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Study the Effect of Fenugreek Seeds on Gastric Ulcer in Experimental Rats

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Abstract: The aim of this study was to investigate the effect of fenugreek seeds on aspegic induced gastric mucosal ulcer in rats. Forty-eight male albino rats of Sprague Dawley strain were classified into normal control group and five aspirin induced gastric ulcer groups which divided into non treated control (+ve) and treated groups that were drug, fenugreek seeds extract, fenugreek oil and fenugreek powder groups. The results revealed that, final weight, weight gain and food efficiency ratio, gastric glutathione, catalase and superoxide dismutase and blood hemoglobin, packed cell volume, glutathione peroxidase, superoxide dismutase and malondialdehyde were significantly decreased in control (+ ve) group compared with normal control group. The volume of gastric juice, total acidity of gastric juice, nitric oxide and malondialdehyde were significantly increased in control (+ ve) group compared with normal control group. On the other side ,the drug, fenugreek extract, fenugreek oil and fenugreek powder rat groups showed a significant increase in final weight, weight gain and food efficiency ratio; gastric glutathione, catalase and superoxide dismutase and blood hemoglobin, packed cell volume, glutathione peroxidase and superoxide dismutase but showed a significant decrease in the values of gastric ulcer index, volume of gastric juice and total acidity, gastric nitric oxide and malondialdehyde and blood malondialdehyde compared with control (+ve) group. Fenugreek extract, fenugreek oil and fenugreek powder rat groups showed a significant decrease in curative ratio percentage and the decrease in total acidity percentage compared with drug group. These observations showed that fenugreek seeds possess antiulcer potential.

Key words: Aspegic · Gastric ulcer · Fenugreek seeds

INTRODUCTION

The gastric ulcer therapy faces a major drawback in modern days due to the unpredictable side effects of the long-term uses of commercially available drugs and affects 5% of the global population [1].Medicinal plants continue to provide valuable therapeutic agents, both in modern medicine and in traditional system [2]. Fenugreek (*Trigonella foenum-graecum*) is a plant in the family fabaceae. Fenugreek is used both as an herb (the leaves) and as a spice. Leaves and sprouts are also eaten as vegetables. Fenugreek is grown wild in India, the Mediterranean and North Africa. Fenugreek seeds is the small stony from the pod of a bean-like plant, yellowish brown and angular [3]. It was used by the ancient Egyptians to combat fever. Fenugreek is most often used to increase milk supply and has also been used to treat arthritis, asthma, bronchitis, improve digestion, maintain a healthy metabolism, treat sore throat and cure acid reflux. Fenugreek also has a long history of use for the treatment of reproductive disorders, to induce labor, to treat hormonal disorders, to help with breast enlargement and to reduce menstrual pain [4].

The active constituents in fenugreek are alkaloids, lysine and L-tryptophan. It also contains steroidal saponins (diosgenin, yamogenin, tigogenin and neotigogenin) and mucilaginous fiber which is believed to be responsible for many of the beneficial effects fenugreek exhibits such as having the ability to aid the digestive process. In the East, beverages are made from the seed to ease stomach trouble. The aqueous extract and a gel fraction isolated from the seeds showed significant ulcer protective effects. It is likely that the antioxidant property of the seeds could be linked to its gastroprotective effect [5,6].

The aim of this study was to evaluate the effect of consumption of extract, oil and powder of fenugreek seeds on aspegic induced gastric mucosal ulcer in experimental rats.

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MATERIALS AND METHODS

Materials: Forty-eight male albino rats of Sprague Dawley strain were purchased from Laboratory Animal Colonies, Helwan, Egypt. The average weight was 145 ± 7g. One gram vials of aspegic drug were obtained from Ameriya Company for Pharmaceutical and Chemical industries, Cairo, Egypt. It was synthesized from salicylic acid, acetic anhydride and non corrosive 12- tungstophosphoric acid. One vial was dissolved in 10 ml distilled water and administered orally as a single dose of freshly prepared aspirin solution in dose 400 mg /kg body weight of rats to induce acute gastric ulcer according to Main and Whittle [7]. Rantidine tablets were obtained from SEDICO Pharmaceutical Company, Giza, Egypt. Each tablet contains 150 mg of ranitidine hydrochloride that inhibits gastric ulcer. Ranitak drug was dissolved in distilled water in dose 30 mg/kg of rat using a stomach tube. The basal diet was performed according to NRC [8]. Fenugreek (*Trigonella foenum-graecum* L.) oil and seeds were obtained from agriculture research center in Giza. Fenugreek seeds were cleaned, dried and finely powdered. Fenugreek seeds powder was added in basal diet as 5% while Fenugreek oil was administered at dose 1.0 ml/kg body weight of rats orally by stomach tube.

