Antiulcerogenic effect of some plants extracts

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Abstract

Methanol extracts were prepared from aerial parts of 8 medicinal plants and evaluated for their potential antiulcerogenic effects using ethanol and Aspirin-induced gastric ulcerations in rats. Oral administration of the methanol extract (400 mg/kg) of Bidens bipinnata Linn., Zygophyllum album Linn. f., Plantago major Linn. (leaves) and Schouwia thebaica Webb. significantly (P< 0.01) decreased the average ulcer index with a curative ratio ranged from 100% for B. bipinnata to 58.3% for S. thebaica in the ethanol-induced gastric ulceration. Mentha microphylla C. Koch., Conyza linifolia Willd., C. dioscoridis (Linn.) Desf., Cynanchum acutum Linn. and P. major (seeds) decreased the ulcer index, however, their curative ratios were below 50% but higher than the reference drug Ranitidine (curative ratio 38.9%). B. bipinnata, Z. album and P. major (leaves) in a dose of 400 mg/kg significantly decreased the number of gastric ulcer and decreased total protein in gastric juice in aspirin-induced gastric ulceration. The total acidity was significantly (P< 0.01) decreased by all tested plant extracts. Based on the decreased ulcer index, increased curative ratio, decreased number of gastric ulcers and decreased total protein and total acidity, B. bipinnata, Z. album and P. major leaves in a dose of 400 mg/kg produced a potent antiulcerogenic effect against alcohol- and aspirin-induced gastric ulcer. Moderate effect was produced by S. thebaica.

Keywords: Antiulcers, Medicinal plants, Mentha microphylla, Cynanchum acutum, Conyza linifolia, Zygophyllum album, Bidens bipinnata, Cynanchum acutum, Plantago major, Schouwia thebaica.

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Introduction

Although there is a large number of products that have been used as antiulcerogenic, but most of these agents produced several side effects including arrhythmias, impotence, gynaecomastia and haematopoetic changes. In addition recurrence rates are high (Ariyoshi et al., 1986). Therefore, there is a need for potent and less toxic antiulcerogenic drugs. Plant extracts are the most attractive source since long time and a large number of plants have been shown to produce promising antiulcerogenic effects (Akhtar et al., 1992; Akhtar & Ahmad, 1995; De Pasquale et al., 1995; Alkofahi & Atta, 1999). In Egypt, a large number of herbs are used for various types of gastrointestinal pain particularly among populations that have little or no medical assistance (Batanouny, 1999; Al-Yousuf et al., 2002). The present work was carried out to investigate the possible antiulcerogenic effect of eight Egyptian plants, known to be used in folk medicine for various types of abdominal pain. These plants are either grown in cultivated lands or found wild in the desert.

Materials and Methods

Plant material

The selected plants were identified by Prof. Dr. N. El-Hadidi, Department of Botany, Faculty of Science, Cairo University and the staff members of the Department of Flora, Ministry of Agriculture, Giza, Egypt. A voucher sample was kept in the Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Egypt. The aerial parts of all the plants were used in this study except Plantago major.
which both seeds and leaves were studied separately to observe difference in the pharmacological actions between the seeds and leaves from previous studies. The air-dried plant material was moderately pulverized, and stored for further use. The powdered plants (200g) were extracted with methanol 95% for at least 24 hours, followed by percolation for 5 to 7 times till complete exhaustion. The methanol extracts were concentrated under reduced pressure using Rotary Evaporator at temperature not more than 50°C. The concentrated extracts were kept at –4°C until used. The extracts were suspended in sterile distilled water with few drops of Tween 80 just before use.

**Animals**

**Ethanol induced gastric ulceration**

Fifty-five male Sprague-Dawley rats (150-200g body wt) were kept under standard conditions before their use. Rats were randomly divided into 11 equal groups. Animals were starved for 48 hour before use to ensure an empty stomach and were kept in cages with raised floors of wide wire mesh to prevent coprophagy (Garg et al., 1993). To prevent excessive dehydration during the fasting period, rats were supplied with sucrose (BDH) 8% (w/v) solution in NaCl (BDH) 0.2% (w/v) which was removed 1 hour before experiments (Glavin & Mikhaeil, 1976).

