suggest that the extract was found to possess anti-ulcerogenic as well as ulcer healing properties, which might be due to its antisecretory activity.

Keywords: *Trigonella foenum graceum*, Honey, Anti-ulcer activity, Ethanol induced ulcer model, Stress induced ulcer model.

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INTRODUCTION

Ayurveda treats not just the ailment but the whole person and emphasizes prevention of disease to avoid the need for cure [1]. Peptic ulcer is a conglomerate of heterogenous disorders which manifests itself as a break in the lining of the gastrointestinal mucosa bathed by acid and/or pepsin or NSAID ingestion is associated with erosions, petechiae type C gastritis, ulceration interference with ulcer healing, ulcer complications and injury to the small and large intestine [2]. Peptic ulcer disease is one of the most common gastrointestinal disorders, which causes a high rate of morbidity [3].

Herbal medicine deals with plants and plant extracts in treating diseases. These medicines are considered safer because of the natural ingredients with no side effects [4]. Screening plants for active drugs is still important and might provide a useful source of new anti-ulcer compounds for developing pharmaceutical drugs or alternatively as simple dietary adjuncts to existing therapies [5].

Although a number of antiulcer drugs such as H2 receptor antagonists, proton pump inhibitors and cytoprotectants are available for ulceration all these drugs have side effects and limitations [6]. Hence herbal medicines are preferred compared to allopathic drugs.

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 [7]. *Trigonella foenum graecum* L.(Leguminosae) or fenugreek is an herbal medicine used in many parts of the world [8]. Its leaves are used for their cooling properties and its seeds for their carminative, tonic and aphrodisiac effects. The seeds are reported to have nutritive properties and stimulate digestive properties. The seeds have been used to treat a number of gastro intestinal disorders [9-12].

Much work has done on the benifitial effects of fenugreek seeds in diabetic, stimulatory effects on immune function and anti-inflammatory [13,14] and hyper cholesterolemia states [15]. The human stomach contains enumerable muscles which carry out the process of digestion and convert the different forms of food into digestive fluids. These digestive fluids are known as pepsin and hydrochloric acid. These fluids make the food digest in the stomach [16]. The ulcer in the stomach might be the result of the disparity in the digestive fluids. The over production of pepsin or hydrochloric acid might cause ulcers.

MATERIALS AND METHOD

Collection of Plant material:

Trigonella foenum-graecum L. dry seeds were collected from the local market and authenticated by Botany department faculty in S.V.University, Tirupati..

Collection of Honey:

The honey used in this study was pure, unprocessed, unboiled commercial honey, obtained from the local market.

Preparation of extract:

T. foenum graecum dried seeds were subjected for extraction with water. Dried seeds (70gms) were taken into a conical flask with distilled water (1400 ml) [in a ratio of 1:20] and boiled at a temperature of 40^{o} c for 3 hours on a heating mantle. After 3 hours, the flask was cooled and then filtration was carried out. The filtrate collected was then subjected to evaporation on a heating mantle at a temperature of 100^{o} c. The residue collected was dark brown in colour.

The product obtained was kept for lyophilisation by a freeze-dryer to produce powdered forms of the extracts. Lyophilisation 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 dark brown colour bottles and refrigerated (4^{o} c) until time of use.

Animals:

A total of 60 male wistar rats were obtained from the animal facility Sugen life sciences Pvt.Ltd. and were used for the study. All rats are certified with good health at the time of receiving. Age of the animals at the start of the treatment was approximately 8-12 weeks.

Preparation of test sample:

Oral pre-treatment of *T. Foenum-graecum* in combination with honey was prepared by mixing *T. Foenum-graecum* seed extract with honey that had been filtered, at a concentration of (10% w/v). A dose of 250 mg/kg body weight it can be taken as test dose 1 and 500mg/kg can be taken as test dose 2, mixtures were mixed homogenously at a temperature of (27^{o}c) over night.