Methods

Preparation of Fenugreek Aqueous Extract: Twenty five grams of fenugreek powdered seeds were extracted with 500 ml boiling distilled water for 5 min. The heated decoction was taken and allowed to cool for 30 min, at room temperature and filtrated twice. The filtrate was lyophilized and stored in refrigerator. The rat dose of aqueous extract was 1.0 g/kg body weight orally by stomach tube.

Chemical Study: Moisture, protein, fat and ash of fenugreek seeds were determined according to the methods of the AOAC [9], while total carbohydrates were calculated by difference as following:

Carbohydrates % = 100 - (moisture % + protein % + fat % +ash %).

Flavonoids and polyphenol were detected according to the method of Geissman [10] and Singleton and Rossi [11], respectively.

Biological Study: Rats were fed the basal diet for five day before starting the experiment for adaptation then the rats were allocated into six equal groups. Normal control group fed on the basal diet only while the other five groups were administered orally a single dose of freshly prepared aspegic solution to induce gastric ulcer then classified into non treated control (+ve) and treated groups that were drug , fenugreek extract , fenugreek oil and fenugreek powder groups. Daily food intake and weekly body weight gain were calculated. Food efficiency ratio (FER) was determined according to the method of Chapman *et al.* [12]. After six weeks of the study, rats were sacrificed. Blood samples were collected for estimation of hemoglobin (HG), packed cell volume (PCV), glutathione peroxidase (GSP), superoxide dismutase (SOD) and malondialdehyde (MDA) according to Vankampen and Ziglstra [13], Mc Inory [14], Tapple [15], Winterbourn *et al.* [16] and Yagi [17], respectively.

Stomachs of each rat were legated around both openings and injected by 3 ml distilled water. The gastric juice was collected in a test tube and centrifuged at 500 rpm for 5 minutes. The gastric juice volume and total acidity of gastric juice was measured by graduated cylinder [9]. The gastric juice decrease percentage was calculated for each group according to Parmar and Desai [18] as following:

The gastric juice decrease percentage = [volume of gastric juice of control positive -volume of gastric juice of treated group/volume of gastric juice of control positive) × 100].

The decrease in total acidity of gastric juice percentage was calculated for each treated group according to Paiva *et al.* [19] as following:

Decrease in total acidity percentage = [total acidity of gastric juice of control positive group - (total acidity of gastric juice of treated group / total acidity of gastric juice of control positive group) × 100].

The stomachs were opened longitudinally, washed with saline and examined under dissecting microscope for gastric ulcer. The length of gastric ulcer was measured for each group to determine of ulcer index (UI) and the curative ratio according to Parmar and Desai [18].

The ulcerative index was calculated by severity of gastric mucosal lesions 1mm or less, 1-2 mm and more than 2 mm and graded as 1, 2 and 3 score, respectively. Then the UI was calculated by using the formula:

UI = 1 × (number of lesions of grade 1) + 2 × (number of lesions of grade 2) + 3 × (number of lesions of grade 3).

Then the overall score was divided by a factor 10, which was designated as ulcer index. The curative ratio was calculated for each group as following:

Curative ratio=(length of gastric ulcer in control positive group - length of gastric ulcer in treated group / length of gastric ulcer in control positive group) ×100.

The mucosa of the glandular stomach of each group was removed by scraping with a blunt knife and prepared 10% homogenate for some biochemical analysis. The stomach glutathione (GSH), catalase, superoxide dismutase (SOD), nitric oxide (NO) and malondialdehyde (MDA) were estimated according to Moron *et al.*[20], Aebi [21], Mc Cord and Fridovich [22], Green *et al.* [23] and Wahlefeld [24], respectively.

