Gastroprotective effect of *Plantago major* L. against gastric injury induced by aspirin in rats.

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**1. Introduction**

*Plantago major* L. is a perennial plant that belongs to the Plantaginaceae family[10] It is renowned as a traditional herbal medicine throughout the world. *P. major*, a popular traditional cure that has been used for many diseases varying from cold to viral hepatitis[11]. The use of *P. major* in wound healing in Scandinavia, ulcers treatments in Turkey[12 ],against skin problems and gastrointestinal disorders in Mexico[10]. *P. major* leaf has been used as a diuretic agent in Guatemala[13]. Furthermore, teas of *P. major* have free radical Scavengers and *P. major* contained active components such as, mucilages, flavonoids, phenolic acids, hydroxycoumarins, tannins and others[14].

Ulcer is a common global problem with increasing incidence and prevalence attributed to several factors encountered during day-to-day life[1]. Gastric ulcer, one of the most widespread, due to an imbalance between aggressive and protective factors. The gastric mucosa is continuously exposed to potentially injurious agents such as acid, 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[2]. Proton pump inhibitors such as omeprazole are extensively used to control increased acid secretion and acid-related disorders including gastroesophageal reflux disease, Zollinger-Ellison syndrome and gastroduodenal ulcer caused by stress (stress related erosive syndrome), nonsteroidal antiinflammatory drugs and by *H. pylori* [4]. Recently, the antiinflammatory, antioxidant and antiapoptotic roles of omeprazole have also been reported[5]. Although histamine-H2 receptor blockers (ranitidine, famotidine etc.) and proton pump inhibitors (omeprazole, lansoprazole etc.) have been used for efficient management of gastric hypersecretion and gastroduodenal ulcers, several adverse effects of these drugs have also been reported[6,7].

The global view is changing towards the development and therapeutic use of non-toxic preparations from traditional medicinal plants for controlling various diseases.

**ARTICLE INFO**

**ABSTRACT**

Ethanolic and water extracts from leaves and seeds of *Plantago major* L. were evaluated for their potential anti-ulcerogenic effects using aspirin induced gastric ulcerations in rats. Oral administration of ethanol and water extracts (1000 mg/kg body weight) of *plantago major* L. Leaves and seeds significantly decreased the ulcer index, total acidity compared with positive control and ethanolic extract of *p.major* leaves at (1000 mg/kg body weight) has significant highest reduction of the ulcer index and total acidity of gastric fluid compared with other extracts. Moreover, serum levels of aspartate transaminase (AST), alanine transaminase (ALT) were significantly (p< 0.05) decreased by all groups which received tested extracts compared to positive control. Also, results of histopathological examination of rats stomach demonstrated the protective effect of the plant extracts under study.

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**Keywords:** Antiulcerogenic effect

**Chemical constituents**

*Plantago major*, leaves extract, seeds extract

Gastroprotective activity

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Thus, novel non-toxic, antisecretory and antiulcer preparations, preferably from medicinal plants are the demand of the day as alternate source of medicine for the efficient management of gastric hypersecretion and gastroduodenal ulcers[8,9]. Therefore, the aim of this work was to study the gastroprotective effect of P. major seeds and leaves extracts in comparison with omeprazole, which is a commonly prescribed drug for gastric ulcer in a model of aspirin-induced gastric ulcer in experimental rats.

2. Materials and methods

2.1 Plant materials:

*Plantago major* L. leaves and seeds were collected from Medicinal and aromatic plants farm in Kanater, Agriculture research center, Giza, Egypt. All samples were cleaned, dried in shade at room temperature for 5.7 days reduced to fine powder and kept for preparing of different extracts.

2.2 Plant extracts

Fifty grams of each plant powder was extracted in 500 ml ethanol (70% v/v) by maceration (48 h). The solvent was removed under vacuum at temperature below 50°C, then the extracts were freeze-dried. A second plant samples from the same bath were extracted with boiling water (10 g powder :100 ml water) according to[30].

2.3 Source of drugs, chemicals and kits

Aspirin and omeprazol was used as the reference for antiulcer drug were obtained from European Egyptian Pharmaceutical Industries, Alexandria, Egypt. All other used reagents and solvents were of analytical grade and kits for serum alanine transferase (ALT) and serum aspartate transferase (AST) were obtained from diagnostic company.

