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Inhibitory Effect of Aqueous Extracts of Barley and Fenugreek on Ulcer Induction in Rats

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Abstract: Aqueous extracts of barley grains (*Hordeum vulgare L.*) *and* fenugreek seeds *(Trigonella foenum-graecum L.)* were administered to a group of Albino rats (n= 42) previously subjected to aspirin at 400mg/kg b.w., for gastric ulcer induction. Two levels of intakes were used 13 ml/kg b.w. (L_1) and 26 ml /kg b.w. (L_2) of each extract as well as a mixture of both (1:1 v:v) of L_1 . Six rats were used for each treatment .Treated rats with aqueous extracts showed significant decline in ulceration severity expressed as determined by ulcer index as well as curative ratios. Moreover, curative ratios ranged between 68.13 and 95.56% in aspirin - induced gastric ulceration groups as related to the control (+ve). Results of total carbohydrate / protein ratio in mucus reduced significantly in the ulcerated group (1.47±0.02) compared to the normal control rats (5.35 ± 0.07). However, this ratio was increased significantly in rats given the aqueous extracts of barley or/ and fenugreek at all levels of intake compared to the +ve control group. When rats were given the aqueous extracts of barley or/ and fenugreek and its mixture at any level of intake, the MDA concentration was decreased significantly, with the lowest value of 3.31±0.04 nmol/g was seen when rats were given the extract as a mixture (G7). On the contrary GSH enzyme concentration in the gastric mucus was significantly reduced in the ulcerated rats (2.40±0.03 nmol/g) compared to the normal rats (3.50±0.03 nmol/g). Concerning the histopathological examination, results revealed that animals treated with barley and fenugreek showed low lesion in stomach compared to ulcerated animals. These results indicated the highly potential effect of these plants against ulcer in rats. In conclusion, this study recommends using barley grains and fenugreek seeds as potentially protective natural compound as a treatment against ulcer. Thus we suggest that barley and fenugreek may have beneficial health effects on human subjects.

Key words: Aspirin-induced ulcer · Gastro protective · Barley · Fenugreek · Extracts

INTRODUCTION

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. As it affects 5% of the global population [1], the treatment of this painful disease and its prevention has become one of the challenging problems. Hence, the search has been focused on natural products with antiulcer properties. From ancient times, plants have been proved to be powerful therapeutic agents for the treatment of various human sufferings, including atherosclerosis, cancer and ulcer. Due to the lack of side effects compared to the synthetic drugs, approximately 60% of the world's population relies almost entirely on plants for medication and natural products have long been recognized as an important source of therapeutically effective medicines Meena *et al*., [2].

Barley (*Hordeum vulgare* L.) is an annual cereal grain, considered as one of the oldest cultivated crops, as a dietary mainstay of ancient civilizations .It is also, an important diet constituent of working class people until the end of the 19th century. The health benefits and medicinal aspects of barley foods are stressed in ancient Egyptian, Greek and Romans [3]. Barley is a nutritious cereal grain that supplies consumers with many bioactive compounds that can improve their health. In many studies, eating whole grains, such as barley, has been linked to protection against atherosclerosis, ischemic stroke, diabetes, insulin resistance, obesity and cancer [4,5] .Whole grains are, also, important as dietary sources of water-soluble, fat-soluble and insoluble antioxidants.

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The long list of cereal antioxidants includes vitamin E, tocotrieonols, selenium, phenolic acids and phytic acid. These multifunctional antioxidants come in immediate-release to slow-release forms and thus are available throughout the gastrointestinal tract over a long period after being consumed [6, 7]. Recent human nutrition studies indicate encouraging health benefits associated with increaced consumption of barley as illustrated by Goldberg *et al*., [8] which contains many nutrients, including dietary fiber, antioxidants, vitamins, minerals (calcium, magnesium, potassium, phosphorus), sphingolipids and unsaturated fatty acid [9].

Fenugreek seeds *(Trigonella foenum-graecum L.)* are used in India as condiment, in Egypt, as a supplement to wheat and maize flour, for bread making, while, in Yemen it is one of the main constituents of the normal daily diet of the general population. Fenugreek leaves are consumed widely in India as a green, leafy vegetable because they are a rich source of calcium, iron, b-carotene and other vitamins [10]. Seeds are reported to have nutritive properties such as stimulating the digestive processes and treating a number of gastrointestinal disorders in the Indian system of medicine [11]. Supplements of fenugreek seeds were shown to lower serum cholesterol, triglyceride and low-density lipoprotein cholesterol in human patients and experimental models of hypercholesterolemia and hypertriglyceridemia [12]. Several intervention trials demonstrated that the antidiabetic effects of fenugreek seeds ameliorate most metabolic symptoms associated with type-1 and type-2 diabetes in both humans and relevant animal models [12, 13]. The aqueous extract and a gel fraction isolated from the seeds showed significant ulcer protective effects. The cytoprotective effect of the seeds seemed to be not only due to the anti-secretory action but also to the effects on mucosal glycoprotein [14]. However, according to the available literature, no experimental data are available regarding the gastric antiulcer potential of barley grains. The present study aimed to examine the effect of barley grains and fenugreek seeds against aspirin -induced gastric damage in rats.