Statistical Analysis: Collected data were presented as mean ±SD and statistically analyzed. Student "t" test was used for significance according to Artimage and Berry [25].

RESULTS AND DISCUSSION

The chemical composition of fenugreek was illustrated in Table 1. The main constituents of fenugreek were carbohydrate (49.81 g %) and protein (27.31 g %). The value of fat was 7.11 g% but the value of ash was 3.67 g%. The value of flavonoids was 399.67 mg/100 while the value of polyphenol was 251.71 mg/100. Data in Table 2 demonstrated that final weight, weight gain and food efficiency ratio (FER) were significantly decreased at $p< 0.01$ and 0.001 in control (+ ve) group compared with normal control group. The fenugreek extract, fenugreek oil and fenugreek powder rat groups showed a significant decrease in weight gain percent and FER at $p< 0.05$ while drug group showed a significant decrease in FER at $p< 0.05$ compared with normal control group. The drug, fenugreek extract, fenugreek oil and fenugreek powder rat groups showed a significant increase in final weight, weight gain and FER compared with control (+ve) group.

The results in Table 3 indicated that volume of gastric juice was significantly increased in control (+ ve) group at $p<0.001$ and treated gastric ulcer rat groups at $p<0.05$ compared with normal control group. The value of total acidity of gastric juice was significantly increased in control (+ ve) group at $p<0.001$, fenugreek oil and fenugreek powder rat groups at $p<0.05$ compared with normal control group. The values of gastric ulcer index, volume of gastric juice and total acidity were significantly decreased in all treated rat groups compared with control (+ve) group. Fenugreek extract, fenugreek oil and fenugreek powder rat groups showed a significant decrease in curative ratio percentage and the decrease in total acidity percentage compared with drug group. The results in Table 4 indicated that the values of gastric glutathione (GSH),catalase and superoxide dismutase (SOD) were significantly decreased but nitric oxide (NO) was significantly increased in control (+ ve) group at $p< 0.001$and in all treated rat groups at $p< 0.05$ and 0.01 compared with normal control group. The value of malondialdehyde (MDA) was significantly increased in control (+ ve) group at $p<0.001$, drug and fenugreek extract rat groups at $p<0.05$ compared with normal control group. Drug, fenugreek extract, fenugreek oil and fenugreek powder rat groups showed a significant increase in GSH, catalase and SOD and a significant decrease in NO and MDA compared with control (+ve) group.

The results in Table 5 indicated that control (+ve) group showed a significant decrease in the values of hemoglobin(HG), packed cell volume (PCV), blood glutathione peroxidase (GSP), blood superoxide dismutase (SOD) and blood malondialdehyde (MDA)

Table 1: Chemical composition of fenugreek seeds.

Variable	Moisture	Protein	Fat	Ash	Carbohydrate	Flovonoids	Polyphenol
Fenugreek	12.11 g %	27.31 g %	7.11 g %	3.67 g %	49.81 g %	399.67 mg/100	251.71 mg/100

Table 2: Mean values ± SD of body weight gain, food intake and FER of the experimental rat groups.

		Gastric ulcer				
				Fenugreek		
Groups						
Variable	Normal control	Control (+ve)	Drug	Extract	Oil	Powder
Initial weight (g)	145.70±5.41^{a}	144.32±3.21^{a}	145.22±4.26^{a}	145.71±5.11^{a}	146.11±6.14^{a}	146.21±6.71^{a}
Final weight(g)	196.86±8.61^{a}	176.53±7.69^{b**}	190.83±9.11^{a}	189.20±8.14^{a}	190.32±10.11^{a}	193.32±11.21^{a}
Weight gain %	51.16±4.21^{a}	32.21±4.11^{c***}	45.61±5.21^{ab}	43.49±4.29^{b*}	44.21±5.11^{b*}	47.11±4.17^{ab}
Food intake(g/d)	0.070±0.003^{a}	0.047±0.002^{a}	0.064±0.002^{a}	0.062±0.004^{a}	0.062±0.004^{a}	0.067±0.001^{a}
FER	14.87±1.14^{a}	10.01±1.61^{c***}	13.41±1.51^{b*}	13.17±1.22^{b*}	13.11±1.43^{b*}	14.27±1.21^{b*}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each raw having different superscript (a, b, c) denote significant difference

Table 3: Mean values ± SD of some gastric parameters of the experimental rat groups.

