Gastroprotective Activity of *Eruca sativa* Leaf Extract on Ethanol-Induced Gastric Mucosal Injury in *Rattus norvegicus*

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Abstract

*Eruca sativa* (Es), known as *jarjeer*, have been used in traditional medicine for the treatment of different diseases. First, its powder was subjected to Energy-Dispersive x-Ray Fluorescence Analysis to determine the mineral content. Then the plant was extracted by 95% of ethanol to evaluate anti-ulcerogenic activity against ethanol induced gastric ulcer. For this purpose, thirty rats were divided into 6 groups n=5. Respectively, all the animals were orally pre-treated with water, 10% Tween 20, omeprazole 20 mg/kg, 250, 500 and 750 mg/kg plant extract one hour before treating with absolute ethanol to generate gastric mucosal injury. After additional hour, rats were anaesthetized and sacrificed; the gastric content was then collected and stomachs were examined to determine mucosal lesions. The results showed that Es contains several beneficial minerals in which potassium showed to be in highest content 22.02±1.2%. Grossly and histologically, the Tween 20 group exhibited severe mucosal injury, whereas the pre-treated rats with plant extract exhibited a significant protection in dose dependent manner. Further, Es caused elevation of pH of gastric content and mucus production. Therefore, it can be concluded that Es-ethanol leaf extract exhibits an anti-ulcer activity against ethanol-induction model through maintaining the acid base balance of gastric content.

Keywords: *Eruca sativa*, Gastric ulcer, Anti-ulcer, Mineral content.

1. Introduction

Gastric ulcer is among the most serious and chronic diseases in the world. It is widely distributed among the world’s population, affecting about 10% of them. It usually occurs in the stomach and near the duodenum (Abdulla et al., 2009). Now, it has been understood that gastric ulcer occurs when there is an imbalance between acid and pepsin together with the protective barrier present in the digestive tract (Mizui et al., 1987; Shaker et al., 2010). Many factors contribute to the etiology of the gastric ulcer. The following causes can significantly decline the defenses of the mucosal barrier of the stomach, which, in turn, raises the probability of getting an ulcer and slows the healing of the existing ulcers. These factors include intake of aspirin, nonsteroidal anti-inflammatory drugs (such as ibuprofen and naproxen), alcohol, stress or emotional, caffeine, cigarette smoking and radiation therapy (Hor et al., 2011).

Although the introduction of proton-pump inhibitors to the classic anti-ulcer therapy revolutionized the treatment of peptic ulcers and other gastrointestinal disorders, there is still no complete cure for this disease. Further, it has been shown that the long-term use of these drugs leads to various adverse and side effects; relapses of the malady, ineffectiveness of different drug regimens and even resistance to drugs are emerging (Al Mofleh et al., 2007).

Nowadays, following the traditional belief, the demand for herbal plants is increasing in the developing countries (Wasman et al., 2011). Traditionally, several plants have been used to treat a variety of diseases, including gastric ulcers.

Iraqi plants have been widely used because of their relevant aromas and tastes that add variety and flavor to foodstuffs. In Erbil- Kurdistan region, many of these plants are used to treat different human diseases but there is no phyto-therapeutic evidence (Naqishbandi, 2014). Here, among Kurdish and Arabian people, Es leaf is widely used in salads. Further, Greek medicine used Es leaf in diuretic, stimulant, and in the treatment of stomach disorders and scurry (Alqasoumi et al., 2009). The seeds and the tender leaves are known in Arabian countries to increase the sexual desire and are considered to be an aphrodisiac (Alam et al., 2007). They are also used as a carminative and to alleviate abdominal discomfort and to improve digestion. Therefore, in the present study, Es leaf

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has been selected for evaluating anti-ulcer ability in laboratory rats.

2. Materials and Methods

2.1. Drug (Omeprazole)

In the present study, omeprazole (OMP) (Charcop, Kandivili, India) was used as a standard positive anti-ulcer drug; it was obtained from a local Pharmacy in Erbil city-Iraq. The drug was dissolved in10% Tween 20 and administered orally to the rats in a concentration of 20 mg/kg body (5 ml/kg) (Wasman et al., 2011).