In the first day, rats of 9 groups were orally given two doses of 400 mg/kg of each extract 6 hours apart. The 400 mg/kg dose was chosen depending on the normal daily consumption of an adult man of the dried plants relatively to their yield of extract. A third dose was given on the 2nd day 1.5 hours before oral administration of ethanol (Merck) 50% (v/v in distilled water) in a dose of 10 ml/kg. The control group was given equal volume of distilled water instead of the plant extracts but received ethanol in the same dose and route. In addition a group was given Ranitidine as a reference drug in a dose of 100 mg/kg by the same route and at the same time intervals. One hour after ethanol administration, all rats were euthanized by an over dose of chloroform and the abdomen was opened and the stomach were rapidly removed, opened along their greater curvature and gently rinsed under running water. Lesions in the glandular part of the stomach were measured under illuminated magnifying microscope (10 x). Long lesions were counted and measured along their greater length. Petechial lesions were counted with the aid of 1-mm squares grid (Ogle et al., 1985). Each five petechial lesions were taken as 1 mm ulcer (Cho & Ogle, 1978). The sum of the total length of long ulcers and petechial lesions in each group of rat were divided by its number to calculate the ulcer index (mm). The curative ratio was determined by the following formula: Curative ratio = (Control ulcer index – Test ulcer index) / (Control ulcer index) ×100

**Aspirin induced ulceration**

Out of eleven groups of five rats each, nine were used as treatment groups and one each for Aspirin and Ranitidine treated group. The modified method of Goel et al (1985) was used for the production of experimental gastric ulceration. Aspirin (200 mg/kg) suspended in carboxymethylcellulose 1% was administered orally using a round tip stainless steel stomach tube. Methanol extract of each plant (400 mg/kg) was given 3 hours prior to and after aspirin administration. Treatment was continued...
for 3 days and the pylorus was ligated on the fourth day under ether anaesthesia. The abdomen was closed and the animals were left to recover. Drinking water was withheld. After 4 hours rats were killed with an overdose of chloroform, the oesophagus was ligated and the stomach was removed. The gastric mucosa was washed with 3 ml distilled water. The gastric juice and the washings were homogenized and centrifuged at 5000 rpm for 5 min. The volume of gastric juice was measured and expressed as ml/100g body weight. The stomach was then cut along the great curvature and the glandular portion was examined under dissecting microscope. The number of ulcers was counted and the total length was measured. The curative ratio was calculated as mentioned before.

**Determination of the total acid output**

Total acid output was determined by titrating 1ml of the gastric juice in 10ml of distilled water with 0.01N NaOH using Phenolphthalein as an indicator. Data were expressed as mEq/ml of the gastric juice (Oser, 1965).

**Determination of protein content**

Total protein (g/dl) in the gastric juice was determined by the Biuret Reagents (Mehl, 1945) using commercial kits.

**Statistical analysis**

The difference between means of treated and non-treated groups was tested for significance using the one-way ANOVA test (Kuchl, 1994).

**Results**

**Alcohol induced ulcers**

*B. bipinnata, Z. album, P. major* (leaves) and *S. thebaica* significantly (*P*≤0.01) decreased the ulcer index with a curative ratio ranged from 100% for *B. bipinnata* to 58.3% for *S. thebaica* in ethanol-induced gastric ulcers (Table 1). *M. microphylla, Conyza dioscoridis, C. linifolia, Cynanchum acutum* and *P. major* (seeds) methanol extracts have decreased the ulcerogenic effect of ethanol with curative ratios below 50%. In reference group, Ranitidine produced a curative ratio of 38.9%.

