Comparative study on the effectiveness of gum arabic Acacia senegal and Moringa oleifera extracts on prevention of peptic ulcer in the experimental rats

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ABSTRACT
Peptic ulcer is one of the world’s major gastrointestinal disorders and affecting 10% of the world population. An estimated 15000 deaths occur each year as a consequence of peptic ulcer. The cost of treatment for peptic ulcers is expensive and has side effects. So, it is necessary to conduct research to develop new therapeutic agents for the treatment of stomach ulcers. It is known that medicinal plants are important sources of biologically active molecules with the genetic ability of anti-ulcer. This study aims to evaluate the effectiveness of using gum arabic Acacia senegal and Moringa oleifera extracts on treatment of peptic ulcer in the experimental rats. Fifty rats were divided into 8 groups (6/each group). One group feeds the basic diet only (positive control group, G+). The remaining 44 rats were infected by aspirin solution to induce peptic ulcer in their stomach. One group of these rats feeds the basic diet only (negative control group, G-). Three groups G1, G2 and G3 feed on the basic diet + gum arabic extract (500, 1000 and 1500 mg/kg of rat weight). The other groups G4, G5, G6 feed the basic diet+ Moringa oleifera extract (800, 1000 and 1200 mg/kg of rat weight). At the end of the experiment (6 weeks), rats were fasted for 12 hours before slaughtering, blood samples were collected to obtain the serum, the stomach was separated for examination and gastric juice was collected to determine the (PH).

The results indicated that rats in groups G6 and G3 had the highest increase in number of their WBC and in their HP as compared to (G+).

Histological study revealed the absence of histopathological changes in G3 and G6, while other groups showed mucosal vascular congestion and submucosal edema with inflammatory cell infiltration.

Key words: Gum Arabic, Acacia senegal, Moringa oleifera, peptic ulcer, risk factors.

INTRODUCTION
Peptic ulcer disease (PUD) occurs due to a breakdown of the gastrointestinal tract’s mucosal defense particularly at a site where the mucosal epithelium is exposed to acid and pepsin (Akomas, et al., 2014; Hemamalini, et al., 2012; Osim, 2002). It affects 4 million people worldwide annually (Zelickson et al., 2011). The most common symptoms are waking at night with the middle or upper abdominal pain that improves with eating. Also include belching, vomiting, heartburn, bloating, weight loss, or poor appetite (Najm, 2011). Complications may include bleeding, perforation, and blockage of the stomach. Bleeding occurs in as many as 15% of people (Milosavljevic et al. 2011). In severe cases, symptoms can include dark or black stool (due to bleeding), vomiting blood (that can look like “coffee-grounds”), weight loss, perforation (a hole through the wall of the stomach) and gastric outlet obstruction from swelling or scarring that...
blocks the passageway leading from the stomach to the small intestine.

The treatment of chronic peptic ulcer disease varies depending on the etiology of the ulcer (HP or Non-steroidal anti-inflammatory drugs, whether the ulcer is initial or recurrent, and whether complications have occurred.

Plants are a rich resource used for centuries to cure various ailments. The literature revealed that many medicinal plants and polyherbal formulations are used for the treatment of ulcer. The first effective drug against gastric ulcer was carbeno-xolone, discovered as a result of research on a commonly used indigenous plant Glycyrrhiza glabra. Sasmal et al. (2007) indicated that cabbage previously employed as an antiulcer agent in folk medicine and it acts by enhancing the gastric mucosal strength.

Gum Arabic (GA) Acacia Senegal is reported to prevent development of indomethacin induced gastric ulcers in rats (Gohar and Zaki, 2014). Also, it is reputed in Arabian medicinal practices to be useful in treating patients with chronic renal failure. Additionally, GA is reported to possess antioxidant (Trommer and Neubert, 2005; Gado and Aldahmash, 2013).

Leaf extracts of Moringa oleifera showed gastric ulcer healing effect in acetic acid–induced chronic gastric ulcers. The acetone extract and methanol extract of the leaves produced gastric anti-secretory effect in pylorus-ligated rats and showed gastric cytoprotective effect in ethanol-induced and indomethacin-induced gastric ulcers. Leaf extracts also produced a significant reduction of stress-induced gastric ulcers. None of the extracts of the fruits showed any significant antiulcer effect. It was concluded that leaves of Moringa oleifera increase healing of gastric ulcers and also prevent the development of experimentally induced gastric ulcers in rats (Devaraj et al., 2007).

This study aimed to evaluate the effect of using extracts of each of gum arabic and Moringa oleifera on treatment of peptic ulcer in rats.

**MATERIALS AND METHODS**

**Materials**

**Animals:** 50 male albino rats (160 to 185g) were obtained from the animal house at the Agricultural Research Centre in Giza (ARC).