2.2. Plant material and preparation of extract

Fresh *Eruca sativa* leaves were purchased from a local vegetable market in Erbil, and the identity of these leaves were confirmed by Dr. Abdullah Shakor, a taxonomist from the Department of Biology College of Education University of Salahaddin-Erbil-Iraq. After identifying Es, the leaf parts of the plant was cleaned, dried in the shaded place for 7-10 days and they were finely powdered using electrical blender; then they were stored in dark glass flasks to protect them from light and molds. An amount of 100g of coarsely pulverized Es leaves were placed in a glass percolator with 1000ml of 95% ethanol and were allowed to stand at room temperature for about 72 h (Wasman et al., 2011). After 3 days, the mixture was filtered using a fine muslin cloth followed by filter paper (Whatman No. 1) and distilled under a reduced pressure in a rotary evaporator (RE 200B/UK) (Donald et al.,1982). The *Eruca sativa* ethanolic leaf extract (ESELE) was then dissolved in Tween 20 (10% v/v) and administered orally to rats in concentrations of different doses (Rouhollahi et al., 2014).

2.3. Determination of Mineral

Energy-Dispersive X-Ray Fluorescence Analyser (CIT-3000 MP)(Sichuan, China) was used to determine the mineral content of Es. For this reason, the amount of 100gm of Es dried powder was weighed and subjected to the (CIT-3000 MP); the amount of each element represents the quantity in 100gm of plant material. This experiment was applied in triple and then the mean was calculated (Bozkalfa et al., 2011).

2.4. Experimental Animals

Healthy thirty adult male rats were obtained from the Experimental Animal House, College of Medicine/Haweler Medical University. The animals were kept at room temperature in humidity rooms on a standard light/dark cycle (12 h light; 12 h dark cycle) at (22±3°C). Rats weighing between 150g - 200g were placed individually in separate plastic cages (56 x 39 x 19), bedded with wooden chips in the animal house of Biology Department /College of Education /Salahadin University-Erbil. The rats were fed with standard rat diet chow and tap water. They were kept under observation for about two weeks before the initiation of the experiment. All the procedures described were reviewed and approved by the Institutional Animal Ethical Committee. Throughout the experiments, all animals received human care according to the criteria outlined in the “Guide for the Care and Use of laboratory Animals,” prepared by the National Academy of Sciences and published by the national Institute of health.

2.5. Antiulcer Experiment

The ethanol ulcer induction experiment of the present study was adopted depending on the method described in previous studies (Garg et al., 1993; Mahmood et al., 2010).

*Rattus norvegicus* rats (150–200 g) were deprived of food for 48 h before the experiment was conducted in order for the stomach to be empty, but they were allowed free access to drink water up until 2 h before starting the experiment (Mahmood et al., 2010). All rats were treated by orogastric intubations. The animals were divided randomly into six groups, each consisting of five rats; they were treated as shown in Table1.

Table 1. Design of antiulcer experiments

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group(1) Normal</td>
<td>Water</td>
<td>-</td>
</tr>
<tr>
<td>Group(2) (negative control group)</td>
<td>Tween 20 10% v/v,</td>
<td>5 mL/kg</td>
</tr>
<tr>
<td>Group(3) (positive control group)</td>
<td>Omeprazole 20 mg/kg,</td>
<td>5 mL/kg</td>
</tr>
<tr>
<td>Group(4) first dose</td>
<td>ESELE 250 mg/kg,</td>
<td>5 ml/kg</td>
</tr>
<tr>
<td>Group(5) second dose</td>
<td>ESELE500mg/kg,</td>
<td>5 ml/kg</td>
</tr>
<tr>
<td>Group(6) third dose</td>
<td>ESELE750mg/kg,</td>
<td>5 ml/kg</td>
</tr>
</tbody>
</table>