MATERIALS AND METHODS

Materials: Barley grains and fenugreek seeds were purchased from the local market and authenticated by agriculture ministry in Geiza- Egypt.

Aspirin (acetylsalicylic acid) Aspegic ® was obtained from el Gomhoriya Company for pharmaceutical industries Cairo- Egypt. It was freshly prepared by dissolving one vial (1g) in 5 ml distilled water. Aspirin solution was orally given to the rats on an empty stomach at a single dose of 2 ml. (equal to 400 mg Aspirin) for gastric ulcer induction in rats.

Commercial kits were used for determining malondialdehyde (MDA), as indicator of lipid peroxidation and total glutathione (GSH) level. All chemicals were obtained from Sigma Chem. Company, Cairo, Egypt. All other chemicals were of analytical grade.

Animals: Forty two adult female Albino rats weighing 150-160 g were obtained from the animal house of the National Research Center, Cairo, Egypt. They were housed individually in stainlesteel cages under a 12 h. light- dark cycle at 20±5°C. Animals were maintained at free access to tap water and were fed a standard pelleted feed according to the National Research Council, [15] for at least 4 days before starting the experiment. Animals were fasted about 17 hour before starting the experiment for ulceration induction, then randomly assigned into seven groups of 6 animals each.

Methods:

Preparation of the Aqueous Extract: Barley grains and Fenugreek seeds were cleaned, dried and grounded into a fine powder. Boiling distilled water was added to the powder (10 g of barley/ or fenugreek /100g water). Powders were soaked in boiled water for 6 hours then filtered through a sieve and stored in dark bottles immediately.

Induction of Gastric Lesion and Treatment Protocol: Animals were randomly divided into seven group's six rats each. The first group (G1) was served as normal control without any treatment (-ve) and the rest of the animals were administered orally with aspirin (400mg/kg b.wt.) The second group (G2) was ulcerogenic control one (+ve). Group3 and 4 (G3 and G4) were treated with aqueous extract of barley at two levels of intake [13 ml/kg b.w. (L_1) and 26 ml /kg b.w. (L_2), respectively]. While the other two groups (G5andG6) were treated with aqueous extract of fenugreek at L_1 and L_2. The last group (G 7) was treated orally with a mixture of both barley and fenugreek extracts(1:1 v/v) of (L_1), one hour (h) prior aspirin administration according to the method of Puscas *et al*., [16].

Six animals were sacrificed after 4h of aspirin administration and the rest of them were used to perform the experiment. Then at the end of the study rats were

killed, stomach was removed after legend the cardiac and pyloric opening and injected with 2ml distilled water. The gastric contents were collected then measured. Gastric juice was centrifuged at 3500 rpm for 15 min and the supernatant were used for determining total acidity by titrating it with 0.01N sodium hydroxide using phenolphthalein according to A.O.A.C., [17]. Each stomach was opened along the greater curvature and washed with a saline solution and examined using magnifying lens to determine the ulcer index according to Parmar and Desai, [18].

Determination of Ulcer Index (UI): The ulcerative index was calculated by severity of gastric mucosal lesions which graded as follows:

Grade1 = less than1mm Erosions
Grade2 = 1-2mm Erosions
Grade3 = More than 2mm Erosions

The UI was calculated using this 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 of 10, which was designated as ulcer index according to Main and Whittle [19].

The curative ratio from the ulcer was calculated for the treated groups according to the method described by Akhtar and Ahamed [20] using the following equation:

$$\text{Curative Ratio} = \frac{\text{LUC-LUT}}{\text{LUC}} \times 100$$

Where:

LUC =length of ulcer in the positive control group.
LUT = length of ulcer in the treated groups.

Analysis of Gastric Mucus: The gastric mucus was analysed for glycoprotein components and antioxidants. The glandular segments from the stomach were removed and immediately homogenized in 10 percent of distilled water. Aliquots were taken from this preparation for quantitative analysis of glycoprotein components: carbohydrate concentration according to Winzler, [38], while protein level was according to Lowry *et al*., [21]. The total carbohydrate to protein ratio, (i.e. mucin activity) was determined. The activities of glutathione (GSH) was determined as described by Moron *et al*., [22] and MDA concentration was according to Ohkawa *et al*., [23].