2.4 Animals

Male albino rats with an average weight of (140-160 g) were provided by the animal house of Food Technology Research Institute, Agriculture Research centre, Giza, Egypt. The animals were housed in standard cages at room temperature (25 ± 3°C) with 12 h dark/12 h light cycles and supplemented with basal diet according to[16], for 7 days as an adaptation period.

2.4.1 Evaluation of antiulcer activity of different extracts from *P. major* against aspirin induced gastric ulcer in rats:

The experiment was performed according to the method of [17] the rats were randomly divided into seven groups of six animals each as follow:

Group (1) (negative control) rats received distilled water contain 1 ml vehicle (1% Tween-80 aqueous solution).

Group (2) (positive control) rats received orally distilled water for 29 days and received aspirin (200 mg/kg b.wt) orally on day 30 after 24 h fasting.

Group (3) rats received omeprazol (10 mg/kg b.wt/day)

Group (4) rats received ethanol extract of plantago leaves (1000 mg/kg b.wt/day)

Group (5) rats received ethanol extract of plantago seeds (1000 mg/kg b.wt/day)

Group (6) rats received water extract of plantago leaves (1000 mg/kg b.wt/day)

Group (7) rats received water extract of plantago seeds (1000 mg/kg b.wt/day)

Group 3, 4, 5, 6, 7 received drug or different extracts for 29 days and all samples were dissolved in 1% Tween – 80 aqueous solution and this dose (1000 mg) were based on a previous study carried out with crude extract of *Plantago major*[17]. All the samples were orally administered orally and at the 29 day of experimental period rats fasted for 24 h and post – orally gavaged with aspirin (200 mg/kg body weight) except group (1). The animals were anesthetized with ether and sacrificed after 6 h from the end of experiment, the blood was collected and centrifuged at 3000 rpm to obtain serum which kept in a deep-freezer until biological analysis.

2.4.2 Determination of acidity of gastric contents

The stomach was opened to collect the gastric contents. Then the gastric contents were centrifuged at 1000 rpm for 10 min. Total acidity were determined according to[18].

2.4.3 Determination of Ulcer index

The stomachs were opened longitudinally, washed with saline and examined under dissecting. The sum of the total length long ulcers in each group of rats was divided by its number to calculate the ulcer index mm [19] Percentage protection index is calculated as follows:

\[ \% \text{ protection} = (Uc - Ut) \times 100 / Uc \]

where: Uc = ulcer index in control group; Ut = ulcer index in treated group.

2.4.4 Determination of liver marker enzymes

Serum aspartate transferase (sAST) and serum alanine transferase (sALT) activities were measured colorimetrically according to[20].

2.5 Histopathological examination of stomachs

The study was performed at Pathology department, Faculty of Veterinary Medicine, Cairo University. Stomachs of sacrificed rats were taken and immersed in 10% (v/v) formalin solution, the fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol then, cleared in xylol and embedded in paraffin then sectioned at 4-6 microns thickness and stained with Haemtoxylin and Eosin according to[21].

2.6 Statistical analysis:

Statistical analysis was carried out according to [22] LSD (Least squares difference) test was used to compare the significant differences between means of treatment[23].
3. Results and discussion