Ranitidine was used as a reference drug and was mixed with filtered honey at a concentration dose of 50 mg/kg body weight.

Ethanol induced gastric ulcer model:

Treatment protocol:

A total of 30 rats were weighed after 48 hrs of fasting with free access to water. They were divided into 5 groups (n=6 in each group) according to their body weights.

Group 1: Rats were referred as sham control here these rats were received distilled water.

Group 2: Rats were referred as vehicle control here these rats were received honey (5ml/kg body weight).

Group 3: Rats were received test dose-1(250mg ml^{-1}/kg body weight).

Group 4: Rats were received test dose-2(500mg ml^{-1}/kg body weight).

Group 5: Rats were referred as reference control here rats were received Ranitidine (50mglkg body weight).

After 15-30 min all groups of Rats were subjected to 1ml of ethanol. 15 minutes later all rats were sacrificed by decapitation and then they were 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 (10x) with a square grid eye piece to access the formation of ulcers (hemorrhagic lesions), the maximum length of each lesion was determined and the sum of the length of each lesions in mm for each stomach was expressed as the ulcer index as recommended by percentage inhibition (% I) was determined by following formula.

$$\% \text{ of ulcer protection} = \frac{\text{Control mean ulcer index - Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

Swimming stress ulcer model:

A total of 15 rats were weighed after 24hrs of fasting with free access to water. They were divided into 5 groups (n=3 in each group) according to their body weights.

Group 1: Rats were referred as sham control here these rats were received distilled water.

Group 2: Rats were referred as vehicle control here these rats were received 1ml of honey

Group 3: Rats were received test dose-1 (250 mg ml^{-1}/kg body weight).

Group 4: Rats were received test dose-2 (500 mg ml^{-1}/kg body weight).

Group 5: Rats were referred as reference control here rats were received Ranitidine (50 mg/kg body weight).

After that animals are forced to swim in a deep concrete tube at 23^0c for 5 hrs, then removed from tube after 5 hrs. Later all rats were sacrificed by decapitation and then they are prepared for dissection.

Each rat stomach was removed and inflated with 10ml 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 (10x) with a square grid eye piece to access the formation of ulcers

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$$\% \text{ of ulcer protection} = \frac{\text{Control mean ulcer index - Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

Common method for evaluation of ulcer index in every model:

The glandular portion of the stomach was opened along the greater curvature and the severity of hemorrhagic erosions in the acid secreting mucosa was assessed on a scale of 0-4.

Mean ulcer score for each animal is expressed as ulcer index.

Ulcer index = $UN+US+UP+100\times10^{-1}$.

Where,

UN = Average number of ulcers per animal.

US = severity of ulcer.

UP = percentage of animals with ulcers.

The percentage protection was calculated using the formula = $U_t/U_c\times100$.

Where,

U_t = Ulcer index of treated group.

U_c = Ulcer index of the controlled group.

Scoring of ulcer ([17])

Normal stomach	-0
Red coloration	-0.5
Spot ulcer	-1
Hemorrhagic streak	-1.5
Ulcers (< 2mm)	-2
Ulcers (>2<4) Perforation	-3
Ulcers (>4mm)	-4

RESULTS AND DISCUSSION

Ethanol-induced gastric ulcer:

In control animal, oral administration of absolute ethanol produced characteristic lesions in the glandular portion of rat stomach which appeared as elongated bands of thick, black & dark red lesions. extract of *Trigonella foenum graceum* has shown significant protection index of 63.38% and 67.84 % with the dose of 250 and 500 mg/kg respectively in comparison to control,

Ranitidine as reference standard drug was showing protection index of 71.07%(Table-2) and is represented graphically in Figure 1.