Histopathology: Autopsy samples were taken from the stomach of the scarified rats and fixed in 10% formalin solution. All samples were sent to the ROYAL SCIENTIFIC SOCIETY, Jordan for histopathological examinations. The obtained tissue sections were collected on the glass slides and stained by hematoxylin and eosin stain for examination by the light microscope [24].

Statistical Analysis: Statistical analysis was carried out using analysis of variance (ANOVA) test with the statistical analysis system SAS, [25], using a microcomputer program. Results were expressed as mean± E at $P << 0.05$ significancy.

RESULTS

Gastric Lesions: The effect of aqueous extract of barley and fenugreek at two levels of intake (L1and L2) and its mixture on the gastric volume, total acidity, ulcer index and curative ratio in aspirin –treated rats are shown in Table (1). Rats given aspirin at 400mg /kg.bw(control +ve) showed marked increase in gastric volume(2.47±0.04 ml), total acidity (254.0± 1.46 mEq) compared with the control -ve, which showed the values of 1.10±0.03 ml and 105.17±1.74 mEq for gastric volume and total acidity, respectively. Data indicated that both parameters were significantly decreased by giving rats the aqueous extract of both barley and fenugreek at all tested levels of intake compared with the control +ve. Results in Table (1), also, showed that the ulceration severity expressed as ulcer index in aspirin treated rats (3.70 ± 0.03) was significantly decreased by consuming of aqueous extract of both barley and fenugreek at all tested levels of intake. The highest reduction in ulcer index was obtained in rats given the mixture of both tested extracts (G7) with a value of 0.20±0.02. Moreover, curative ratios in treated rats with aqueous extracts ranged between 68.13 and 95.65% in aspirin - induced gastric ulceration groups as related to the control +ve. The highest value of the curative ratio was 95.56% for rats given the mixture of both barley and fenugreek extract (G7).

Results in Table (2) showed the effect of aqueous extract of barley and fenugreek at the two levels of intake and its mixture on the concentration of carbohydrate, protein and total carbohydrate/protein of gastric mucus and gastric juices of aspirin –treated rats. Carbohydrate

Table 1: Effect of aqueous extract of barley* and fenugreek* at two levels of intake (L1and L2) and its mixture on the gastric volume, total acidity, ulcer index and curative ratio in aspirin –treated rats.

Treatments	Gastric volume (ml)	Total acidity mEq	Ulcer index	Curative Ratio %
G1 (normal –ve)	1.10±0.03 ^{f}	105.17±1.74 ^{d}	0.00±0.00 ^{f}	-
G2 (400mg aspirin +ve)	2.47±0.04 ^{a}	254.00±1.46 ^{a}	3.70±0.03 ^{a}	-
G3 (400mg aspirin+ L1 barley)	1.77±0.04 ^{b}	103.33±1.17 ^{d}	1.20±0.04 ^{b}	68.13
G4 (400mg aspirin + L2 barley)	1.58±0.04 ^{c}	83.83±1.01 ^{e}	1.10±0.03 ^{c}	71.03
G5 (400mg aspirin+ L1 fenugreek)	1.50±0.03 ^{dc}	127.67±2.1 ^{b}	0.60±0.03 ^{d}	85.16
G6 (400mg aspirin+ L2 fenugreek)	1.28±0.02 ^{e}	117.17±1.57 ^{c}	0.50±0.02 ^{e}	87.90
G7 (400mg aspirin+ mix. of L1 0f barley + fenugreek)	1.37±0.02 ^{de}	123.33±1.54 cb	0.20±0.02 ^{f}	95.56

Table 2: Effect of aqueous extract of barley * and fenugreek* at two levels of intake (L1and L2) and its mixture on the concentration of carbohydrate, protein and total carbohydrate/protein of gastric mucus and gastric juices of aspirin –treated rats

