ANTI-ULCER ACTIVITY OF OREGANO (Origanum syriacum L.) AGAINST GASTRIC ULCER IN RATS

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ABSTRACT

Ethanol extract of oregano (Origanum syriacum L.) and oregano volatile oil were evaluated for their antiulcer activity in Wistar albino rats (150-170 g) by oral administration of indomethacin suspension. The antiulcer activity was assessed by determining and comparing the ulcer index in the test drug groups of rats with that of the vehicle control and standard omeprazole. The volatile oil and ethanol extracts showed significant reduction of ulcers in a dose-dependent manner. The parameters taken to assess antiulcer activity were volume of gastric juice, total acidity, and ulcer index. The extracts and volatile oil significantly decreased the gastric secretion, total acidity on gastric ulcers in the indomethacin-induced rats, and the effects were compared with omeprazole.

KEYWORDS: Oregano, anti-ulcer

1 INTRODUCTION

Traditional medicinal practitioners have claimed for centuries that extracts from plants can be effectively used for the evaluation of different type of ulcers. Except for the use of appropriate vaccine for the treatment ulcers caused by infection, some treatments are available today to cure ulceration. It is not surprising that a considerable interest has been taken by researchers to examine these numbers of traditional plant remedies, used for treating ulcers [1].

Oregano is an annual, perennial and shrubby herb that is native of the Mediterranean, Euro-Siberian and Irano-Siberian regions. Oregano plants are widely used in agriculture as well as in pharmaceutical and cosmetic industries, but also used as a culinary herb, and flavoring substance of food products, alcoholic beverages and perfumery products for their spicy fragrance [2].

The cause of ulceration in patients is mainly due to hyper-secretion of gastric juice, and also due to hyper-secretion of pepsin. An ulcer is basically an inflamed break in the skin, or the mucus membrane lining the alimentary tract. Ulceration occurs when there is a disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance. About 19 out of 20 peptic ulcers are duodenal. Gastric ulcers, found in the stomach wall, are less common. The gastric mucosa is continuously exposed to potentially injurious agents, such as acids, pepsin, bile acids, food ingredients, bacterial products (Helicobacter pylori), and drugs. These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow, and gastric motility [3]. Some previous studies have reported antioxidant and antimicrobial activities of oregano extracts in the inhibition of Helicobacter pylori growth [4].

The worldwide market of oregano has increased with exceptional examples, such as USA where the procapita consumption has augmented over 3800% between the years 1940 to 1985 [5], but European countries importation as well. In this market context, oregano is the most widespread species [6]. The objective of study was to evaluate the effectiveness of leaf extract in preventing the formation of gastric ulcer by indomethacin-induced gastric damage in rats.

2 MATERIAL AND METHODS

2.1 Plant material

Samples of oregano (Origanum syriacum L.), were collected at harvest period from the area Sinai, Egypt in September, 2010, and identified by Hassna Ahmed. Specimens were deposited in the Herbarium of the Faculty of Science (Giza: 12613), Cairo University.

2.2 Preparation of ethanol extract

Oregano powder was extracted by dipping into 80% ethanol, and after 72 h under refrigerated conditions (4 °C), the mixtures were filtered. The filtrate was evaporated under vacuum, and percentage yield (w/w) of alcoholic extract was determined.

2.3 Preparation of volatile oil

50 g of the aerial plant leaves were hydro-distilled for 3 h in an all-glass Clevenger apparatus [7]. Heat was supplied to the heating mantle and the essential oil was ex-
tracted with 4 L of water for 3 h until no more essential oil was recovered.

### 2.4 Experimental animals

Albino Wistar strain rats weighing 150-170 g were obtained from animal house in Food Technology Research Institute. They were maintained under well-ventilated room temperature with natural day/night cycle, in polypropylene cages. Albino rats were fed on basal diet (70% corn starch, 10% corn oil, 10% casein, 5% fiber, 4% minerals, and 1% vitamins) [8], and tap water throughout the experimental period. Animals were housed for one week prior to the experiments to adapt them to laboratory conditions. The animals were randomly distributed into 6 different groups with 6 animals in each group. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC).

