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RAPID COMMUNICATION

Beneficial effects of *Foeniculum vulgare* on ethanol-induced acute gastric mucosal injury in rats

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Abstract

AIM: To examine the anti-ulcerogenic and antioxidant effects of aqueous extracts of *Foeniculum vulgare* (FVE) on ethanol-induced gastric lesions in rats.

METHODS: FVE was administered by gavage at doses of 75, 150 and 300 mg/kg, and famotidine was used at the dose of 20 mg/kg. Following a 60 min period, all the rats were given 1 mL of ethanol (80%) by gavage. One hour after the administration of ethanol, all groups were sacrificed, and the gastric ulcer index was calculated; whole blood malondialdehyde (MDA) and reduced glutathione (GSH), serum nitrate, nitrite, ascorbic acid, retinol and β-carotene levels were measured in all the groups.

RESULTS: It was found that pretreatment with FVE significantly reduced ethanol-induced gastric damage. This effect of FVE was highest and statistically significant in 300 mg/kg group compared with the control (4.18 ± 2.81 *vs* 13.15 ± 4.08, $P < 0.001$). Also, pretreatment with FVE significantly reduced the MDA levels, while significantly increased GSH, nitrite, nitrate, ascorbic acid, retinol and β-carotene levels.

CONCLUSION: FVE has clearly a protective effect against ethanol-induced gastric mucosal lesion, and this effect, at least in part, depends upon the reduction in lipid peroxidation and augmentation in the antioxidant activity.

Key words: *Foeniculum vulgare*; Ethanol; Rat; Ulcer; Antioxidant

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INTRODUCTION

Peptic ulcer is a common disorder of the gastrointestinal system and millions of people suffer from this disease in the world. The medical cost of treating peptic ulcer and its complications amounts to billions of dollars annually. The pathogenesis of peptic ulcer disease is multifactorial, including chronically using non-steroid anti- inflammatory drugs, cigarette smoking, alcohol, and reactive oxygen species (ROS). ROS are generated by cells in some physiological and pathological circumstances. Any derangement between pro-oxidants and antioxidants, in which pro-oxidants prevail is known as oxidative stress[1]. Insufficient antioxidant protection or excess production of ROS can result in this condition. ROS can react with all macromolecules, such as lipids, proteins, nucleic acids, and carbohydrates, particularly polyunsaturated fatty acids on cell membranes. After the beginning of an initial reaction with ROS, a continuing chain reaction is started and cell injury and, ultimately, cell death occur[2]. Peptic ulcer is produced by the imbalance between gastroduodenal mucosal defense mechanisms and offensive factors. Some studies have revealed that ROS and lipid peroxidation are implicated in the pathogenesis of ethanol-induced gastric lesions and gastrointestinal damage, and they attack and damage many biological molecules such as prostaglandins[3-5]. Therefore, treatment with antioxidants and free radical scavengers can decrease ethanol-induced gastric mucosal damage.

Foeniculum vulgare (FVE) is a well-known umbelliferous plant. For centuries, FVE fruits have been used as traditional herbal medicine in Europe and China. It is native to southern Europe and the Mediterranean area. The seeds of this plant have been known to be able to regulate menstruation, alleviate the symptoms of female climacteric syndrome, and increase libido[6]. FVE also

possesses emnenagague and galactagogue properties[7]. It has been reported that FVE could be used in the pediatric colic and some respiratory disorders due to its anti-spasmodic effects[8,9]. Seeds of it are used in folk remedies for treatment of dysmenorrhea. FVE (in Turkish "Rezene") is natively found in North and West regions of Turkey. It is cultivated for the herb as a spice (flavouring salads) and medicine in Turkey. Powders or tablets (0.5-1 g) of seeds, or its infusion forms (2%) are taken 2-3 times per day. As a medicinal plant, FVE has been used as an antispasmodic, carminative, diuretic, lactation stimulant, and as dressings for wounds in Turkish traditional medicine[10]. It contains 1%-3% of a volatile oil, which is composed of 50%-85% of anethole and about 20% of d-fenchone[11,12]. Other compunds present in FVE are d-a-pinene, d-a-phellandrene, dipentene, methyl chavicol, feniculun, anisaldehyde, and anisic acid[11,13].

The aim of this work was to assess the gastroprotective activity of FVE in rat models of experimentally ethanol-induced gastric lesions. In particular, we investigated the effects of aqueous extracts of FVE on gross mucosal lesions in the stomach, glutathione (GSH), nitrite, nitrate, ascorbic acid, retinol and β-carotene levels, and changes in lipid peroxidation determined by measuring malondialdehyde (MDA) levels in the blood.

MATERIALS AND METHODS

Plant material

The aerial parts of FVE were collected in June 2003 from Bursa. The plant was identified by the Department of Botany of Science and Arts Faculty, Atatürk University, Erzurum, Turkey, where a voucher specimen is kept.