Treatments	Carbohydrate in gastric mucus (mg/g)	Carbohydrate in gastric juice (mg/ml)	Protein in gastric mucus (mg/g)	Protein in gastric juice (mg/ml)	Total Carbohydrate/ protein in mucus
G1 (normal –ve)	13.27±0.18 ^{c}	12.08±0.46 ^{c}	2.48±0.04 ^{c}	1.78±0.04 ^{d}	5.35±0.07 ^{b}
G2 (400mg aspirin+ve)	6.82±0.10 ^{d}	2.70 ±0.06 ^{e}	4.63±0.04 ^{a}	2.96±0.03 ^{a}	1.47±0.02 ^{c}
G3 (400mg aspirin+L1 barley)	19.13±0.28 ^{b}	27.06±0.44 ^{b}	3.53±0.04 ^{b}	2.45±0.04 ^{b}	5.40±0.11 ^{b}
G4 (400mg aspirin+L2 barley)	22.27±0.42 ^{a}	56.66±0.98 ^{a}	2.52±0.04 ^{c}	2.45±0.04 ^{b}	8.86±0.18 ^{a}
G5 (400mg aspirin+L1 fenugreek)	19.53±0.57 ^{b}	8.51±0.18 ^{d}	3.47±0.03 ^{b}	2.48±0.03 ^{b}	5.60±0.17 ^{b}
G6 (400mg aspirin+L2 fenugreek)	14.60±0.36 ^{c}	10.28±0.29 ^{dc}	2.60±0.03 ^{c}	2.38±0.03 ^{b}	5.62±0.17 ^{b}
G7 (400mg aspirin+mix. of L1 barley+fenugreek)	13.82±0.20 ^{c}	11.86±0.36 ^{c}	2.43±0.04 ^{c}	2.16±0.04 ^{c}	5.68±0.06 ^{b}

Aqueous extract of barley and fenugreek were given 4h. after aspirin administration L1=13 ml/kg b.w and L2 =26 ml /kg b.w.
Values are means ±SE n = 6 rats in each group. Means with different superscript letters are significantly ($p < 0.05$) differ.

Table 3: Effect of aqueous extract of barley* and fenugreek* at two levels of intake (L1and L2) and its mixture on the concentration of glutathione (GSH) and Malondialdehyde (MDA) in gastric mucus

Treatments	MDA (nmol/g)	GSH (nmol/g)
G1 (normal –ve)	10.98±0.06 ^{b}	3.50±0.03 ^{c}
G2 (400mg aspirin+ve)	16.45±0.07 ^{a}	2.40±0.03 ^{e}
G3 (400mg aspirin+L1 barley)	7.33±0.06 ^{c}	3.20±0.03 ^{d}
G4 (400mg aspirin+L2 barley)	6.20±0.04 ^{d}	3.70±0.07 $^{c\,b}$
G5 (400mg aspirin+L1 fenugreek)	5.43±0.05 ^{e}	3.20±0.04 ^{d}
G6 (400mg aspirin+L2 fenugreek)	4.38±0.05 ^{f}	3.75±0.05 ^{b}
G7 (400mg aspirin+mix. of L1 barley+fenugreek)	3.31±0.04 ^{g}	4.00±0.03 ^{a}

Aqueous extract of barley and fenugreek were given 4h. after aspirin administration
L1=13 ml/kg b.w and L2 =26 ml /kg b.w.
Values are means ±SE n = 6 rats in each group.
Means with different superscript letters are significantly ($p < 0.05$) differ.

Table 4: Effect of aqueous extract of barley * and fenugreek * at two levels of intake (L1and L2) and its mixture on the histopathological changes in stomach of rats

Treatments	Necrosis	Sloughing and desquamation of hemeia epithelialis	cells infiltration in lamina propria	submuscol edema
G1 (normal –ve)	-	-	-	-
G2 (400mg aspirin +ve)	+++	+++	++	+++
G3 (400mg aspirin+ L1 barley)	-	-	+	++
G4 (400mg aspirin+ L2 barley)	-	-	+	++
G5 (400mg aspirin+ L1 fenugreek)	-	-	-	-
G6 (400mg aspirin+ L2 fenugreek)	-	-	-	+
G7 (400mg aspirin+ mix. of L1 barley + fenugreek)	-	-	-	-

- Normal + Little effect ++ Moderate effect +++ Sever effects

levels in gastric mucus as well as in gastric juice were significantly lower in ulcerated group (6.82±0.1mg/g, 2.70±0.06 mg/ml, respectively) compared to the normal rats (13.27± 0.18 mg/g and 12.08±0.46 mg/ml). When rats were given a mixture of both extracts (G7) animals showed normal levels of carbohydrate concentration in the gastric mucus and in the gastric juice with values of 13.82±0.02 mg/g and 11.86±0.36 mg/ml, respectively. However, protein concentration in both gastric mucus and in gastric juice were significantly increased in the ulcerated group (4.36 ± 0.04 mg/g, 2.96 ± 0.03 mg/ml) compared to the normal rats (control -ve) with values of 2.48± 0.04 mg/g and 1.78±0.04 mg/ml, respectively. As a result of giving rats the extract of barley and fenugreek at the two levels of intake, protein concentration in mucus as