2.5.1. Gastric Ulcer-Induction by Ethanol and Tissue Sample Collection

The rats were starved for 48 h before the experiment, but they were allowed free access to drinking water up till 2 h before the experiment. Gastric ulcer in *Rattus norvegicus* was induced by orogastric intubation of absolute ethanol (5 ml/kg) (Alrdahe et al., 2010). All animals were anaesthetized by intraperitoneal injection with ketamine (100 mg/ml) and xylazine (100mg/ml) in a ratio 4:1 (v/v) (HIKMA pharmaceuticals, Amman-Jordan). The animals were sacrificed and their stomachs were tied from up and down to preserve the gastric juice for measuring the gastric acid; then the stomachs were excised and fixed in formalin 10% for histological examination.

2.5.2. Measurement of Acid in Gastric Juice

Each stomach was opened along the greater curvature. Samples of gastric contents were analyzed for hydrogen ion concentration by pH-meter using 3540 pH Conductivity Meter (JENWAY-Japan).

2.5.3. Measurement of Mucus Production

The gastric mucus production was measured in all the experimental rats that were subjected to absolute ethanol-induced gastric mucosal injury. The gastric mucosa of each rat was gently scraped using a glass slide and the mucus obtained was weighed using a precision electronic balance (Tan et al., 2002).
2.5.4. Histological Preparation

A histological examination was performed after the assessment of ulcer lesion. The stomachs were fixed in 10% of buffered formalin solution. Tissue processing (dehydration, cleaning and infiltration) was done automatically using automated tissue processor (Leica TP1020). Then, the tissues were embedded in paraffin wax using Leedo HISTOEMBEDDER. The embedded tissues were sectioned with microtome to produce 5 μm paraffin wax tissue sections. Then, the sections were stained with haematoxylin and eosin followed by mounting with DPX mounting media. Next, the mounted sections were evaluated for microscopic examination using light microscope (AmScope microscope eyepiece camera. China).

2.6. Statistical Analysis

All data were analyzed by Statistical Package Social Science (SPSS) version 17.0. One-way ANOVA is used to show the mean differences between all samples (* \( p \leq 0.05 \)).

3. Results

3.1. Determination of Mineral

In this experiment, different minerals have been detected in *Eruca sativa* leaf powder using Energy-Dispersive X-Ray Fluorescence Analyser, as shown in Table (2). The results show that potassium, sulphur and calcium are in large quantity while cerium, iodine, phosphorus, bismuth and selenium are relatively low compared to the other elements. Interestingly, the potassium was detected in a large percentage: 22.02±1.2%.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Eruca sativa Leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium Ca</td>
<td>5.23± 0.9</td>
</tr>
<tr>
<td>Potassium K</td>
<td>22.02±1.2</td>
</tr>
<tr>
<td>Sulfur S</td>
<td>6.58±0.7</td>
</tr>
<tr>
<td>Cerium Ce</td>
<td>0.0060±0.1</td>
</tr>
<tr>
<td>Minerals (%)</td>
<td></td>
</tr>
<tr>
<td>Iodine I</td>
<td>0.001±0.3</td>
</tr>
<tr>
<td>Phosphorus P</td>
<td>0.0017±0.4</td>
</tr>
<tr>
<td>Bismuth Bi</td>
<td>0.0036±0.3</td>
</tr>
<tr>
<td>Selenium Se</td>
<td>0.0024±0.6</td>
</tr>
</tbody>
</table>

This experiment has been applied in triple and then the mean±SE was calculated

3.2. Gross Evaluation of Gastric Lesions

Results showed that the rats pre-treated with *Eruca sativa* ethanolic leaves extract (ESELE) had significantly reduced the areas of gastric ulcer formation compared to the rats pre-treated with only 10% Tween 20 (ulcer control group). As shown in Figure 1, the rats pre-treated with 10% Tween 20 showed severe damage and injuries of gastric mucosa, as shown in Figure (1B). The rats pre-treated with plant extract significantly suppressed the formation of the mucosal injuries but some folds were still noticed in the rats pre-treated with 250 and 500 mg/kg (Figure 1D&E). On the other hand, for the rats pre-treated with750 mg/kg of ESELE, a complete protection of gastric mucosa was observed with the flattening of gastric mucous wall as in (Figure 1F).