		Gastric ulcer				
				Fenugreek		
Groups						
Variable	Normal control	Control (+ve)	Drug	Extract	Oil	Powder
Ulcer index (mm)	--	6.65±1.14 [a]	2.69±0.34[c]	3.21±0.55[c]	3.45±0.56[b]	4.11±0.48[b]
Curative ratio %	--	--	59.54±6.18[a]	51.72±5.17[b]	48.12±4.11[b]	38.19±4.71[c]
Volume of gastric juice (ml)	0.91±0.01[c]	2.63±0.31[a***]	1.14±0.11[b*]	1.11±0.02[b*]	1.20±0.13[b*]	1.35±0.12[b*]
Decrease in volume of gastric juice %	--	--	56.65±7.17 [a]	57.79±6.11 [a]	54.37±5.14[a]	48.66±5.21 [b]
Total acidity of gastric juice	0.12±[c]	0.40±0.02 [a***]	0.15±0.01[c]	0.19±0.03[c]	0.21±0.01[b*]	0.23±0.02[b*]
Decrease in gastric total acidity %	--	--	62.51±8.14 [a]	52.53±5.16 [b]	47.52±5.18[b]	42.50±4.33 [c]

Significant with control group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each raw having different superscript (a, b, c) denote significant difference

Table 4: Mean values ± SD of gastric GSH, catalase, SOD, NO and MDA of the experimental rat groups.

		Gastric ulcer				
				Fenugreek		
Groups						
Variable	Normal control	Control (+ve)	Drug	Extract	Oil	Powder
GSH (n mol/ mg protein)	31.14±3.16[a]	15.33±2.11[c***]	22.16±3.10[b*]	23.24±3.12[b*]	20.11±2.10[b*]	21.14±3.01[b*]
Catalase (µ/mg protein)	18.71±1.18[a]	9.81±1.16[c***]	13.22±1.14[b*]	14.34±1.81[b*]	13.12±1.17[b*]	15.61±1.61[b*]
SOD (µ/mg protein)	11.10±1.14[a]	5.98±1.01[c***]	7.20±1.21[b**]	7.11±1.13[b**]	8.14±1.16[b*]	8.34±1.20[b*]
NO (n mol/ mg protein)	6.88±0.55[c]	19.35±2.11[a***]	11.42±1.16[b*]	12.11±1.14[b*]	13.12±1.03[b*]	10.14±1.20[b*]
MDA (n mol/ mg tissue)	1.50±0.21[c]	3.91±0.23[a***]	2.11±0.18[b*]	2.01±0.17[b*]	1.99±0.20[bc]	1.81±0.11[bc]

Significant with control group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each raw having different superscript (a, b, c) denote significant difference

Table 5: Mean values ± SD of blood HG, PCV, GSP, SOD and MDA of the experimental rat groups.

		Gastric ulcer				
				Fenugreek		
Groups						
Variable	Normal control	Control (+ve)	Drug	Extract	Oil	Powder
H G(gm/dl)	13.16±1.11[a]	9.01±0.88[c**]	12.34±1.14[a]	12.20±1.17 [a]	11.78±1.21[ab]	12.60±1.28 [a]
PCV%	38.36±3.61[a]	25.67±3.21[b**]	35.66±3.16[a]	34.24±4.10[a]	33.14±4.11[a]	36.61±3.17[a]
GSP (µg /mg)	9.11±1.21[a]	3.14±0.37[c***]	6.81±1.21[b*]	7.11±0.99[b*]	5.99±0.52[b**]	7.23±1.03[b*]
SOD (µg /mg)	25.14±2.16[a]	9.33±1.15[d***]	19.21±1.25b[c*]	22.17±3.11[ab]	18.99±1.14b[c*]	21.61±2.03[ab]
MDA (n mol/ml cells)	1.12±0.36[c]	6.33±0.51[a***]	3.67±0.44[b*]	2.68±0.66[b*]	2.88±0.58[b*]	2.11±0.44[b*]