Extraction and preparation of test samples

Air-dried FVE was pulverized with a blender. Obtained plant material (230 g) was mixed with boiling distilled water and stirred on the hot plate for 15 min. Subsequently, it was filtered over Whatman No.1 paper. Finally, the filtrate was frozen and lyophilized in a lyophilizator (Labconco, Freezone 1L, USA) at a 5 μmHg pressure and -50℃ (14.9 g).

Animals

Thirty-five Sprague-Dawley rats with a weight range of 190-225 g were used for the experimentation. The rats were fed with standard laboratory chow and water before the experiment. Rats were divided into 5 equal groups (n = 7) and housed in cages. Twenty-four hours before the experiment, the rats were fasted and allowed access to water *ad libitum*. The investigation was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication no. 85-23, revised 1996) and approval has been received from our institutional Animal Ethics Committee.

Chemicals

Chemicals used in this investigation, GSH, thiobarbituric acid, phosphate buffer, butylated hydroxytoluene, trichloroacetic acid, EDTA, [5,5-dithiobis-(2-nitrobenzoic acid)], phenylendiamine, sodium azide, 2,4-dinitrophe nylhydrazine, ethanol, hexane, sodium nitrite, sodium nitrate, sulfanilamide, N-(1-Naphthyl) ethylenediamine dihydrochloride and vanadium (Ⅲ) chloride were purchased from Sigma. All the other chemicals and reagents used in this study were of analytical grade.

Ulcer study

The anti-ulcerogenic effect of FVE was investigated with the ethanol-induced ulcer model. On the first day of the experiment, groups 1, 2 and 3 were administered with 75, 150 and 300 mg/kg FVE, group 4 was administered with 20 mg/kg famotidine, and group 5 was administered with saline solution. All of drugs were administered by gavage at the same volume (0.5 mL). Following a 60 min period, all the rats were given 1 mL of ethanol (80%) by gavage. One hour after the administration of ethanol, rats were injected with a high dose of ketamine (100 mg/kg), blood samples were taken by cardiac punctures, and stomachs were removed and opened along the greater curvature and washed in physiological saline solution. For measurement of the gross gastric mucosal lesions, freshly excised stomachs were laid flat and the mucosal lesions were traced on clear acetate paper. Gross mucosal lesions were recognised as hemorrhage or linear breaks (erosions) with damage to the mucosal surface. The area of stomach tissue and gross lesions were approximately calculated by planimetry using a simple magnifier. The results were translated to the term of "total ulcer area/total gastric area" and these were expressed as an ulcer index (%).

Biochemical analysis

Fasting blood samples were drawn into heparin-free tubes during routine blood sampling for biochemical analysis. After immediate centrifugation (1000 g for 10 min at 4℃), the serum was stored in polystyrene plastic tubes at -70℃ until analysis. Whole blood was collected into heparinized tubes and whole blood MDA and GSH levels were studied on the same day of admission.

Whole blood MDA (as an important indicator of lipid peroxidation) levels were measured according to a method of Jain *et al*[14]. The principle of the method was based on the spectrophotometric measurement of the color developed during the reaction of thiobarbituric acid with MDA. Concentrations of thiobarbituric acid reactive substances (TBARS) were calculated by the absorbance coefficient of malondialdehyde–thiobarbituric acid complex and expressed as nmol/mL. Whole blood GSH concentrations were also measured by the spectrophotometric method[15]. The concentrations of nitric oxide (nitrate and nitrite) were detected by the methods of Miranda *et al*[16]. Nitrite and nitrate calibration standards were prepared by diluting sodium nitrite and sodium nitrate in pure water. After loading the plate with samples (100 μL), addition of vanadium (Ⅲ) chloride (100 μL) to each well was rapidly followed by addition of the Griess reagents, sulfanilamide (50 μL) and N-(1-Naphthyl) ethylenediamine dihydrochloride (50 μL). The Griess solutions may also be premixed immediately prior to application to the plate. Nitrite mixed with Griess

Table 1 Effects of aqueous extracts of *Foe* FVE and famotidine on ethanol-induced gastric mucosal injury in rats

Groups (n = 7)	Ulcer index (%) (mean ± SD)	Inhibition (%)
Control (ethanol)	13.15 ± 4.08	-
75 mg/kg FVE + Ethanol	8.18 ± 2.66	37.8
150 mg/kg FVE + Ethanol	9.48 ± 3.78	27.9
300 mg/kg FVE + Ethanol	4.18 ± 2.81[b]	68.2
20 mg/kg Famotidine + Ethanol	8.68 ± 2.63[a]	34

[a]$P < 0.05$, [b]$P < 0.001$, *vs* ethanol.

reagents forms a chromophore from the diazotization of sulfanilamide by acidic nitrite followed by coupling with bicyclic amines, such as N-1-(naphthyl) ethylenediamine. Sample blank values were obtained by substituting diluting medium for Griess reagent. Nitrite was measured in a similar manner except that samples and nitrite standards were only exposed to Griess reagents. The absorbance at 540 nm was read to assess the total level of nitrite and nitrate in all samples[16]. Serum vitamin C (ascorbic acid) level was determined after derivatization with 2,4-dinitr ophenylhydrazine[17]. The levels of β-carotene at 425 nm and vitamin A (retinol) at 325 nm were detected after the reaction of serum: ethanol: hexane at the ratio of 1:1:3, respectively[18].