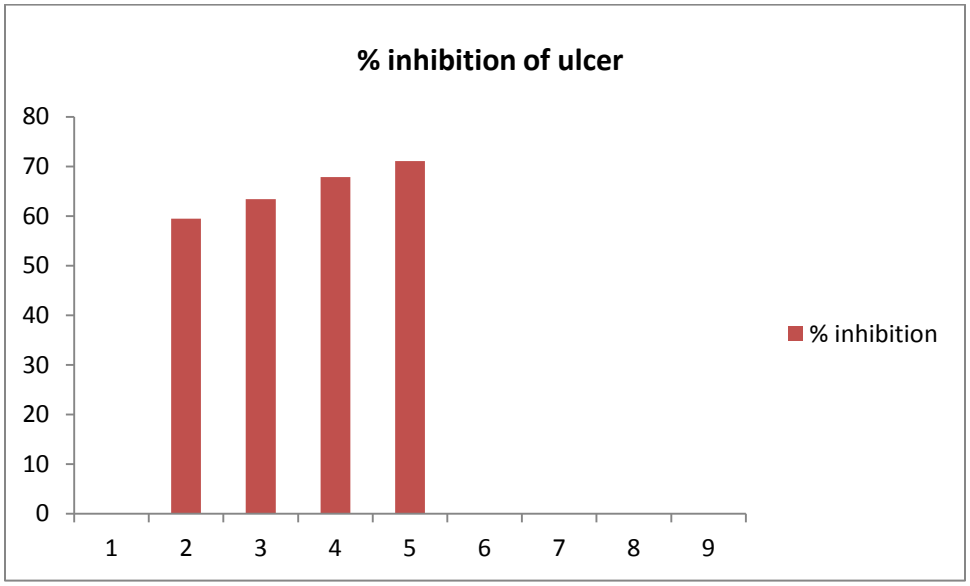

Figure 1: Graphical representation of % inhibition of ulcer in ethanol induced model

Table 1: Mean Tabular Column of Ethanol Induced Ulcer Showing Body Weight, Ulcer index, Ulcer protection.

Group	Body Weight Mean±S.D	Mean±S.D. Ulcer index	% inhibition of ulcer
Control	182.76±6.02	72.95±1.02	0.00
Honey alone	183.6±5.95	29.58±0.91	59.45
Aqueous Extract	184.16±6.11	26.38±1.58	63.38
Low dose+Honey Aqueous Extract	183.83±5.84	23.46±1.44	67.84
High dose+Honey Honey+Ranitidine	184.28±6.31	21.1±1.51	71.07

Stress- induced gastric ulcer:

In control animal stress produced characteristic lesions in the glandular portion of rat stomach which appeared as elongated bands of thick, black & dark red lesions. extract of *Trigonella foenum graceum* has shown significant protection index of 59.05% and 60.77 % with the dose of 250 and 500 mg/kg respectively in comparison to control, Ranitidine as reference standard drug was showing protection index of 68.95 %(Table-2) and is represented graphically in Figure 2.