3.3. pH of Gastric Content and Mucus Production

The effect of ESELE on gastric acidity and mucus production in the ethanol-induced gastric lesion model is shown in Table (3). The acidity of gastric content significantly \((P \leq 0.05)\) decreased in experimental animals pre-treated with 500 and 750 mg/kg of ESELE and the omeprazole group compared with that of the ulcer control group. While rats pre-treated with 250 mg/kg of ESELE did not show any effect in the pH level of gastric juice and mucus production, as shown in Table 3, that there is no significant differences \((P \leq 0.05)\) compared with the negative control group. Rats pre-treated with 250 mg/kg of ESELE did not show any effect in the pH level of gastric juice and mucus production, as shown in Table 3, that there are no
significant differences (P≤ 0.05) compared with the negative control group.

Table 3. Effect of ESELE on pH of gastric content and mucus in rats.

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Pre-treatment (5 ml/kg dose)</th>
<th>pH of gastric content</th>
<th>Mucus content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>6.68 ±0.2*</td>
<td>0.67±1.1*</td>
</tr>
<tr>
<td>2</td>
<td>10% Tween 20 (Ulcer control)</td>
<td>3.6 ± 0.10**</td>
<td>0.31±0.8</td>
</tr>
<tr>
<td>3</td>
<td>Omeprazole (20 mg/kg)</td>
<td>6.84 ± 0.17*</td>
<td>0.62±0.9*</td>
</tr>
<tr>
<td>4</td>
<td>LD ESELE (250 mg/kg)</td>
<td>4.3 ± 0.23**</td>
<td>0.37±1.9</td>
</tr>
<tr>
<td>5</td>
<td>MD ESELE (500 mg/kg)</td>
<td>6.9 ± 0.20*</td>
<td>0.55±0.5*</td>
</tr>
<tr>
<td>6</td>
<td>HD ESELE (750 mg/kg)</td>
<td>7.2 ± 0.4*</td>
<td>0.60±1.7*</td>
</tr>
</tbody>
</table>

All data expressed in mean ± SEM. * Significant difference (p<0.05) with 10% Tween 20 (Ulcer control), ** Significant difference (p<0.05) with omeprazole (positive control).

4. Discussion

Normally, there is a balance between the protective factors (e.g., mucus, bicarbonate, prostaglandins, nitric oxide and normal blood flow) and aggressive factors (e.g., acid plus pepsin, active oxidants, leukotrienes, endothelins, bile or exogenous factors including non-steroidal anti-inflammatory drugs and ethanol). Gastric ulcer develops when the aggressive factors overcome the protective mechanisms (Borrelli and Izzo, 2000).

It is known that gastric lesions, produced by ethanol administration, appear as multiple-hemorrhagic red bands of different size along the glandular stomach. Absolute ethanol is commonly used for inducing ulcer in experimental rats and lead to intense gastric mucosal damage (Abdulla et al., 2010). Studies suggest that the ethanol damage to the gastrointestinal mucosa starts with microvascular injury, namely disruption of the vascular endothelium resulting in increased vascular permeability, edema formation and epithelial lifting (Szabo et al., 1995). Ethanol produces necrotic lesions in the gastric mucosa by its direct toxic effect, reducing the secretion of bicarbonates and production of mucus (Marhuenda et al., 1993). The exposure to ethanol increases the extension of the cellular damage in a dose-dependent way (Mutoh et al., 1990).