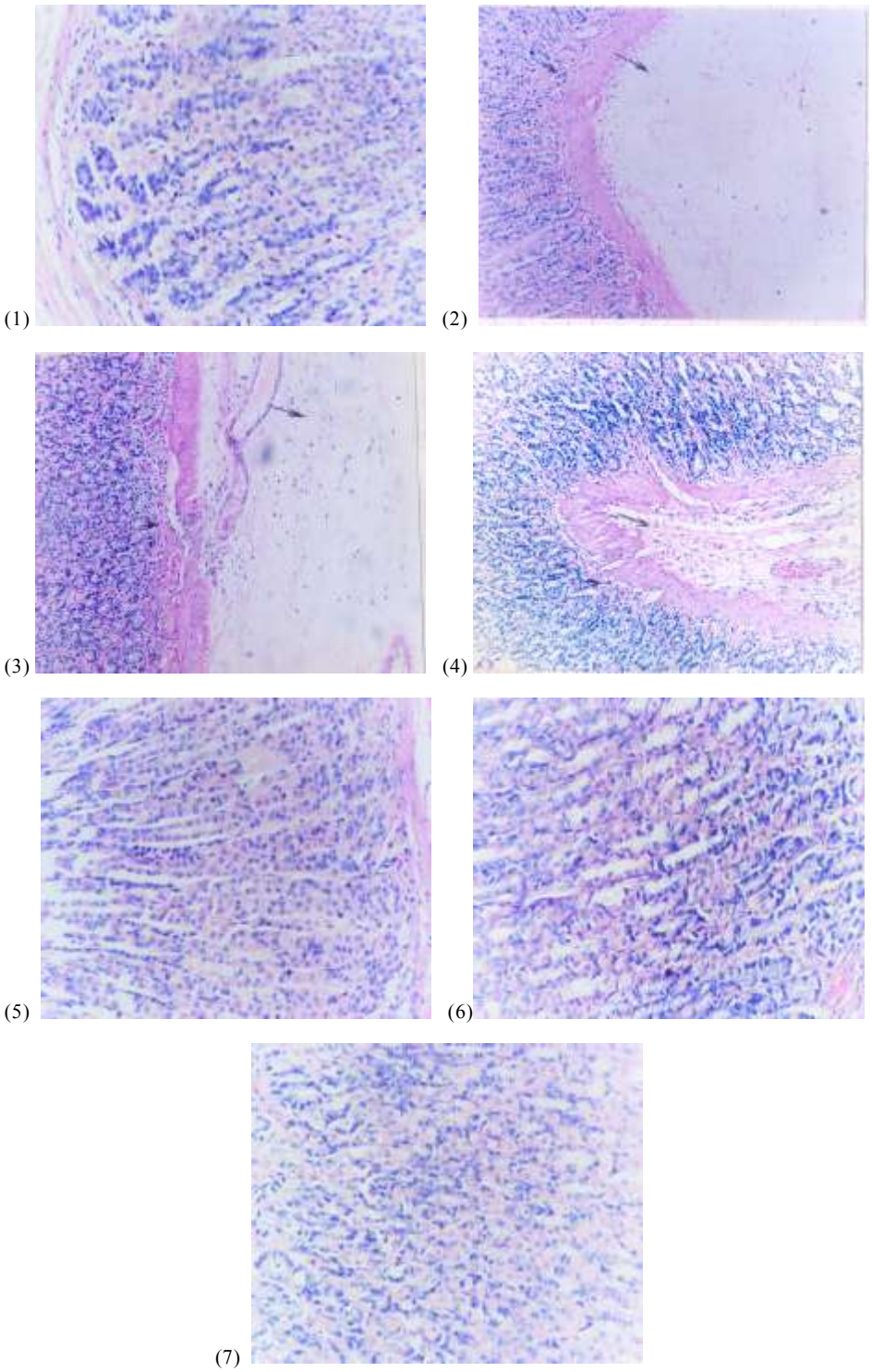

Fig. 1: Stomach of–ve (normal) rats .Showing normal histology. (H& E × 200)
Fig. 2: Stomach of +ve control .Showing focal desquamation of liming epithelium of gastric mucosa, Inflammatory cells infiltration in lamina propria and submucosal edema. (H& E × 100)
Fig. 3: Stomach of rats treated with L_1 barley. Showed a few leucocytic cells infiltration in lamina Propria associated with submucosal edema. (H&E × 100)
Fig.4: Stomach of rats treated with L2 of barley. Showed a few leucocytic cells infiltration in lamina propria associated with submucosal edema. (H&E × 100)
Fig. 5: Stomach of rats treated with L1 of fenugreek .Showing normal histology (H& E × 200)
Fig. 6: Stomach of rats treated with L2 of fenugreek. Showing normal histology (H& E × 200)
Fig. 7: Stomach of rats treated with a mixture of barley + fenugreek at L1. Showing normal histology (H& E × 200)

well as in gastric juice were reduced compared to the ulcerated group (G2) as shown in Table (2).