Statistical analysis

All values were expressed as mean ± SD. Statistical analyses of data were performed using a one-way analysis of variance (ANOVA) and Tukey's posttest. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Ulcer study

Ulcer indices (UI) are shown in Table 1. Per-oral administration of 80% ethanol produced multiple mucosal lesions in the rat stomach. Pre-treatment with FVE and famotidine were found to inhibit ethanol-induced gastric mucosal injury. This inhibitor effect of FVE was highest and statistically significant in the 300 mg/kg group and higher than that of famotidine group. In 75 mg/kg and 150 mg/kg of FVE groups, the inhibitor effects on ethanol-induced gastric mucosal injury were similar to famotidine group, which were not significant statistically. Famotidine also significantly inhibited ethanol-induced gastric lesions compared with the control.

Biochemical analysis

MDA levels of whole blood are shown in Table 2. The administration of ethanol increased the MDA level in whole blood. In contrast, pretreatment with FVE significantly decrased the MDA levels at doses of 150 and 300 mg/kg, compared with ethanol administered alone. Additionally, famotidine was found to prevent the rise in MDA level.

GSH level in whole blood was decreased in the ethanol-administered group. In contrast, GSH levels significantly increased at doses of 150 and 300 mg/kg FVE and in famotidine groups (Table 2). Nitrite and nitrate levels in serum were decreased in the ethanol administered group, while increased in the FVE groups. This increase was significant only in 75 and 150 mg/kg FVE groups, but not in 300 mg/kg FVE or famotidine groups (Table 2). All doses of FVE and famotidine increased the serum ascorbic acid levels, whereas only 300 mg/kg of FVE induced a significant increase (Table 3). On the other hand, serum β-carotene and retinol levels in FVE groups were higher than that of control, while the difference was significant only in the 150 mg/kg FVE group (Table 3).

Table 2 Effects of aqueous extracts of FVE and famotidine on whole blood MDA and GSH, and serum nitrite and nitrate levels (mean ± SD) in rats

Groups (n = 7)	MDA (nml/mL)	GSH (mg/dL)	Nitrite (mg/L)	Nitrate (mg/L)
Control (ethanol)	5.29 ± 0.6	44.46 ± 3.1	1.33 ± 0.7	4.55 ± 2.6
75 mg/kg FVE + Ethanol	4.51 ± 1.0	48.87 ± 2.5	2.56 ± 0.9[a]	8.81 ± 2.7[a]
150 mg/kg FVE +Ethanol	4.05 ± 0.3[a]	56.79 ± 3.3[d]	2.73 ± 0.5[a]	8.88 ± 1.9[a]
300 mg/kg FVE + Ethanol	4.12 ± 0.6[a]	51.34 ± 3.1[b]	1.64 ± 0.5	5.52 ± 1.7
20 mg/kg Famotidine + Ethanol	3.98 ± 0.7[a]	51.68 ± 3.2[b]	2.10 ± 0.8	6.82 ± 2.1

[a]$P < 0.05$, [b]$P < 0.01$, [d]$P < 0.001$, *vs* ethanol.

Table 3 Effects of aqueous extract of FVE and famotidine on serum antioxidant vitamins levels (mean ± SD) in rats

Groups (n = 7)	Ascorbic Acid (mg/dL)	β-Carotene (μg/dL)	Retinol (μg/dL)
Control (ethanol)	0.81 ± 0.2	26.09 ± 1.5	57.42 ± 3.4
75 mg/kg FVE + Ethanol	0.85 ± 0.2	27.48 ± 1.9	58.79 ± 4.6
150 mg/kg FVE + Ethanol	0.95 ± 0.2	31.13 ± 2.1[b]	68.45 ± 4.9[b]
300 mg/kg FVE + Ethanol	0.98 ± 0.2[a]	28.76 ± 1.8	63.61 ± 4.5
20 mg/kg Famotidine + Ethanol	0.97 ± 0.3	26.55 ± 1.6	58.57 ± 3.6

[a]$P < 0.05$, [b]$P < 0.01$, *vs* ethanol.