### 2.5 Administering drugs

Group 1 was control (vehicle), group 2 was treated with ethanol extract (125 mg/kg), group 3 (ethanol extract 250 mg/kg), group 4 (volatile oil 125 mg/kg), group 5 (volatile oil 250 mg/kg), and group 6 with standard drug (omeprazole 20 mg/kg). With a gavage needle attached to the filled 3-ml syringe, drugs were administered orally for 14 days. Similarly, test compound at different concentrations and vehicle control were fed to appropriate animals. Each animal was checked twice daily for discomfort, diarrhea, lethargy or other general signs of illness [9].

### 2.6 Study of antulcer activity using indomethacin-induced ulcers

Animals were treated with respective doses for 14 days as mentioned in a previous model. After 14-days treatment, animals were fasted for 24 h. Ulcer was induced by administration of aqueous suspension of indomethacin (30 mg/kg), and 6 h later, the animals were sacrificed [10].

### 2.7 Evaluation of antulcer activity

Under weak diethyl ether anesthesia, the abdomen was opened and the pylorus was ligated. The abdomen was then sutured, and at the end of 6-h ligation, the animals were sacrificed. After decapitation or deep anesthesia, the abdomen was opened and ligature placed around esophagus, close to diaphragm. Stomach was dissected out, and gastric juice was collected in graduated tubes. Then, the contents were subjected to analysis of free and total acid-secreting factors against irritation of the stomach by gastric acid [16, 17]. Inhibition of prostaglandins results in early damage to the mucosal, parietal, and endothelial cells [12], thus leading to the formation of an ulcer. Moreover, the occurrence of an ulcer is also mediated by free radicals.

### 2.8 Determination of total gastric output

Total acidity of gastric output was determined according to Parvez et al. [13]. 1 ml of gastric juice was pipetted into a 100-ml conical flask, 2 to 3 drops of phenolphthalein solution were added before titration with 0.01 N NaOH (which was previously standardized with 0.01 N oxalic acid) until all traces of the red color disappeared, and the color of solution was turned into yellowish-orange. The volume of alkali added corresponding to total acidity was noted. Total volume of gastric juice was also observed.

Acidity was calculated as follows:

\[
\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH}}{0.1} \times 100 \text{ (meq/l/100 g)}
\]

### 2.9 Histological evaluation

The gastric mucosal tissue from control and experimental groups were fixed in 10% buffered formalin. The fixative was removed by tap water-washing overnight. After dehydration through ascending grades of alcohols, the tissues were cleaned in methyl benzoate, and embedded in paraffin wax. Sections were cut into 3-5 µm thickness and stained with haematoxylin and eosin. After dehydration and cleaning, the sections were mounted and observed under the light microscope. The sections obtained, were checked and confirmed by a pathologist. The slides were examined microscopically for pathomorphological changes, such as congestion, hemorrhages, oedema and erosions using anarbitrary scale for the assessment of severity of these changes [14].

### 3 RESULTS AND DISCUSSION

Gastric and duodenal ulcers are illnesses that affect a considerable number of people in the world. Stress, smoking, and ingestion of non-steroidal anti-inflammatory drugs contribute to the gastric ulcer and the infection of Helicobacter pylori, a spiral-shaped bacterium found in stomach, which is also implicated in the gastric ulcer [15]. Administration of nonsteroidal anti-inflammatory drugs (NSAIDs), including aspirin and indomethacine, inhibits the biosynthesis of prostaglandins, particularly of PGE2 and PGI2, which are protective factors (mucosal resistance factors) against irritation of the stomach by gastric acid [16, 17]. Inhibition of prostaglandins results in early damage to the mucosal, parietal, and endothelial cells [12], thus leading to the formation of an ulcer. Moreover, the occurrence of an ulcer is also mediated by free radi-
cals from the conversion of hydroxyperoxyl into hydroxyl fatty acids, causing destruction of the cells. These hydroxyperoxyl compounds, in turn, are produced by the degranulation of mast cells, and the complete lipid peroxidation accompanying cellular damage [18].

We evaluated effects of volatile oil and ethanol extracts obtained from oregano leaves in animals using different standard experimental models of induced gastric ulcers with indomethacin. The volume and the total acid output of the gastric juice were increased. Circular and linear lesions were frequently observed in the stomach of all the control animals. Administration of oregano extracts resulted in a significant reduction in ulcer index, in dose-dependent manner, with regard to control (Tables 1, 2).

