Possible protective effect of gum arabic on experimentally induced gastric ulcer in adult male albino rats: a histological and immunohistochemical study
Omayma K. Helal, Mohamed M. Yousef and Mona Elnaa

Background
Peptic ulcer is a serious disease with a high incidence of occurrence in our community. Gum arabic (GA) is an edible, dried, gummy exudate from the stems and branches of *Acacia senegal*. It has been claimed to act as an antioxidant.

Aim of the study
The aim of this study was to evaluate the protective effect of GA on stress-induced peptic ulcer in rats.

Material and methods
In this study, 30 adult male rats were divided equally into three groups: the control group (group I), the stress ulcer group (group II), and the GA group (group III); the GA group received GA 7.5 g/kg/day through an orogastric tube for 10 days. After 10 days, the rats were fasted for 24 h. Cold immobilization stress was induced in groups II and III. The animals of all groups were then anesthetized with diethyl ether, and their stomachs were isolated immediately, opened from the greater curvature, and washed with saline. Ulcer severity, ulcer score, and ulcer index were determined. Stomach specimens were fixed in 10% neutral-buffered formalin for histological, hematoxylin and eosin, histochemical, Periodic Acid Schiff’s (PAS), and Masson’s trichrome stainings and for immunohistochemical detection of proliferating cell nuclear antigen (PCNA).

Results
Pretreatment with GA significantly decreased the gastric lesions. Both ulcer severity and score were significantly decreased (32.8% and 39.58%, respectively) compared with the stress-untreated group. Ulcer index was significantly decreased (49%) compared with the stress group (*P* < 0.05). The stress group showed atrophic gastric mucosa with loss of glandular tissue (hematoxylin and eosin), positive PAS reaction in the mucus neck cells, many collagen fibers between the atrophic glands, and moderate PCNA reaction in the glandular cells. In the GA group, there was nearly normal gastric mucosa with a small area of atrophied surface epithelium, PAS-positive reaction in surface and neck mucus cells, some collagen fibers between the near normal gastric glands, and mild PCNA reaction in the gland cells.

Conclusion
It can be concluded that GA exerts a protective effect against stress-induced gastric mucosal lesions in rats.

Keywords:
gum arabic, stomach, stress ulcer
and sympathetic pathways forming the brain–gut axis [6]. Various types of stress may play a role in the onset and modulation of acute or chronic peptic ulcer disease [7].

Exudate gums are produced by many trees and shrubs as a natural defense mechanism, particularly in semiarid regions. When the plant bark is injured, an aqueous gum solution is exuded to seal the wound, preventing infection and dehydration of the plant [8].

Gum arabic (GA) is a dried exudate obtained from the stems and branches of Acacia seyal or Acacia seyal [9]. GA is a branched-chain complex polysaccharide, either neutral or slightly acidic, found as a mixed calcium, magnesium, and potassium salt of a polysaccharidic acid [10]. It can be considered as an arabinogalactan containing less than 5% glycoprotein [11].

GA is used as an emulsifier and stabilizer in food and pharmaceutical industries as, for example, an encapsulation agent [12].

GA is reputed in Arabian medicinal practices to be useful in treating patients with chronic renal failure. The mechanisms of this nephroprotection are unclear [13].

The aim of this study was to evaluate the possible protective effect of GA on stress-induced peptic ulcer in rats.

Material and methods

In this study, 30 adult male albino rats with an average weight of 120–150 g, purchased from a holding company for biological products and vaccine (Vascera, Agouza, Giza, Egypt), were used. Animals were housed in an animal house, fed a commercial standard pellet diet (El-Nasr Pharmaceutical Chemicals Co, Cairo, Egypt), and allowed free access to tap water.

Rats were divided into three groups of \( n = 10 \) each:

Group I (the control group) received sterile water through an orogastric tube for 10 days.

Group II (the stress ulcer group) received sterile water through an orogastric tube for 10 days before stress exposure.

Group III (the GA group) received GA through an orogastric tube for 10 days before stress exposure. GA was dissolved in sterile water just before use at a dose of 7.5 g/kg/day [14].

