THE EFFECTS OF *HIPPOPHAE RHAMNOIDES L.* EXTRACT ON ETHANOL-INDUCED GASTRIC LESION AND GASTRIC TISSUE GLUTATHIONE LEVEL IN RATS: A COMPARATIVE STUDY WITH MELATONIN AND OMEPRAZOLE

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SUMMARY

Objective: To investigate and compare the effects of a hexanoic extract obtained from fresh fruit of *Hippophae rhamnoides L*., (HRe-1) melatonin and omeprazole on ethanol-induced gastric ulcer and on the levels of gastric tissue glutathione (GSH).

Methods: Fifty albino Wistar male rats were used. Gastric lesion was produced by ethanol. GSH levels of gastric tissue were determined according to Griffith method.

Results: Mean number of ulcer foci was 12.3+0.8 in ethanol group, 3.1+0.5 in HRe-1 and 4.3+0.67 melatonin groups. Mean ulcer area was 5.4+0.86 mm² in HRe-1, 20.5+0.72 mm² in omeprazole, 7.0+0.93 mm² in melatonin and 29.3+1.32 mm² in ethanol groups (p< 0.001: ethanol group vs other groups). Gastric tissue GSH levels of HRe-1 and melatonin groups were fairly close to the normal values. Additionally, this level was significantly reduced in omeprazole and ethanol groups. While there was no difference in terms of mean ulcer area and number of ulcer foci, between melatonin and HRe-1 groups, gastric tissue GSH levels were found significantly higher in HRe-1 than in melatonin groups.

Conclusion: HRe-1 has some benefical effects, even more potent than melatonin, on gastric tissue GSH levels and on the prevention of ethanol-induced ulcer formation in rats.

KEYWORDS   *Hippophae rhamnoides L.*   melatonin   omeprazole   ethanol   gastric lesion   glutathione

INTRODUCTION

It is known that ethanol is among many factors increasing risk of gastric ulcer formation such as stress, use of steroids and non-steroidal anti-inflammatory drugs[1,2]. Ethanol is widely used to induce experimental gastric ulcer in animals[3]. For the purpose of lesion formation, per-os administration of ethanol was utilised since it easily and rapidly penetrates into the gastric mucosa[4]. By increasing mucosal permeability and release of vasoactive products, ethanol causes vascular damage, and gastric cell necrosis which, in turn, leads to ulcer formation[4,5]. It is claimed that oxygen free radicals play a role in the pathogenesis of gastric damage caused by ethanol[3]. For the prevention of such a damage, there are some protective mechanisms in the cellular level. Endogenous glutathione (GSH) is one of the protective mecha-

nisms. GSH protects the cell against oxidative damage by interacting with oxygen free radicals. Some studies reported that GSH level is decreased in ulcerated gastric tissue of rats[2,6].

Hippophae rhamnoides L., a member of the Elaeagnaceae family, is a perennial plant which grows up to 3-10 m height and is distributed in the fields of north and east Anatolia at an altitude of 900-1850 m. Fruits of *H. rhamnoides L.* have been used extensively in traditional medicine in Turkey to treat constipation, skin wounds and influenza infections. Its fruits are orange colored, sour to taste, single-seeded and 3-7 mm in diameter[7]. They also contain carotenes (α, β, δ), vitamins C, E, riboflavin, folic acid, tannins, sugar, glycerides of palmitic, stearic and oleic acids, polyphenols and some essential amino acids (Lys, Thr, Met, Val and Ile)[8,9]. Some studies have shown

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that the extract of *H. rhamnoides L.* (HRe-1) scavenges superoxide radicals and prevents lipid peroxidation, perhaps due to the polyphenols in the extract[10]. *H. rhamnoides L.* pollen is used as an active medical remedy for gastric ulcer, burns, some skin and allergic diseases[8,11]. In our previous study, HRe-1 had been found to be protective against stress-induced gastric lesion[12].