3.1. Antiulcer activity of different extracts of *P. major*

The obtained results from Table 1 showed that, ulcer index for positive control was 13.6±0.54mm to zero compared with the negative control. Also the ulcer index was significantly (p<0.05) decreased by all tested extracts, the ethanol extracts of *P. major* leaves at dose 1000 mg/kg body weight has significant highest reduction of the ulcer index (3.2±0.83) exhibiting a highest protection ratio (76.47%) compared with all tested extracts. In reference group, omeperazol produced a protection ratio of (83.82%). This due to the gastric protective effect of *P. major* ethanolic extracts of seeds and leaves is probably due to their high content from flavonoids, phenols, tannins, mucilage and leaves has high content from these compounds than seeds [14]. Also water extract of *P. major* seeds at dose (1000mg /kg) has high gastric protective effect than leaves, this may be due to high content from mucilage (19.5%) [14]. The obtained data was in accordance with [24] shows that *plantago major* contain five classes of biologically active compounds namely flavonoids (baicalein, baicallin, luteolin), phenolic compounds (cafeic acid, chlorogenic acid, ferulic acid, p-coumaric acid), benzoic compound (vanillic acid), iridoid glycoside (aucubin) and triterpenes (oleanolic acid, ursolic acid). Also [17] pointed out that the botanical compounds with antiulcer activity include flavonoids, tannins, gums, and mucilage in the plant kingdom and also [25] showed that no signs of toxicity were observed with doses up 2g/kg body weight from methanolic extract of *plantago major* in an acute toxicity assay. On the other hand, there was a significant increase (p<0.05) in total acidity of gastric fluid in the positive control group compared with the negative control.

The total acidity was significantly (p<0.05) decreased by all tested plant extracts compared to positive control. In addition, the highest reduction in the total acidity of gastric fluid was observed in rats orally administered with ethanolic extract of *P. major* leaves (1000mg/kg body weight) followed by ethanolic and extracts of *P. major* seeds. This may be due to different extracts possessed gastroprotective and anti-ulcerogenic effects which are related to the inhibition of the gastric acid secretion and an increase of mucosal defensive factors [26].

Also [27] reported that naturally occurring the Phytoconstituents like flavonoids, tannins, gums and mucilage are functioned to cure the peptic ulcer. Phytoconstituents is very cheap with economic and with out side effect produce when compare to allopathic drugs. Also natural products found in the extracts of the bark of *Excoecaria agallochalis* decreases the acidity and increases the mucosal defense in the gastric areas [9]. In this respect [28] shows that different alcohol extracts of *Glycyrrhiza glabra* showed a significant reduction in the ulcer index. In addition ALP and TBARS were significantly reduced due to different extracts has antioxidant effects on the gastric mucosa in rats.

### Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcer index (mm)</th>
<th>Protection (%)</th>
<th>Acidity (m Eq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (G1)</td>
<td></td>
<td></td>
<td>16.05±0.86</td>
</tr>
<tr>
<td>Positive control (G2)</td>
<td>13.60±0.54</td>
<td>0</td>
<td>76.80±2.48</td>
</tr>
<tr>
<td>Omeprazole (G3)</td>
<td>2.20±0.44</td>
<td>83.82</td>
<td>21.00±0.81</td>
</tr>
<tr>
<td><em>P. leaves</em> ethanolic extract (G4)</td>
<td>3.20±0.83</td>
<td>76.47</td>
<td>26.00±1.22</td>
</tr>
<tr>
<td><em>P. seeds</em> ethanolic extract (G5)</td>
<td>4.00±0.70</td>
<td>70.59</td>
<td>28.08±0.9</td>
</tr>
<tr>
<td><em>P. leaves</em> water extract (G6)</td>
<td>7.60±0.50</td>
<td>44.12</td>
<td>42.20±1.09</td>
</tr>
<tr>
<td><em>P. seeds</em> water extracts (G7)</td>
<td>4.40±0.44</td>
<td>68.38</td>
<td>28.60±2.07</td>
</tr>
<tr>
<td>L.S.D. at 5%</td>
<td>0.76215</td>
<td>1.8960</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD of three measurements.
Means in the same column with different letters are significantly different (p < 0.05)

3.2. Effect of oral administration of plant extracts on liver marker enzymes

Serum levels of AST and ALT were assayed in this study and the results in Table 2 showed that serum levels of AST and ALT (p<0.05) were significantly increased in rats received aspirin alone (54.2±2.16 and 60.08±2.05, respectively) compared to negative control. Aspirin alone showed acute elevation of serum AST and ALT as indicators of hepatic injury since elevated levels of these hepatocellular enzymes are signs of aspirin-induced tissue damage.

In contrast, the rats pretreated with omeprazole and ethanolic extract of *P. major* leaves prior to aspirin inhibited (p < 0.05) the acute increase in serum AST and ALT, which may shows its action in preventing the acute tissue damage.