**Table 1 : Effect of some plant extracts on Ethanol- and Aspirin-induced-gastric ulceration in rats (mean ± SD, n = 5)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Alcohol-induced ulcers</th>
<th>Aspirin-induced ulcer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ulcer index</td>
<td>Curative ratio%</td>
</tr>
<tr>
<td>Control</td>
<td>14.40±4.10</td>
<td>0.00</td>
</tr>
<tr>
<td>Mentha microphylla</td>
<td>11.60±2.40*</td>
<td>19.40</td>
</tr>
<tr>
<td>Conyza dioscoridis</td>
<td>8.00±0.70**</td>
<td>44.40</td>
</tr>
<tr>
<td>Conyza linifolia</td>
<td>11.00±1.20*</td>
<td>23.60</td>
</tr>
<tr>
<td>Zygophyllum album</td>
<td>0.75±0.30**</td>
<td>94.80</td>
</tr>
<tr>
<td>Bidens bipinnata</td>
<td>0.00**</td>
<td>100.00</td>
</tr>
<tr>
<td>Cynanchum acutum</td>
<td>9.40±2.00**</td>
<td>34.70</td>
</tr>
<tr>
<td>Plantago major</td>
<td>11.60±4.70*</td>
<td>19.40</td>
</tr>
<tr>
<td>Plantago major</td>
<td>1.80±0.80**</td>
<td>87.50</td>
</tr>
<tr>
<td>Schouwia thebaica</td>
<td>6.00±1.30**</td>
<td>58.30</td>
</tr>
<tr>
<td>Ranitidine(100mg/kg-1)</td>
<td>8.80±1.50</td>
<td>38.90</td>
</tr>
</tbody>
</table>

* Significant at *P* ≤ 0.05; ** Significant at *P* ≤ 0.01
Aspirin-induced ulceration

*B. bipinnata, Z. album, P. major* (leaves) and *S. thebaica* in an oral dose of 400 mg/kg, significantly ($P<0.05, P<0.01$) decreased the number of Aspirin-induced gastric ulcer (Table 1, Fig. 1A). *M. microphylla, C. linifolia, C. dioecoidis, Cynanchum acutum and P. major* (seed) did not affect the number of Aspirin-induced gastric ulcer. *B. bipinnata, Z. album and S. thebaica* significantly ($P<0.05, P<0.01$) increased the volume of gastric juice in Aspirin-induced gastric ulcers (Fig. 1B). *M. microphylla, C. linifolia, C. dioecoidis, Cynanchum acutum, P. major* (seed and leaves) in an oral dose of 400 mg/kg, did not affect the volume of gastric juice (Table 1, Fig. 1B). The total acidity was significantly ($P<0.01$) decreased by all of the tested plant extracts (Fig. 1C). The total protein was significantly decreased by oral administration of 400mg/kg of *B. bipinnata* only (Fig. 1D).
**Discussion**

The present data demonstrated the efficacy of some plant extracts against gastric ulceration induced by two experimental models, viz. ethanol and Aspirin-induced gastric ulcers. The results showed that the highest protective effect against ethanol induced gastric damage was produced by the methanol extract of *B. bipinnata* (curative ratio 100%, three times more than Ranitidine), *Z. album* (94.8%), *P. major* leaves (87.5%) and *S. thebaica* (58.3%). Although *Conyza dioscoridis* (Linn.) Desf., and *Cynanchum acutum* methanol extracts decreased the ulcer index, curative ratio below 50%, they were as effective as the reference drug, Ranitidine. Preliminary phytochemical screening of these plants showed that they contain variable amounts of triterpenes and flavonoids (Atta & Mounir, 2004). Triterpens are the active constituents that have been claimed to be effective as an anti-ulcer agent because it protects the mucosa against acid effects by selective inhibition of PGF$_{2\alpha}$ (De Pasquale et al, 1995). Sodium carbenoxolone is a triterpenoid related compound that has been shown to possess an antiulcerogenic effect by protecting the mucosa from acid effect by selective inhibition of PGF$_{2\alpha}$ (Aguwa & Okunji, 1986). Moreover a flavonoid fraction was isolated from another species of *Bidens* i.e. *B. aurea* (Ait.) Sherff, to which the antiulcerogenic effect of this plant was attributed (Matrin et al, 1996). Therefore, the gastric protective effect of these extracts is probably due to their content of flavonoids and or triterpenes. *M. microphylla, Conyza dioscoridis, C. linifolia* methanol extracts have increased the ulcerogenic effect of ethanol. This is probably due to an irritant effect or synergistic effect with Aspirin.