In the present study, we observed a flattening in the mucosal wall, which suggests that the anti-ulcer effect of ESELE might be due to a decrease in the gastric motility. It is reported that the changes in the gastric motility may play a role in the development and prevention of experimental gastric lesions (Garrick et al., 1986; Takeuchi et al., 1988; Abdulla et al., 2010). The relaxation of the circular muscles may protect the gastric mucosa through flattening the folds. This increases the mucosal area exposed to necrotizing agents and reduce the volume of the gastric irritants on rugal crest (Takeuchi and Nobuhara, 1985). Rats treated with ESELE (250mg/kg, 500mg/kg and 750mg/kg) and those treated with omeprazole displayed a better protection of their gastric mucosa as seen by the reduction of the ulcerated areas. The reduced submucosal edema and the inflammatory reactions were also observed in these groups.

The results of this study showed that the ESELE possesses significant anti-secretory, anti-ulcer and cytoprotective properties in rats. Pre-treatment with ESELE produced a dose-dependent decrease in the gastric acidity and an increase in mucus content. The anti-ulcerogenic activity of the extract was also confirmed histologically. Histology studies confirmed the efficacy of ESELE supplementation in preventing ethanol-induced hemorrhage and necrosis in the superficial layer of the gastric mucosa. The cytoprotective effect of the extracts could be partially due to their flavonoid content and to
their reactive oxygen species scavenging property (Sanchez et al., 2001).

Since ESELE markedly inhibited a gastric acid secretion and ruminal ulcers in ethanol induced rats, this observed effect could be related, at least in part, to the ability of ESELE to reduce gastric acid secretion. It is now accepted that the gastric acid secretion plays an important role in the progression from an erosive mucus layer to a gastric lesion. On the other hand, substances, which have the ability to suppress gastric acid secretion, such as proton pump inhibitors (Omepazole) and histamine H2-receptor antagonists, are believed to accelerate the healing process of the gastric lesions or inhibit the formation of mucosal injury (Brzozowski et al., 2000).

The preliminary phytochemical screening of Es revealed the presence of flavonoids, steroids and/or triterpenes. Moreover, quercetin and its derivatives were also reported in Es leaves. Previous studies have shown that flavonoids may be related to the anti-ulcer activity (Hiruma-Lima et al., 2006), and play a major role in the mechanism of gastro-protection through the rising pH of gastric juice (Havsteen, 2002; La Casa et al., 2000).

Elements play a crucial role in the medicinal value of a plant, in health and in curing disease. They play a nutritive, catalytic and balancing function in plants. Plants take them from the ground and incorporate them into organic compounds that we consume through eating either the plants or the animals that eat them (Joyo et al., 1997). In the present study, K⁺, S and Ca²⁺ were detected in a large quantity while Ce, I, P, Bi and Se are relatively low compared to the other elements. The K⁺ is found in a large percentage (22.02%). Our findings are in accordance with the study of Bozokalfa et al. (2011) who showed that the leaves of *Eruca sativa* contain a large amount of important mineral elements for human nutrition, particularly K⁺, Ca²⁺ and P concentrations. Due to the deficiency of these minerals in human diet, most of these minerals are often taken as supplements for their important role in human health (Agarwal et al., 2011). Some of these elements are directly related to the anti-ulcer ability, as it was reported previously by Kim et al. (2012), who demonstrated that selenium inhibits the formation of ethanol-induced gastric mucosal lesions through the prevention of lipid peroxidation and the activation of enzymatic radical scavenging. Chai (2011) also reported that compounds that contain bismuth are often used in the three-drug treatment programs of gastric ulcer; they destroy the cell walls of *Helicobacter pylori* bacteria.

5. Conclusion

In conclusion, *Eruca sativa* leaf extracts could significantly protect the gastric mucosa against ethanol-induced injury. Such protection was shown to be dose-dependent as ascertained by the reduction of the ulcer areas in the gastric wall as well as by the reduction or the inhibition of edema and the leucocytes infiltration of submucosal layers. Particularly at a dose of 750 mg/kg leaf extract, this protection could be due to the balance between acid-base production in stomach and the mineral content of the plant itself.

Further studies are required to determine the phytochemical compounds responsible for the mechanism of antulcer of *Eruca sativa* leaf extracts.

Acknowledgment

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References