The aim of this study was to investigate and compare the effects of a hexanoic extract obtained from fresh fruit of *H. rhamnoides L.*, melatonin and omeprazole on ethanol-induced gastric ulcer and on the levels of gastric tissue GSH.

MATERIALS AND METHODS

Plant material: The ripe fresh fruit of *H. rhamnoides L.* were collected from Erzurum-Tortum (altitude of 1600 m) in December 1997. The plant was identified by Dr. Ali Aslan in the department of Pharmaceutic Botany of Pharmacy Faculty, Atatürk University, Turkey.

Extraction and preparation of test sample: Fruits of the plant were removed from the branches and washed with tap water and dried. Fruits were crushed in a mortar and mixed. Fruit mash was placed in a glass jar and hexane was added in equal volume. 48 h later, juice was obtained from the mixture by squeezing and centrifuging at 1000 xg for 15 min; clear supernatant was removed by a drip. Hexane was evaporated from liquid by evaporator (Büchi, Rotavapor, R 110, Switzerland).

Animals and ulcer study: Anti-ulcerogenic effect of HRe-1 was investigated with the ethanol-induced ulcer model. Fifty albino Wistar male rats with a weight of 190-200 g were used for the experiment. The rats were fed with standard laboratory chow and water before the experiment. The laboratory was windowless with automatic temperature (22 ± 1 °C) and lighting controls (14 h light /10 h dark). Forty rats were divided into 4 equal groups and housed in cages. Twenty-four hours before the experiment, the rats were fasted and allowed access to water ad libitum. On the day of the experiment, group 1 received orally HRe-1 (500 mg/kg pure extract), while group 2 received omeprazole (20 mg /kg)[13] per-os. Group 3 was injected with melatonin (10 mg/kg, *i.p.*)[14] and group 4 (the control group) received only 0.5 mL of distilled water by gavage. An optimal dose of HRe-1

(500 mg/kg) for antiulcerogenic effect had been determined in our previous study[12]. Additionally, ten animals (group 5) which received none of the agent at the same conditions, were used to determine normal gastric tissue GSH levels. Following a 30-minute-period, all the animals except group 5, were given 1 mL of ethanol (50%) by gavage. One hour after the administration of ethanol, animals were sacrificed by decapitation. The stomach was removed and opened along the greater curvature and washed in physiological saline solution. For the measurement of the gross gastric lesions, the freshly excised stomach was laid flat and the mucosal lesions were traced on clear acetate paper. Gross mucosal lesions were recognised as haemorrhage or linear breaks (erosions) with damage to the mucosal surface. The area of gross lesions was approximately calculated by planimetry using a simple magnifier. After this evaluation, biochemical analysis was performed.

Biochemical analysis (GSH assay): GSH levels of gastric tissue of animals were determined in according to Griffith method[15]. 0.5 g of gastric tissue taken from greater curvature was homogenized in a dilution reagent that contains 5% Triton X-100 and 1 mM EDTA. After centrifugation at 10000 x g for 10 min at 4 °C, 400 µL from supernatant, 700 µL of 0.3 mM NADPH, 100 µL of 6 mM 5,5-dithio-bis-2-nitrobenzoic acid (DTNB) and 500 µL buffer (0.2 M sodium phosphate plus 10 mM EDTA, pH 7.5) were mixed carefully in a cuvette. Then 10 µL of glutathione reductase (E.C. 1.6.4.2, Boehringer Mannheim, 120 U/mg) was added and incubated for 10 min at room temperature. The absorbance of colour developed was detected at 412 nm (Shimadzu spectrophotometer, Japan). The reference cuvette contained the same concentrations of DTNB, NADPH and enzyme but no sample. Exogenous GSH levels was used as a standard and values were presented as (µmol/g wet weight of tissue.

Chemicals: Omeprazole was purchased from (Ilsan-Iltas, Istanbul, Turkey); melatonin, EDTA, DTNB, Triton X-100, and sodium phosphate were purchased from Sigma; glutathione reductase was purchased from Boehringer Mannheim.