### TABLE 1 - Changes of volume gastric juice and total acidity in rats treated with *Oregano syriacum* ethanol extract and volatile oil, compared with omeprazole.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Volume of gastric juice (ml)</th>
<th>Total Acidity (meq/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (positive control)</td>
<td>7.65±0.23</td>
<td>131±5.33</td>
</tr>
<tr>
<td>G2 (ethanol extract 125 mg)</td>
<td>5.99±0.18</td>
<td>65.83±1.75</td>
</tr>
<tr>
<td>G3 (ethanol extract 250 mg)</td>
<td>5.26±0.58</td>
<td>40.2±2.35</td>
</tr>
<tr>
<td>G4 (volatile oil 125 mg)</td>
<td>4.87±0.26</td>
<td>25.20±1.95</td>
</tr>
<tr>
<td>G5 (volatile oil 250 mg)</td>
<td>3.22±0.36</td>
<td>22.25±3.36</td>
</tr>
<tr>
<td>G6 (standard omeprazole 20 mg)</td>
<td>2.88±0.46</td>
<td>39.83±2.75</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. for 6 rats per group; significant at P<0.05 compared to respective control group; LSD = 1.491.

### TABLE 2 - Change of ulcer index and ulcer protection in rats treated with *Oregano syriacum* ethanol extract and volatile oil, compared with omeprazole.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcer index (mm)</th>
<th>Ulcer protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 control</td>
<td>6.83±0.28</td>
<td>------------</td>
</tr>
<tr>
<td>G2</td>
<td>5.166±0.25</td>
<td>9.76</td>
</tr>
<tr>
<td>G3</td>
<td>4.166±0.56</td>
<td>39.03</td>
</tr>
<tr>
<td>G4</td>
<td>1.8±0.42</td>
<td>73.657</td>
</tr>
<tr>
<td>G5</td>
<td>3.1±0.23</td>
<td>54.631</td>
</tr>
<tr>
<td>G6</td>
<td>3.6±0.59</td>
<td>47.31</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. for 6 rats per group; significant at P<0.05 compared to respective control group; LSD = 0.828.

#### 3.1 Histopathological results

Treatments with the total ethanolic extract and volatile oil reduced the volume of gastric secretion and total acidity. Decreased ulcer index and gastric contents as well as secretory parameters can be implicated with protective effects of the drug.

Microscopically, stomach of rats from group 1 (G1) revealed focal necrosis of gastric mucosa associated with leucocytic cell infiltration (Figs. 1-3), desquamation of mucosa with exposed muscularis mucosa (Fig. 2), and necrosis of optical mucosa associated with haemorrhage (Fig. 4). Stomach of rats from group 2 showed mucosal blood vessels and submucosal oedema (Figs. 5 and 6) associated with few leucocytic cell infiltration (Fig. 6). Groups 4-6 showed no histopathological changes of rat stomach (Figs. 7-10). The etiology of peptic ulcer is unknown in most of the cases, but yet it is generally accepted that it results from an imbalance between aggressive factors and the mainte-
FIGURE 4 - Stomach of rat from group 1 (control) showing necrosis of optical mucosa associated with haemorrhage (H and E X200).

FIGURE 5 - Stomach of rat from group 2 (treatment: ethanolic extract 125mg/kg) showing congestion of mucosal blood vessels and submucosal oedema (H and E X200).

FIGURE 6 - Stomach of rat from group 2 (treatment: ethanolic extract 125mg/kg) showing submucosal oedema associated with few leucocytic cells infiltration (H and E X200).

FIGURE 7 - Stomach from group 3 (treatment: ethanolic extract 250 mg/kg) showing no histopathological changes (H and E X200).

FIGURE 8 - Stomach from group 4 (treatment: volatile oil 125mg/kg) showing no histopathological changes (H and E X200).

FIGURE 9 - Stomach from group 5 (treatment: volatile oil 250 mg/kg) showing no histopathological changes (H and E X200).
cells, or interfering with the prostaglandin synthesis [19]. These results indicate that the doses of ethanol extract and volatile oil of *O. syriacum* have anti-secretory effects of gastric acid. The present study showed that treatment with *O. syriacum* extracts caused beneficial effects on indomethacin-induced gastric ulcer in rats as evidenced by reduction in the ulcer index.

**REFERENCES**