Data of total carbohydrate / protein ratio in mucus reduced significantly in the ulcerated group (1.47±0.02) compared to the normal control rats (5.35±0.07). However, this ratio was increased significantly in rats given the aqueous extract of barley or/ and fenugreek at all levels of intake compared to the +ve control group. Moreover, all values of the carbohydrate / protein ratios of all treated animals with the extracts reached the normal levels except for group 4 having L_2 of barely which significantly increased (8.86±0.18) vs. normal+ve rats (5.35±0.07).

Oxidative and Antioxidant: Data in Table (3) revealed that ulcerated rats (G2) had significantly the highest concentration of MDA in the gastric mucus (16.45±0.07 nmol/g) compared to the normal rats (10.98±0.06 nmol/g). When rats were given the aqueous extract of barley or/ and fenugreek and its mixture at any level of intake, the MDA concentration was decreased significantly, with the lowest value of 3.31±0.04 nmol/g was seen when rats were given the extract as a mixture (G7).On the contrary GSH enzyme concentration in the gastric mucus was significantly reduced in the ulcerated rats (2.40±0.03 nmol/g) compared to the normal rats (3.50±0.03 nmol/g). In general the treated rats with the aqueous extract of barley or/ and fenugreek and its mixture at any level of intake showed increased levels of GSH enzyme compared to the ulcerated animals.

Histopathological Results: Table (4) showed the effect of aqueous extract of barley and fenugreek at two levels of intake and its mixture on some histopathological changes or parameters in stomach of rats. Microscopically slides examination of stomach of rats from group (1) showed normal histology of gastric mucosa (Fig.1). Mean while, stomach of rats treated with aspirin (400mg/kg b.wt.) had shown focal desquamation of liming epithelium of gastric mucosa, necrosis, sloughing of lamina epithelialis of gastric mucosa, inflammatory cells infiltration in lamina propria associated with marked submuscol edema as shown in Fig.2 . However, rats treated with barley at L1and L2, showed few leucocytic cells infiltration in lamina propria associated with submucosal edema (Fig.3 and 4) .Examined stomach of rats treated with fenugreek at the two levels of intake (G5and6) showed no histopathological changes and normal gastric mucosa (Fig.5 and 6), respectively. However, group 6 only observed submucosal stomach edema .Mean while, stomach of rat from group (7) having a mixture of barley and fenugreek at level 2 revealed no histopathological changes (Fig. 7)

DISCUSSION

Results obtained in this study showed that there was a significant increase in gastric volume, total acidity of gastric juice and ulcer index values as a result of giving aspirin to the animals. These results could be supported by the findings of Ivey, [26] who stated that the non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin and indomethacin, which commonly used as pain relief agents in the treatment of rheumatoid arthritis and many other acute and chronic inflammatory conditions, can cause gastric mucosal damage. In another study by Langman *et al*., [27] who reported that aspirin causes many changes such as inhibiting prostaglandin synthesis, interferes with protective mechanisms, (i.e. mucus, bicarbonate secretion, surface epithelial hydrophobicity and mucosal blood flow). These changes permit back diffusion of acid through the breached surfaces to destroy the capillaries cells and vein causing hemorrhagic ulcer. Enhancement of leukotriene synthesis by NSAIDs results in damaging effect. Aspirin also decreases mucosal ATP synthesis and cell turnover process. The changes brought about by NSAIDs, as described above, can induce gastric damage through the generation of reactive oxygen species(ROS) and inhibiting cell proliferation as illustrated by Yoshikawa *et al*., [28] and Rao *et al*., [29] and Vaananann *et al*., [30] who support our results concerning ulcer.

Results of the present study revealed that protein concentration in both gastric juice and in mucosa as well as the level of MDA were significantly increased in the ulcerated group compared to the other treated ones. Also, data showed significant reduction in both total carbohydrate and the levels of GSH in gastric mucosa. Similar finding was recorded by Banerjee, [31] who reported that NSAIDs inhibit gastric peroxidase and increase mucosal oxidation. In another study by Pihan *et al*., [32] they reported that some radicals such as OH- causes lipid peroxidation and increases gastric lesions induced by aspirin .

In treated groups of animals with barley and fenugreek at the two levels of intake and its mixture, results showed significant decrease in gastric volume, ulcer index values and total acidity and curative ratio compared to the ulcerated group. Also, there was

significant increase in gastric mucus (carbohydrate/ protein ratio in mucosa) production and glutathione value which revealed the potent cytoprotective activity of these extracts against the inhibition of prostaglandin synthesis, enhancement of leukotriene synthesis, decrease in the mucosal ATP synthesis by aspirin .Compounds in barley that have astroprotective effects include polyunsaturated fatty acids, oligosaccharides, plant sterols, stanols, saponins and beta-glucan soluble fiber (5.5 g/100 g grains), insoluble fiber and antioxidants.