Statistical analysis: Results were given as mean $\pm$ SEM. Data were analysed by Mann-Whitney U-test. A p value lower than 0.05 was regarded as statistically significant.

Table 1. The effects of *Hipophae rhamnoides L.* extract, omeprazole and melatonin on ethanol induced gastric injury.

Groups	Drugs	Ulcer foci number	p	Ulcer area (mm²)	p
1	500 mg/kg HRe-1+1 mL of ethanol (50%)	3.1 ± 0.5	< 0.001	5.4 ± 0.86	< 0.001
2	20 mg/kg omeprazole+1 mL of ethanol (50%)	9.3 ± 0.7	> 0.05	20.5 ± 0.72	< 0.001
3	10 mg/kg melatonin+1 mL of ethanol (50%)	4.3 ± 0.67	< 0.001	7.0 ± 0.93	< 0.001
4	1 mL distilled water+1 mL of ethanol (50%)	12.3 ± 0.8	-	29.3 ± 1.32	-

P- when compared to group 4; n=10 in each group.

RESULTS

The effects of HRe-1, omeprazole, and melatonin on ethanol-induced gastric ulcer (macroscopic results): There are damaged areas in the stomach of ethanol-administered rats. Lesions (of various size and shape) were distributed throughout the gastric mucosa. Hyperemia was also seen on gastric mucosa with blister on around the ulcer edge. Margins of ulcer areas were sharply demarcated. Ethanol-given group had more evident hyperemia on the gastric mucosa than groups given HRe-1, omeprazole and melatonin. As seen in the Table 1, mean number of ulcers was 12.3±0.08 in ethanol group, while it was 3.1±0.50 (p <0.001), 9.3±0.7 (p>0.05) and 4.3±0.67 (p <0.001) in HRe-1-, omeprazole-, and melatonin administered groups, respectively. Ulcer area was found as 29.3±1.32 mm² in ethanol-given group, while it was 5.4±0.86 mm² (p <0.001) in HRe-1, 20.5±0.72 mm² (p <0.001) in omeprazole and 7.0±0.93 mm² (p <0.001) in melatonin groups.
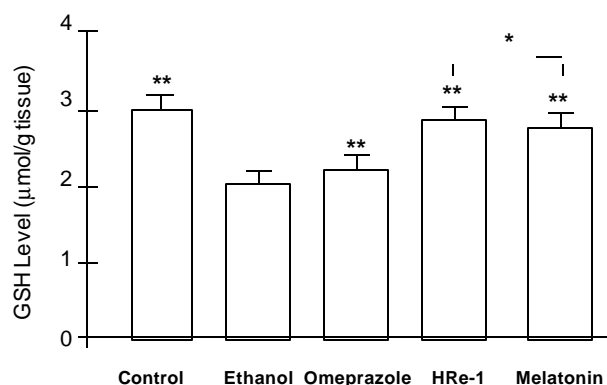
The effects of HRe-1, omeprazole and melatonin on gastric tissue glutathione levels (biochemical results): Normal GSH level of gastric tissue was 0.3±0.05 µmol/g tissue (p<0.001). When compared to the gastric tissue GSH levels of the ethanol group (1.86±0.008 µmol/g tissue), this level was found to be 2.84±0.009 µmol/g tissue in HRe-1 (p <0.001), 1.97±0.007 µmol/g tissue in omeprazole (p <0.001) and 2.79±0.007 µmol/g tissue in melatonin groups (p <0.001). The results (mean±SEM) are given in Figure 1. There was a significant difference between melatonin and HRe-1 group in terms of gastric tissue GSH level (p <0.01).