In addition data demonstrated in Table 2 reveal that indicated that serum levels of AST and ALT were significantly (p<0.05) decreased by all groups which received tested plant extracts compared to positive control.
Table 2 Effect of oral administration of plant extracts on liver marker enzymes

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (IU/l)</th>
<th>ALT (IU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (G1)</td>
<td>22.20 ±1.92</td>
<td>18.80 ±1.9</td>
</tr>
<tr>
<td>Positive control (G2)</td>
<td>54.20 ±2.16</td>
<td>60.08 ±2.05</td>
</tr>
<tr>
<td>Omeprazole (G3)</td>
<td>23.80 ±1.48</td>
<td>20.02 ±1.79</td>
</tr>
<tr>
<td>P. leaves ethanolic extract (G4)</td>
<td>26.32 ±1.35</td>
<td>22.28 ±1.75</td>
</tr>
<tr>
<td>P. seeds ethanolic extract (G5)</td>
<td>29.13 ±1.78</td>
<td>25.00 ±1.58</td>
</tr>
<tr>
<td>P. leaves water extract (G6)</td>
<td>42.04 ±1.74</td>
<td>44.80 ±1.9</td>
</tr>
<tr>
<td>P. seeds water extract (G7)</td>
<td>28.84 ±2.06</td>
<td>25.80 ±1.48</td>
</tr>
<tr>
<td>L.S.D. at 5%</td>
<td>2.30287</td>
<td>2.17640</td>
</tr>
</tbody>
</table>

Values are means ± SD of three measurements.
Means in the same column with different letters are significantly different (p < 0.05)

Figure 1 Histological section of rat stomach in negative control group show normal mucosal layers (H&E 40X).

3.3 Histopathological examination:

The histological examination was done to confirm the previous results and the histological examination of stomach of rat in negative control (Group1) revealed normal mucosal layers Figure 1. Meanwhile, stomach of rat from (group 2) positive control, which received aspirin alone, showed marked necrosis of gastric mucosa associated with hemorrhage and inflammatory cell infiltration Figure 2. However, stomach of rat from group 3 which received omeprazole showed no histopathological changes Figure (3) No histopathological findings were noticed in the examined sections from group 4 which received plantago leaves ethanol extract (1000 mg/kg) slight submucosal oedema Figure (4).

Figure 2 Stomach of rat from group 2 showing marked necrosis of (1) gastric mucosa associated with (2) hemorrhage and (3) inflammatory cells infiltration.

Figure 3 Stomach of rat from (group 3) which gave Omeprazole (10 mg/kg) shows no significance change.

Also the same changes were observed in the examined sections from group 5 which received plantago seeds ethanol extract (1000mg/kg). Figure(5) this due to plantago leaves ethanolic extract contain high content from flavonoid, phenols, tannis (6.41, 13.05, and 5.63 mg/g on dry weight) compare with plantago seeds (3.03, 7.43, and 2.43 mg/g) these compounds has potential antiulcerogenic. [14,17]
Figure 4 Histological section of rat stomach from group 4 showing apparent normal gastric mucosa.

Also, tannin has anti-inflammatory effects which help to control all indications of gastritis and irritating bowel disorders by its ability to bind to the proteins of the pathogenic[3].

Figure 5 Histological section of stomach of rat from group 5 revealed submucosal oedema.

In this respect [15] show that tannins in plantago extract enhanced presence of goblet cells would insure an improved synthesis of mucous which improve the intestinal barrier and functioning and thereby increases host protection against infections. Also plantago major with specific polysaccharides (mucilage) improves wound and has anti-inflammatory, analgesic antioxidant and anti-ulcerogenic activity of this plant has a protective effect on stomach in ulcerative rats[29] and also[10] reported that p. major has been used in Turkey in the treatment of ulcer. The powder dried leaves were taken together with honey daily before breakfast.

4- Conclusion

In this study, we have investigated the potential antiulcerogenic effects of ethanolic and water extracts of leaves and seeds from plantago major against gastric ulcer model in rats induced by aspirin. This work shows that all tested plant extracts gave a significant decrease in ulcer index, total acidity of gastric fluid, activity of ALT and AST compared with positive control. Also histopathological examination of rats stomach confirmed the biochemical changes and demonstrated the protective effects of plant extracts under study.

References