The antioxidants include vitamin E, tocotrieonols, selenium, phenolic acids and phytic acid. These multifunctional antioxidants come in immediate-release to slow-release forms and thus are available throughout the gastrointestinal tract over a long period after being consumed [33, 34, 8, 9]. In another study by Anuradha and Ravikumar, [35], who revealed that aqueous extracts of fenugreek seeds posses significant antioxidant activity in vitro which in turn could be linked to its gastroprotective effect.

Fenugreek seeds are reported to contain flavonoids (100 mg/100 g seeds) as reported by Nair and Nagar, [36]. It could be conceivable that the fenugreek seeds exert their antiulcer activity through its content from flavonoids since flavonoids are reported to protect the mucosa by preventing the formation of lesions by various necrotic agents [39, 14].

Concerning the histopathological examination, results of study showed that animals treated with barley and fenugreek showed low lesion in stomach compared to ulcerated animals by aspirin and the animals treated with a mixer of the two extracts in high level showed normal stomach tissues, these results indicated the highly potential effected of these plants against ulcer in rats.

This study recommends using barley grains and fenugreek seeds because they are potentially protective natural compound against ulcerated experimental rats. Thus we suggest that barley and fenugreek may have beneficial health effects on human subjects.

REFERENCES

1. Debashis, B., B. Kaushik, B. Mrinnalini, J. Russel, Reiter and K.B. Ranajit, 2002. Involvement of reactive oxygen species in gastric ulceration: protection by melatonin. Indian J. Exp. Biol., 40: 55-60.
2. Meena, K., S. Sobhana and M.A. Anatanandga, 1997. Bioassay for antitumor compounds in callus extracts of *Phyllanthus* spp. and Euphorbia spp. In: Pareek, L.K., Swarnakar, P.L. (Eds.), Trends in Plant Tissue.
3. Wood, Rebecca, 1988. The Whole Foods Encyclopedia. New York, NY: Prentice-Hall Press.
4. Behall, K.M., D.J. Scholfield and J. Hallfrisch, 2004. Diets containing barley significantly reduce lipids in mildly hypercholesterolemic men and women. Am. J. Clin. Nutr., 80: 1185-93.
5. Food and Drug Administration, FDA, 2002. Food labeling: health claims, soluble dietary fiber from certain foods and coronary heart disease, Intl. rule. Fed. Reg., 67: 61773-61783.
6. Tsai, C.J., M.F. Leitzmann, W.C. Willett and E.L. Giovannucci, 2004. Long-term intake of dietary fiber and decreased risk of cholecystectomy in women. Am. J. Gastroenterol. Jul., 99(7): 1364-70.
7. Webster, F.H., 2002. Whole-grain oat and oat products: In Whole- Grain Foods in Health and Disease, eds L. Marquart, J. Slavin *et al*.American Association of Cereal Chemists, St. Paul, MN, pp: 83-124.
8. Goldberg, R.J., J. Ciampa and D. Lessard, 2007. Long-term survival after heart failure, a contemporary population-based perspective j. Arch Intl. Med. Mar 12: 167(5): 490-6.
9. Murtaugh, M.A., D.R. Jacobs and B. Jacob, 2003. Epidemiological support for the protection of whole grains against diabetes. J. Proc. Nut. Soc., 62: 143-9.
10. Sharma, R.D., A. Sarkar, D.K. Hazra and B. Misra, 1996. Hypolipidaemic effect of fenugreek seeds, a chronic study in noninsulin dependent diabetic patients. J. Phytotherapy Res., 10: 332-334.
11. Puri, D., 1998. Therapeutic potentials of fenugreek. Indian J. Physiol. Pharmacol., 42: 423-424. 332-334.
12. Basch, E., 2003. Therapeutic applications of fenugreek, J. Altern Med. Rev., 8(1): 20-27.
13. Srinivasan, K., 2005. Plant foods in the management of diabetes mellitus, spices as beneficial antidiabetic food adjuncts. Intl. J. Food Sci. Nut., 56(6): 399-414.
14. Suja Pandian, R., C.V. Anuradha and P. Viswanathan, 2002. Gastroprotective effect of fenugreek seeds (*Trigonella foenum graecum*) on experimental gastric ulcer in rats, J. Ethnopharmacol., 81: 393-397.
15. National Research Council, 1995. Nutrient Requirement of Laboratory Animals, General Consideration for Feeding and Diet Formulation: National academy press.
16. Puscas, I., C. Puscas, M. Coltau, R. Pasca, J. Towers, M. M´arquez, E. Herrero, O. Fillat and J.A. Ortiz, 1997. Comparative study of the safety and efficacy of ebrotidine versus ranitidine and placebo in the prevention of piroxicaminduced gastroduodenal lesions. Journal Arzneimittelforschung 47: 568-572.