After 10 days, the cold immobilization restrained method was used to induce peptic ulcer in groups II and III, as performed by some researchers [15]. Rats were fasted for 24 h before induction of ulcer stress by tying rats to a wooden plank and keeping them in a refrigerator for 4 h at 2–4°C.

The animals of all groups were then anesthetized with diethyl ether, and their stomachs were isolated immediately, opened from the greater curvature, and washed with saline.

Ulcer severity [16] (the total ulcer surface area was measured by a transparent graph paper), ulcer score [16]; (gastric lesions were scored according to their severity between 1 and 6, as in Table 1, and ulcer index [16] (ulcer index = mean ulcer score of the similarly treated group × percentage of ulcerated animals of the same group) were determined. Values were expressed as mean ± standard error of mean. Comparisons between different groups were carried out by one-way analysis of variance, followed by the Tukey–Kramer test. The level of significance was set at \( P < 0.05 \).

Thereafter, stomach specimens were fixed in 10% neutral buffered formalin for histological; hematoxylin and eosin (H&E) [17], histochemical; Periodic Acid Schiff’s (PAS) [18] and Masson’s trichrome [19] stains and for immunohistochemical: detection of proliferating cell nuclear antigen (PCNA). A three-step immunoperoxidase staining technique was performed; the mouse monoclonal anti-PCNA/cyclin antibody (clone PC-10, Dako, Heliopolis, Cairo, Egypt) was used. Sections were counterstained with hematoxylin [20]. Negative control slides were prepared.

The mean area percentage for collagen fiber accumulation (Masson’s trichrome) and PCNA reaction was quantified in 10 images of each group using Image-Pro Plus program version 6.0 (Media Cybernetics Inc., Bethesda, Maryland, USA).

Results

Statistics

Ulcer severity, ulcer score, and ulcer index

No visible sign of ulceration was observed in the animals in the control group.

Cold restraint stress resulted in obvious gastric lesions in all animals. The average ulcer severity and score in group II (stress group) were 28.40 ± 1.39 and 4.70 ± 0.15, respectively (Table 2 and Histograms 1 and 2). Ulcer index was 0.5 ± 0.019 in animals exposed to stress (Table 1 and Fig. 3).

The average ulcer severity and score in group III (GA group) were 7.71 ± 0.88 and 1.9 ± 0.23, respectively (Table 2 and Histograms 1 and 2). Ulcer index was 0.23 ± 0.07 in animals pretreated with GA and then exposed to stress (Table 2 and Histogram 3).

Pretreatment with GA significantly decreased the gastric lesions. Both ulcer severity and score were significantly decreased (32.8% and 39.58%, respectively) compared with the stress-untreated group. Ulcer index was significantly decreased (49%) compared with the stress group \( (P < 0.05) \).

Table 1. Ulcer severity and ulcer score

<table>
<thead>
<tr>
<th>Ulcer severity [ulcerated area (mm)]</th>
<th>Ulcer score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No damage</td>
<td>–</td>
</tr>
<tr>
<td>Blood at the lumen</td>
<td>1</td>
</tr>
<tr>
<td>Pin-point erosions</td>
<td>2</td>
</tr>
<tr>
<td>One to five small erosions &lt; 2 mm</td>
<td>3</td>
</tr>
<tr>
<td>More than five small erosions &lt; 2 mm</td>
<td>4</td>
</tr>
<tr>
<td>One to three large erosions &gt; 2 mm</td>
<td>5</td>
</tr>
<tr>
<td>More than three large erosions &gt; 2 mm</td>
<td>6</td>
</tr>
</tbody>
</table>

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Histological results

**Hematoxylin and eosin stain**
In the control group (group I), H&E staining revealed gastric mucosa with surface epithelium, lamina propria containing gastric glands, and muscularis mucosa (Fig. 1). In the stress group (group II), it showed atrophic gastric mucosa with loss of glandular tissue (Fig. 2). In the GA group (group III), it showed near normal gastric mucosa with a small area of atrophied surface epithelium (Fig. 3).

**Periodic Acid Schiff’s stain**
In the control group, PAS staining revealed a strong positive PAS reaction in surface and neck mucus cells (Fig. 4). In the stress group, it showed an interrupted mucosal layer and weak PAS reaction in mucus neck cells (Fig. 5). In the GA group, it showed near normal mucosa with PAS-positive reaction in surface and neck mucus cells (Fig. 6).