Significant with control group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each raw having different superscript

at p<0.01and 0.001 compared with normal control group. The value of GSP was significantly decreased but MDA was significantly increased in all treated rat groups at p<0.05 compared with normal control group. The value of SOD was significantly decreased in drug and fenugreek oil rat groups at p< 0.05 compared with normal control group. The values of HG, PCV, GSP and SOD were significantly increased but the value of MDA was significantly decreased in all treated rat groups compared with control (+ve) group.

DISCUSSION

It is clear that, aspegic drug causes gastric mucosal damage as it inhibits prostaglandin synthesis, interferes with protective mechanisms, such as mucous and bicarbonate secretion, surface epithelial hydrophobicity and mucosal blood flow. The changes permit back diffusion of acid through the breached surfaces to destroy cells, capillaries and vein-causing hemorrhagic ulcer [26, 27]. Aspegic drug induces an ulceration

model that shows severe erosion of gastric mucosa with necrotic patches, sub-mucosal edema and neutrophil infiltration [28]. Numerous publications were reported on the chemical composition of fenugreek seeds. It was reported that fenugreek seeds are a rich source of the polysaccharide galactomannan and also a source of saponins such as diosgenin, yamogenin, gitogenin, tigogenin and neotigogens. Other bioactive constituents of fenugreek include mucilage, volatile oils and alkaloids such as choline and trigonelline [29]. Fenugreek seed also contains 5.5-7.5% lipids constituting mainly of neutral lipids (85%) followed by phospholipids (10%) and glycolipids (5%). Unsaturated acids comprising mainly of linoleic (40%), linolenic (25%) and oleic (14%) acids dominate the fatty acid profile [30](Sulieman et al., 2000). Fenugreek seeds are reported to contain flavonoids. Fenugreek seeds have been found to contain protein, vitamin C, niacin and potassium and also alkaloids, lysine and L-tryptophan, as well as steroidal saponins as diosgenin, yamogenin, tigogenin and neotigogenin [31]. Fenugreek has 45-65% total carbohydrates, with 15% of galactomannan and also contains flavonoids, coumarins, saponins and more calcium, phosphorous, iron, zinc, manganese, silicon, sodium and thiamine [32]. Fenugreek is also an excellent source of selenium, an anti-radiant which helps the body utilize oxygen. The above mentioned chemical compositions of fenugreek have important role in improving the obtained nutritional results because fenugreek seeds is assumed to have a stimulating effect on the digestive process. In recent studies, it has received much scientific attention as a potential source of antioxidant property of the seeds could be linked to its gastroprotective effect. Fenugreek seeds also appear to influence free radical formation and associated with increased antioxidant enzyme activities [33]. Aqueous extract of the seeds possess significant antioxidant activity *in vitro* [34]. Replenishment of sulfhydryl levels in mucosa may contribute to the anti-ulcer activity of the seeds so increasing the levels of SH groups inhibits ulcer formation [5]. Polyphenol-rich extract of fenugreek seeds protect erythrocytes from oxidative damage. Flavonoids of fenugreek seeds exert their antiulcer activity through protection of the mucosa by preventing the formation of lesions by various necrotic agents [35].

The polysaccharide fraction of fenugreek seeds form a mucin like gel layer of galactomannan on the surface of the mucosa, or form a protecting complexes between gel and mucus as a barrier against the agents introduced into the stomach or against endogenously formed acid and pepsin in the stomach. It is speculated that the polysaccharide composition of the gel and/or the flavonoids are responsible for the gastroprotective and anti-secretory activities of the seeds [36]. The antisecretory activity of the fenugreek seeds might be important in protecting the gastric mucosa against the ulcerogenic

It is concluded that fenugreek seeds have ability to treat gastric ulcer in rats and further studied investigation are needed to exert the active principle of fenugreek seeds as pharmacological therapy.

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