17. A.O.A.C., 1995. Official Methods of Analysis of the Association of Analytical Chemists. 16th Edition page 936, Washington DC., USA.
18. Parmar, N.S. and J.K. Desai, 1993. A review of the current methodology for the evaluation of gastric and duodenal antiulcer agents. Indian J. Pharmacol., 25: 120-135.
19. Main, L.H. and B.J.R. Whittle, 1975. Investigation of the vascodilator and antisecretory role of prostaglandin in the rat gastric mucosa by use of non-steroidal anti-inflammatory drugs. British J. Pharmacol., 53: 217-224.
20. Akhtar, A.H. and K.U. Ahamed, 1995. Anti-ulcerogenic evaluation of the methanolic extracts of some indigenous medicinal plants in Pakistan in aspirin-ulcerated rats. J. Ethanopharmacol., 46: 1-6.
21. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin-Phenol reagent. J. Biological Chem., 193: 265-275.
22. Moron, M.A., J.W. DePierre, and B. Mannervick, 1979. Levels of glutathione, glutathione reductase and glutathione-*S*-transferase activities in rat liver. J. Biochimica et Biophysica Acta, 582: 67-78.
23. Ohkawa, H., W. Ohishi and K. Yagi, 1979.Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. Anal Biochem., 95: 351-358.
24. Banchroft, J., A. Stevens and D. Turner, 1996. Theory and practice of histological techniques: Fourth Ed. Churchil Livingstone, New York, London, San Francisco, Tokyo.
25. SAS (Statistical Analysis System), 1996. User Guide Statistics .SAS Institute Inc. Editors, Cary, NC.
26. Ivey, K.J., 1988. Mechanisms of non-steroidal anti-inflammatory druginduced gastric damage.Actions of therapeutic agents. American J. Med., 84: 41.
27. Langman, M.J.S., P. Brok, C.J. Hawkey, F. Silverstein and N. Yeomans, 1991. Non-steroidal anti-inflammatory drug associated ulcer; epidemiology,causation and treatment. J. Gastroenterology and Hepatology, 6: 442-448.
28. Yoshikawa, T., Y. Naito, A. Kishi, T. Tomii, S. Iinuma, H. Ichikawa, M. Yasuda, S. Takahashi and M. Kondo, 1993. Role of active oxygen lipid peroxidation and antioxidation in the pathogenesis of gastric mucosal injury induced by indomethacin in rats. J. Gut., 34: 732-739.
29. Rao, Ch.V., K. Sairam, and R.K. Goel, 2000. Experimental evaluation of Bacopa monniera rat gastric ulceration and secretion. Ind. Journal Physiol. Pharmacol., 44: 35-41.
30. Vaananann, P.M., J.B. Medding and J.L. Wallace, 1991. Role of oxygen derived free radicals in indomethacin-induced gastric injury. American J. Physiol., 261: G470-475.
31. Banerjee, R.K., 1990. Non-steroidal anti-inflammatory drugs inhibit gastric peroxides activity. J. Biochim. Biophysica Acta pp: 1034-275.
32. Pihan, G., C. Regillo and S. Szabo, 1987. Free radicals and lipid peroxidation in ethanol- and aspirin-induced gastric mucosal injury. Digestive Diseases in Sciences, 32: 1395-1399.
33. Norbaek, R., K. Brandt and T. Kondo, 2000. Identification of flavone C-glycosides including a new flavonoid chromophore from barley leaves (*Hordeum vulgare L.*) by improved NMR techniques. J. Agri. Food Chem. May, 48(5): 1703-7.
34. Nilsson, A., Y. Granfeldt, E. Östman, T. Preston and I. Björck, 2006. Effects of GI and content of indigestible carbohydrates of cereal-based evening meals on glucose tolerance at a subsequent standardised breakfast. European J. Clin. Nutr., 60: 1092-1099.
35. Anuradha and Ravikumar, 1998. Anti lipidperoxidative activity of seeds of fenugreek (Trigonella foenum graecum). Med. Sci. Res., 26: 317-321.
36. Nair, S. and R. Nagar, 1997. Anti-oxidant flavonoids in common Indian foods. South Asian J. Prev. Cardiol., 1: 33-35.
37. Saurez, J., M.D. Herrera and E. Marhuenda, 1996. Hesperidine and neohesperidin dihydrochalcone on different experimental models of induced gastric ulcer. Phytotherapy Res., 10: 616-618.
38. Winzler, R.J., 1955. Determination of serum glycoprotein . In Glick, D.P. (Ed.) (Methods of Biochemical Analysis): Interscience Publishers Inc., New York, pp: 279-311.