### Table 2. Effect of gum arabic on stress-induced gastric ulceration in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Ulcer severity</th>
<th>Ulcer score</th>
<th>Ulcer index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>–</td>
<td>28.40 ± 1.39</td>
<td>–</td>
</tr>
<tr>
<td>Group II</td>
<td>28.40 ± 1.39</td>
<td>7.71 ± 0.88*</td>
<td>4.70 ± 0.15</td>
</tr>
<tr>
<td>Group III</td>
<td>7.71 ± 0.88*</td>
<td>1.9 ± 0.23</td>
<td>0.5 ± 0.019</td>
</tr>
<tr>
<td></td>
<td>0.5 ± 0.019</td>
<td>0.23 ± 0.07*</td>
<td>–</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM, n = 10.

*Significant difference from stress group (P < 0.05).

### Histogram 1.

Effect of gum arabic on stress-induced ulcer severity in rats. Significant decrease (a) of average ulcer severity in group III compared with group II.

### Histogram 2.

Effect of gum arabic on stress-induced ulcer score in rats. Significant decrease (a) of average ulcer score in group III compared with group II.

### Histogram 3.

Effect of gum arabic on stress-induced ulcer index in rats. Significant decrease (a) of average ulcer index in group III compared with group II.
**Masson’s trichrome stain**

In the control group, Masson’s trichrome staining revealed a few collagen fibers in the lamina propria between the basal parts of fundic glands (Fig. 7).

**Immunohistochemical stain**

In the control group, immunohistochemical staining revealed mucosa with weak PCNA reaction in the gland neck cells (Fig. 10). In the stress group, it showed moderate PCNA reaction in the glandular epithelium (Fig. 11). In the GA group, it showed mild PCNA reaction in the gland neck cells (Fig. 12).
Figure 8. A photomicrograph of a section of the stomach fundus of an affected rat (group II) showing many collagen fibers (arrowhead) in the lamina propria and between the atrophic fundic glands. Masson’s trichrome, \(\times\) 200.

Figure 9. A photomicrograph of a section of the stomach fundus of a gum arabic-treated rat (group III) showing some collagen fibers (arrowhead) between gastric glands. Masson’s trichrome, \(\times\) 200.

Table 3. Mean area percentage of collagen fibers accumulation in groups I, II, and III

<table>
<thead>
<tr>
<th>Group</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean area percentage of collagen fibers accumulation</td>
<td>0.02</td>
<td>0.13</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Histogram 4.

The mean area percentage of collagen fiber accumulation in groups I, II, and III.

Figure 10. A photomicrograph of a section of the stomach fundus of a control rat (group I) showing gastric mucosa with weak proliferating cell nuclear antigen (PCNA) reaction in the gland neck cells (double arrows). PCNA, \(\times\) 200.

Figure 11. A photomicrograph of a section of the stomach fundus of an affected rat (group II) showing gastric mucosa with increased proliferating cell nuclear antigen (PCNA) reaction in the glandular epithelium (double arrows). PCNA, \(\times\) 200.

Figure 12. A photomicrograph of a section of the stomach fundus of gum arabic-treated rats (group III) showing gastric mucosa with mild proliferating cell nuclear antigen (PCNA) reaction in the gland neck cells (double arrows). PCNA, \(\times\) 200.
The mean area percentage of the PCNA reaction for the three groups is represented in Table 4 and Histogram 5.

### Table 4. Mean area percentage of proliferating cell nuclear antigen (PCNA) reaction in groups I, II, and III

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean area percentage of PCNA reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.003</td>
</tr>
<tr>
<td>II</td>
<td>0.018</td>
</tr>
<tr>
<td>III</td>
<td>0.007</td>
</tr>
</tbody>
</table>

### Histogram 5.

The mean area percentage of proliferating cell nuclear antigen reaction in groups I, II, and III.