DISCUSSION

For a long time, peptic ulcer has been one of the important causes of morbidities and mortalities. Several factors such as increased vascular permeability, gastric motility and vagal activity, decreased gastric blood flow and protective prostaglandin levels play an important role in gastric ulcer pathogenesis. The treatment of peptic ulcers is still a big challenge and development of new drugs is urgent. There are a number of medicinal plants that have been shown to be effective against ulcer diseases in traditional medicine[19]. Because of the folkloric uses, these medicinal plants may be a good source for the development of potential drugs. In recent years, many efforts have been done to explore new anti-ulcer drugs from natural resources, and antiulcer activity of a variety of chemical compounds isolated from medicinal plants have been determined[20,21].

Ethanol is a commonly used ulcerogenic agent and when given by gavage to rats, it produces severe gastric hemorrhagic lesions. The mechanism of ethanol-induced gastric lesions is varied, including the depletion of gastric mucus content, damaged mucosal blood flow and mucosal cell injury. In addition, ethanol-induced gastric mucosal damage is associated with overproduction of free radicals, which lead to an increased lipid peroxidation[22]. Increase in lipid peroxide content and oxygen-derived free radicals results in marked changes in cellular levels and causes membrane damage, cell death, exfoliation and epithelial erosion. Accumulation of activated neutrophils in the gastric mucosa may be a source of free radicals. Several studies revealed that some antioxidant drugs such as melatonin and dantrolene have protective effects against ethanol-induced acute gastric injury in rats[23-25]. Results of the present study showed that all doses of FVE prevented gastric tissue damage against ethanol-induced stress, only significantly in the highest dose group. Furthermore, FVE decreased the lipid peroxidation and increased the non-enzymatic antioxidant. Antioxidative properties may, at least partially, be one of the possible mechanisms by which FVE ameliorated the ethanol-induced gastric lesions.

GSH is a well-known antioxidant, which is usually present as the most abundant low-molecular mass thiol in most organisms. It has various functions in the defense against oxidative stress and xenobiotic toxicity. It can act as an electron donor for glutathione peroxidase in animal cells, and also directly reacts with ROS. GSH is readily oxidized to glutathione disulfide (GSSG) by glutathione peroxidase, as well as by the reaction with ROS[26], which may subsequently cause the reduction in GSH levels.

Nitrate and nitrite [a marker of endogenous nitric oxide (NO) production], as a free radical, seems to be a potential antioxidant. It takes part in termination of lipid peroxidation (LPO) reactions. NO is an effective chain-breaking antioxidant in free radical-mediated LPO. It reacts rapidly with peroxyl radicals as a sacrificial chain-terminating antioxidant. The antioxidant effect of NO on LPO has been explained by terminating the radical chain reaction through the reaction of NO with lipid peroxy radical (ROO·) to form adducts by equation[27-29]. The protective effect of NO on LPO has also been shown[30,31].

$$4\ NO + 2\ ROO\cdot + H_2O \rightarrow 2\ ROONO + 2\ NO + H_2O \rightarrow RONO_2 + RONO + 2\ HNO_2$$

Aerobic organisms are protected against ROS by enzymatic antioxidant (superoxide dismutase, glutathione peroxidase and catalase) and non-enzymatic antioxidant (such as β-carotene, retinol, vitamin C and GSH) defense systems. Antioxidant vitamins, such as ascorbic acid, retinol and β-carotene play an important acute and chronic role in reducing or eliminating the oxidant damage produced by ROS[32]. In the present study, we also measured serum antioxidant vitamin capacity and, levels of all of the antioxidant vitamins were increased in FVE, but not in famotidine treated groups. Increase in vitamin levels in FVE groups may be related to vitamin content in FVE.

The preliminary phytochemical screening of FVE showed the presence of up to 8% volatile oil (including about 85% of anethole, up to 5% of estragole, and fenchone), flavonoids (rutin, quercetin and kaempferol glycosides), coumarins (bergapten, imperatorin, xanthotoxin and marmesin), sterols and sugars. These are also present in oil of FVE, d-a-pinene, d-a-phellandrene, dipentene, methyl chavicol, anisic acid, anisaldehyde and limonene[11-13,33]. Previous studies proved that anethole possesses significant antioxidant, anti-inflammatory and ulcer healing activity in experimental models[34]. Additionally, flavonoids, sterols, tannins and coumarins of some plants are also known to possess antiulcer activity[35-38]. Therefore, the presence of flavonoids content and other bioactive compounds in FVE may be associated with the ulcer preventing action.

In conclusion, our data show that FVE has an obvious gastroprotective effect and antioxidant properties. Although it is unclear about the exact mechanism underlying these actions, the effects on acute gastric lesions suggest a multifactorial mechanism, involving the antioxidant properties of FVE. FVE may be a new alternative for clinical management of gastric ulcer diseases and/or an antioxidant against oxidative stress. Further studies are required to clarify the anti-ulcer and antioxidant actions of FVE.

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