DISCUSSION

The effects of HRe-1, omeprazole and melatonin on ethanol-induced gastric injury and on the gastric tis-

Figure 1. The effects of *Hipophae rhamnoides L.* extract, (500 mg/kg), omeprazole (20 mg/kg), melatonin (10 mg/kg) and ethanol (1 mL of 50%) on gastric tissue GSH (glutathione) levels in rats. * p< 0.01 (comparison between HRe-1 and melatonin), ** p< 0.001 when compared to the ethanol groups (group 4).

sue GSH levels were investigated in this study. To induce gastric injury, 1mL of ethanol (50%) was administered to each starved rat by gavage. Starvation leads to a decrease in ATP production, glycogen storage, cytoprotective prostaglandins and GSH levels[16]. Ethanol administration to starved-rats causes mucosal damage[4,17].

As a preliminary study, in fed animals which were given 1mL of ethanol (50%), no gastric mucosal lesion was detected but only hyperemia on gastric mucosa was observed. In the light of this finding, it might be suggested that gastric mucosal barrier protects the mucosa against ulcer-inducing agents.

HRe-1 and melatonin significantly decreased the ethanol-induced gastric injury. The number of lesion area was approximately four times less in HRe-1 and three times less in melatonin administered groups

than that of ethanol group. Although omeprazole significantly prevented the expansion of lesion area, it reduced the number of lesion foci, but not in a statistically significant manner. The imbalance between aggressive and protective factors is important in peptic ulcer formation. In addition, hyperacidity is also one of the important aggressive factors for ulcerogenesis[18]. Omeprazole, as a proton pump inhibitor, powerfully decreases H^+ secretion throughout the day. Effect of omeprazole against ulcer formation was also seen in the present study. GSH levels in omeprazole-administered rats were found lower than those of the control group. HRe-1 and melatonin were more effective in reducing ulcer area than omeprazole. However, there was no statistically significant difference between the effects of HRe-1 and melatonin.

It was found that the mean gastric tissue GSH level of the ethanol-administered rats was lower than that of the control and other drug administered groups. GSH levels of HRe-1 and melatonin groups were slightly lower than those of the control group. Some studies have shown that the oxygen free radicals play a role in ethanol-induced gastric injury[3,19] and decreased GSH levels of the gastric tissue[2]. Body et al. suggested that GSH levels were found to be decreased in gastric ulcer tissue[6]. Ethanol-induced generation of free radicals reduces the cysteine which is required for GSH synthesis, which is, therefore, decreased[2]. Data from this study indicated that depletion of gastric GSH is associated with generation of gastric lesion in the rats. GSH is a tripeptide and a superoxide radical scavenger and it protects thiol protein groups required for maintaining the integrity of cell against oxidation[20].

It is suggested that the scavenging of superoxide radicals and antilipoperoxidant activity of HRe-1 mainly depend on its polyphenol content[11]. Additionally, β-carotenes, which are found in HRe-1, scavenge superoxide radical and suppress singlet oxygen[6]. Ethanol-induced gastric injury has also been prevented by melatonin due to its antioxidant effect which has been observed in some *in vivo* and *in vitro* studies[5,21]. Our findings agree with the recent studies reported by other authors who found that GSH levels were low in highly-injured gastric tissue and high in slightly-injured gastric tissues[22].

Ca^{2+} plays a role in peptic ulcer pathogenesis and calcium channel blockers, such as verapamil and nitrendipine prevent the gastric ulcer formation in rats[23,24]. Total flavones of *H. rhamnoides L.* weakened the contractile force of cultured rat myocardial cells and suppressed strophantin G-evoked guinea pig papillary muscle arrhytmias. These effects may result mainly from its inhibition of Ca^{2+} influx and its interference with the intracellular Ca^{2+} reservoir[25]. Therefore, antiulcerogenic effects of HRe-1 may partly occur *via* its antagonistic action to calcium.

In conclusion, our results indicate that the effects of HRe-1 and melatonin on ethanol-induced gastric injury might be related to their antioxidant activity and also indicate that HRe-1 has more potent activity than melatonin in terms of prevention of ethanol-induced lesion and gastric tissue GSH content.

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