### Discussion

Physical and psychological stresses are widely accepted as modifiers of the clinical course of different gastrointestinal disorders such as peptic ulcer, irritable bowel syndrome, or inflammatory bowel disease. Growing experimental evidence from a variety of models, such as immobilization, thermal injury, or early maternal deprivation in laboratory animals, uniformly supports the ability of stress to induce the development of gastric ulcers [21]. Stress-related gastric ulcer is a typical, serious, stress-induced injury occurring as a complication in severely ill patients after burns, sepsis, major surgery, or trauma of the central nervous system [22].

In this study, gastric ulcer was experimentally induced in rats using the cold immobilization restrained method. This agreed with the results of some researchers [23,24] who have stated that a combination of the body restraint procedure with exposure to cold temperatures drastically increases the occurrence of gastric ulcer over a shorter period.

The stress group (group II) of this study showed atrophic gastric mucosa with loss of glandular tissue (H&E), an interrupted mucosal layer with positive PAS reaction in the mucus neck cells (PAS), many collagen fibers between the atrophic fundic glands, and moderately expressed PCNA reaction in the glandular epithelium. These results agreed with researchers [22,25] who have stated that, on exposure to stress for 4 h, rats developed severe gastric mucosal lesions. PAS-stained stomach tissues showed an evidence of loss of gastric mucosa and mucin-synthesizing cells with reduced mucin content in ulcer-induced groups. Further, some studies [26,27] have reported that myofibroblasts reside beneath the epithelium. When the stomach is ulcerated, myofibroblasts migrate toward the ulcer bed to form granulation tissue. Later, they produce collagen fibrils and other extracellular matrixes and form ulcer scars. They also stated that the expression of PCNA in ulcerated tissues increased.

It is important to understand the mechanism of formation of stress ulcers, because it might suggest a means for the prevention and treatment of stress-related organ injury. Several factors including reactive oxygen species (ROS), cytokines, and bioactive amines have been implicated in stress ulcer formation [28]. Stress causes both sympathetic (causes direct arteriolar vasoconstriction) and parasympathetic (induces an increased motility and muscular contraction) stimulation of the stomach, leading to local hypoxia and near or actual ‘ischemia.’ The ischemic condition caused an increase in the level of H2O2, which, in conjugation with O2 generates OH, OH oxidizes important cellular constituents [29]. Oxidative stress and caspases have been implicated as potential mediators of cell death [30]. ROS have a key role in the activation of caspases [31]. Lipid peroxidation induced by oxygen-free radicals seems to play a critical role in the pathogenesis of acute gastric mucosal injury in both experimental animals and humans [32]. Lipid peroxidation destroys cell membranes with the release of intracellular components such as lysosomal enzymes, leading to further tissue damage. The damage of membrane proteins decreases the membrane’s permeability, the activities of enzymes and receptors, and the activation of cells [33]. Immobilization stress was reported to increase the susceptibility to mucosal damage through the induction of nitric oxide synthesis (NOS-2) activity and expression [34]. Nitric oxide participates in the modulation of gastric emptying and antral motor activity. It also regulates acid and gastric mucus secretion and alkaline production and is involved in the maintenance of mucosal blood flow [35]. Increasing the concentration of NO results in the production of ROS and ultimately in oxidative stress [36]. However, some researchers [37] have demonstrated that NO enhanced gastric mucus production.

GA refers to a group of closely related polysaccharides including arabinose and galactose, and arabinogalactan [38]. Plant polysaccharides have been reported to have antiviral, antitumoral, immunostimulatory, anti-inflammatory, anticomplementary, antimigratory, hypoglycemic, and antitumor activities [39].

In this study, pretreatment of rats with GA polysaccharide 10 days before induction of stress resulted in significantly decreased gastric lesions. Ulcer severity, score, and index were significantly decreased (32.8%, 39.58%, and 49%, respectively) compared with the stress-untreated group. This agreed with the conclusions drawn by some researches [39,40] who reported that arabinogalactan (CPP) significantly inhibited induced gastric lesions in rats.