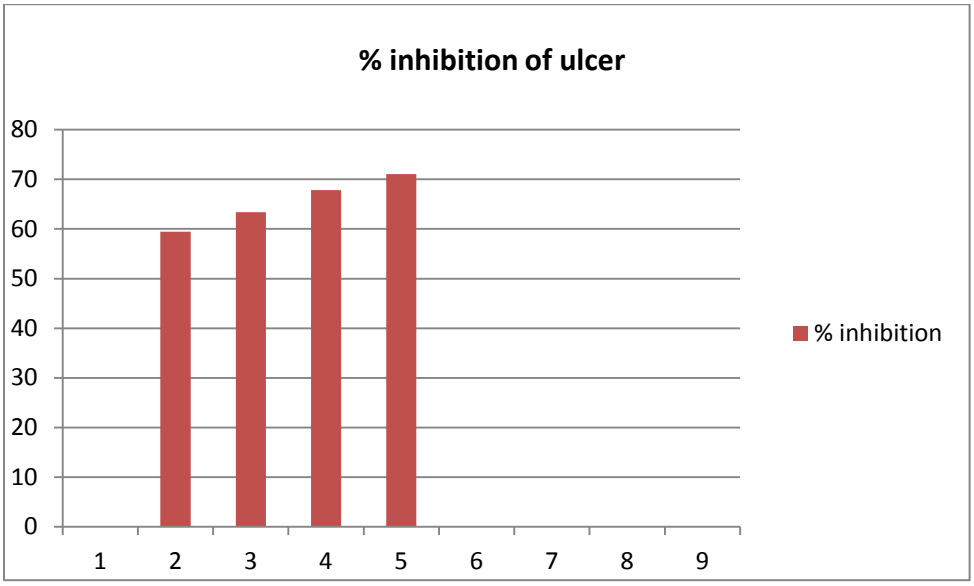

Figure 2: Graphical representation of % inhibition of ulcer in swimming stress model

Table 2: Mean Tabular Column of Stress Induced Ulcer Showing Body Weight, Ulcer index, Ulcer protection.

Group	Body Weight Mean±S.D	Mean±S.D. Ulcer index	% inhibition of ulcer
Control	184.4±5.99	70.625±1.43	0.00
Honey alone	181.25±5.61	29.81±1.54	57.79
Aqueous Extract	182.38±5.83	28.92±1.2	59.05
Low dose +Honey Aqueous Extract	179.95±5.44	27.7±0.82	60.77
High dose +Honey Honey +Ranitidine	183.40±5.93	21.92±1.32	68.95

DISCUSSION:

Different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defence mechanisms by increasing mucosal production, stabilizing the surface epithelial cells or interfering with the prostaglandin synthesis [18, 19]. Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the haemorrhage and necrotic aspects of tissue injury [20]. Alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intra cellular membrane permeability to sodium and water. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium [21].

The aqueous extract of *Trigonella foenum graecum* L or fenugreek in honey is used to study the anti-ulcer activity by ethanol induced ulcer model and stress induced ulcer model. Absolute

ethanol induces gastric lesions is rapid and it is convenient way of screening plant extracts for anti-ulcer potency and cytoprotection in macroscopically and microscopically visible lesions. Animals pretreated with honey and test drug significantly ($p<0.05$) lower the ulcer index in rats compared with the control.

The cytoprotective effect of the seeds seemed to be not only due to the anti-secretary 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 anti-oxidant potential of gastric mucosa there by lowering mucosal injury.

Stress causes both sympathetic and parasympathetic stimulation of stomach leading to local hypoxia. The ischemic condition caused an increase in the levels of H_2O_2 which in conjugation with O_2 generates OH^- ions which oxidized various cellular constituents such as proteins, membrane lipids. Lipid peroxidation causes loss of membrane fluidity and leads to production of ulcers. The present study found that the aqueous extract of *Trigonella foenum graecum* L. or fenugreek in combination with honey markedly reduced the ethanol and stress induced gastric mucosal ulcers.

CONCLUSION:

The present study involves the investigation of anti-ulcer property of aqueous extract of *Trigonella foenumgraecum* (fenugreek) seeds in combination with honey on Ethanol induced and stress induced ulcer models in rats. From the study the following conclusions are made. Pre-treatment with *Trigonella foenum graecum* seed extract in combination with honey protect the gastric mucosa against the ulcerogenic actions of ethanol and stress. In Ethanol induced model, administration of Ethanol causes Gastric damage in fasted rats by increasing gastric acid secretion. *Trigonella foenum graecum* seeds aqueous extract in higher doses (500mg/kg) markedly inhibited the ulcer index indicating the gastro protection against Ethanol. In stress induced ulcer model, there was significant inhibition of gastric acid secretion and ulcer index by the *Trigonella foenum graecum* seed extract in higher doses showed promising anti-ulcer activity more or less than that of Ranitidine The present study indicates aqueous extract of *Trigonella foenum graecum* seeds in combination with honey have potential anti-ulcer activity in the two models tested.

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