In ethanol-induced gastric ulcers, three of the tested plants showed curative ratio of over 75%. These plants are *B. bipinnata* (100%), *Z. album* (94.8%) and *P. major* leaves (87.5%). On the other hand, these plant extracts decreased the number of ulcers, total acidity and total protein in gastric juice but did not decrease the volume of gastric juice in Aspirin-induced ulcers indicating that these plants are antilulcerogenic but not antisecretory. Inhibition of endogenous prostaglandin renders the stomach more susceptible to damage. These eicosanoids inhibit acid secretion by the stomach and promote the secretion of cytoprotective mucous (Robert, 1981). The mechanism of Aspirin-induced ulcerogenic effect is the inhibition of the synthesis of endogenous prostaglandins (Whittle & Vane, 1983) which consequently decrease the cytoprotective effect by prostaglandins. The antilulcerogenic effect and lack of antisecretory effect of the tested plants points out their possible cytoprotective effect as a mechanism of action. Similar conclusion has been reported for other plant extracts (Deshpande et al, 2003).

The antilulcerogenic active constituent(s) of these plants appeared to be extracted by methanol. Similar conclusion was obtained for other plants (Akhtar et al, 1992). However, the water extract of some of these plants like *P. major* leaves, seeds or whole plant have shown to possess antilulcerogenic effect (Bustos et al, 1996; Guillen et al, 1997; Joshi et al, 1982). Although, *P. major* decreased the number of ulcers and total acidity and increased the curative ratio, it did not affect the volume of gastric juice or its protein content. Yesilda et al (1993) concluded that although *P. major* combined water and methanol extract inhibited water immersion-stress-induced ulcer by 40%, it was not among the most active plants tested. Moreover, *P. major* leaves are proved to contain the triterpenoids urosolic acid, which inhibits cyclooxygenase-2 and cyclooxygenase-1 catalysed prostaglandin biosynthesis in vitro (Ringbom et al, 1998). In this study *B. bipinnata* comes on the top of the gastric protective plant extracts. This is probably due to its high flavonoid content. The present results confirm the traditional use of some of these plants for treatment of gastrointestinal disturbances. Additional experiments are required to isolate and test the active constituents to explore the exact mechanism of action of these plants.

**References**

1. Aguwa CN and Okunji CO, Gastrointestinal studies of *Pyrenacantha staedtii* leaf extracts, J Ethnopharmacol, 1986, 15, 45-55.
Anti-ulcerogenic effect of Momordica charantia L. fruits

The Bitter Gourd, Momordica charantia Linn. are used externally for the rapid healing of wounds and internally for the treatment of peptic ulcers in Turkish folk medicine. Hence, the scientists at Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey evaluated the anti-ulcerogenic effect of the mature fruits on ethanol-induced ulcerogenesis model in rats. The olive oil extract of the material as well as dried-powdered fruits in filtered honey showed significant and dose-dependent anti-ulcerogenic activity against this model. A potent and dose-dependent inhibitory activity was also observed by the administration of ethanol extract of the fruits. For the bioassay-guided fractionation, the material was first extracted with hexane and then by ethanol and both extracts was found active against the same ulcer model. Further, ethanol extract of the fruits showed significant activity against HCl–EtOH induced ulcerogenesis in Indomethacin-pretreated rats and Diethylthiocarbamate-induced ulcer models [Gürbüz İhan, Akyüz Çidem, Yeilada Erdem and Ener Bilge, Anti-ulcerogenic effect of Momordica charantia L. fruits on various ulcer models in rats, J Ethnopharmacol, 2000, 71(1-2), 77-82].