Furthermore, histological examination of the stomach isolated from rats pretreated with GA in this study showed near normal gastric mucosa with atrophied surface epithelium, some collagen fibers in the lamina propria and between the near normal gastric glands, and mild PCNA reaction in the gland cells. These agreed with...
the results of many researchers [41,40] who reported gastroprotective properties in several studies when polysaccharide was administered to rats before experimentally induced gastric ulcer. The potential activity of GA to act as a direct cytoprotective agent has been explained by researchers [39] who have stated that many mechanisms suggested for antulcer effects of polysaccharides lie in their ability to bind to the mucosal surface and to function as a protective coating, by diminishing the secretory activities of acid and peptic and protecting the mucosa by increasing mucus synthesis or scavenging radicals. Others [42] have reported that plant polysaccharides have attracted attention because of their therapeutic potential as antiulcer agents. Most of these actions are thought to involve components of the innate immune system, such as the complement system, and the release of ROS, nitric oxide, and cytokines by macrophages, dendritic cells, and granulocytes. Diverse plant polysaccharides are shown to interact specifically with pattern recognition receptors on innate leukocytes such as lectin-like receptors, toll-like receptors, and scavenger receptors on leukocytes.

It can be concluded that GA exerts a protective effect against stress-induced gastric mucosal lesions in rats.

References

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تمت إحصاء الصمغ العربي على قرحة المعدة المستحثة تجريبياً في ذكور الفئران البيضاء البالغة (دراسة هستولوجية و هستوكيماوية مناعية).

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قسم الأنسجة و بيولوجيا الخلية كلية طب بنيا2

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تعتبر قرحة المعدة مرض خطير و داه معدل كبير الحدو. و يعتبر الصمغ العربي هو الاتصال الصمغي الجاف الصالح لأكاد لفروع و سيفان الأكاذيبا السنغالية و قد عرف أنه يعمل كمضاد للأكسدة.

استخدم لهذه الدراسة 30 ذكرًا بالغًا قسمت إلى 3 مجموعات متساوية: المجموعة الأولى ضابطة و الثانية ذات القدرة الإجهاد و الثالثة أعيت الصمغ العربي. بعد 10 أيام من تعاطى الصمغ العربي تم تجويع الفئران لمدة 24 ساعة ، تم إجهاد المجموعة الثانية و الثالثة بتقييدها في جو من البرودة ، ثم تم تخدير الحيوانات في كل المجموعات بالإثرب. و تم استخراج المعدة و فتحها عند الانتهاء الأكبر ثم غسلها بملح البرق كما تم تحديد شدة القرحة و درجتها و معاملها. تم تثبيت العينات و صبغها ثم فحصها نسيجاً بالهيماتوكسيلين والإيوسين و نسجوج - كيمياءً بصبغة حامض البيبر أيوديك شيف و ماسون ثلاثي الألوان، و نسجوج- مناعياً لإظهار تفاعل بي بي إن آن.

و قد أوضح المتحدث أن الإصابة بقرحة المعدة قلت كثيراً في المجموعات التي تم علاجها مسبقًا بالصمغ العربي عن تلك التي لم تعالج. و قد قلت شدتها و معاييرها بشكل ملحوظ (32.8% و 39.58% على التوالي) عندما قورنت بالمجموعة المصابة. و كذلك قل مؤشر القرحة بشكل ملحوظ (49%) عندما قورن بالمجموعة المصابة.

و قد أظهرت صبغة الهيماتوكسيلين والإيوسين ضموريًا في الغشاء المخاطي للمعدة مع فقد للنسيج الغدي. و كان تفاعل حمض البيبر أيوديك شيف موجباً في خلايا الغشاء المخاطي للمعدة مع تجمع متوسط لائييف الكولاجين في صبغة ماسون ثلاثي الألوان. و قد أظهر النسيج الغدي تفاعلًا متوسطًا للبي بي إن آن.

أما في المجموعة التي أعطيت الصمغ العربي من قبل فقد أظهرت اقتراب الغشاء المخاطي للمعدة الطبيعية مع بقاء ضمور في النسيج الطلائي للسطح و تفاعل موجب لحمض البيبر أيوديك شيف في خلايا السطح و المخاطية العنقية. و قد وجد ترسب ملحوظ لألياف الكولاجين في الصفة الأولية و بين الغدد التي تم إصلاحها و كذلك تفاعل قوي للبي بي إن آن في النسيج الطلائي للسطح و الخلايا العنقية للغدد.

نتخليص من ذلك أن للصمغ العربي دور وقائي ضد قرحة المعدة الإجهادية في الفئران البيضاء.