**Herbs:** Gum arabic" Acacia senegal" (1kg) and Moringa oleifera (1kg) were purchased from HARRAZ "Agricultural seeds, spices and medicinal plants" from Cairo Governorate, Egypt.

**Drugs:** Aspirin (Aspirin Revo at a concentration of 320 mg) was purchased from pharmacies. Vitamins and mineral salts were purchased from "Al Gomhoreya Company for Chemicals"-Cairo-Egypt.

**Methods**

**1. Preparation of aqueous extract**

The aqueous extracts of Gum Arabic and Moringa oleifera were prepared using 10g dried material/100 ml distilled water and boiling for 5 min at 100°C. Then they were filtrated, concentrated at 50°C under reduced pressure using a Rota vapor. The extracts were kept at -15 °C until it was used in the experiment (Kassi et al., 2004).

**2. Preparation of basal diet**

The basal diet was formulated to meet recommended nutrients levels for rats according to Hegested et al. (1941) as shown in Table (1).

| Ingredients       | Weight |
|------------------|--------|
| Casein           | 15%    |
| Corn oil         | 10%    |
| Cellulose        | 5%     |
| Vit. Mixture     | 1%     |
| Salt mixture     | 4%     |
| Corn starch      | 65%    |
| **Total**        | **100%** |
Experimental design

All Animals were fed on the prepared basal diet. Water is performed all the time. Rats were maintained under healthy conditions of humidity and temperature 22±2°C and maintained in air-conditioned room on a 12h light/dark cycle. They were acclimatized under the test condition for a week before treatments.

After acclimatization period, 50 rats were divided into 8 groups (6/group). Negative control group fed on the basal diet. Each of rats in the other groups were induced gastric ulcer by giving orally 4 ml aspirin solution on an empty stomach daily for a week according to Al Dalian et al. (2008) method. Sample of 2 rats from each group was taken to examine the stomach for gastric ulcers according to Agrawal et al. (2000) method.

Groups G1, G2 and G3 fed the basic diet + gum arabic extract (500, 1000 and 1500 mg/kg of rat weight), respectively. While groups G4, G5 and G6 were fed the basic diet + Moringa oleifera extract (800, 1000 and 1200 mg/kg of rat weight), respectively.

The experiment lasted for 6 weeks, during which the daily intake of the food was calculated by weight calculation once a week. At the end of the experiment, all rats were sacrificed after using an overdose of diethyl ether and their stomachs removed and washed by saline. The following items were investigated:

1- White Blood Cells (WBC) and Lymphocytes: Blood samples from each rat were collected separately count of WBC and lymphocyte.

2- pH and volume of gastric juice: Gastric juice was collected in a test tube to measure its volume and the pH of the stomach juice was determined using the pH meter (hi9021). Gastric juices were centrifuged at 500 rpm for 5 minutes, then separated and measured volume by graduated cylinder.

3. Histopathological examination

The stomachs of the scarified rats were taken and immersed in 10% formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. Specimens were then cleared in xylem, embedded in paraffin, sectioned at 4-6 microns thickness and stained with Heamtoxylin and Eosin stain for examination of the stomach as described by Carleton (1979).

4. Statistical analysis

The obtained results were expressed as Mean ± SE. Data were evaluated statistically using one-way analysis of variance (ANOVA). Significant difference between means was estimated at p<0.05 (STATSOFT, 2006).

RESULTS

1- Effect of gum arabic and Moringa oleifera extracts on WBC and lymphocytes in rats

WBC and lymphocytes in rats given orally gum arabic extract at doses 500, 1000 and 1500 mg/kg demonstrated significant increase in those of G1, G2 and G3 when compared with positive control group (G+) with the highest significant increase was in G3. On the other hand, WBC and lymphocytes were significantly decreased in rats of G1, G2 and G3 as compared with negative control group (G-) with lowest one recorded at G3.

The results of the effect of using Moringa oleifera extract at doses of 800, 1000 and 1200 mg/kg orally on WBC and lymphocytes in rats of G4, G5 and G6 showed significant increase in G4, G5 and G6 when compared with positive control group (G+), while there was a significant decrease in G4 and increase in G5 and G6 as compared with negative control group (G-). The highest significant increases in
WBC and lymphocytes were found in G6. As shown in Table (2).

Table 2: Effect of gum arabic and *Moringa oleifera* extract on WBC and lymphocytes in rats.

| Treat                                    | WBC count Mean ± SD | Lymphocytes count Mean ± SD |
|------------------------------------------|---------------------|----------------------------|
| (G+) Control Positive                    | 3,167.34 i ± 95.11  | 0.58 g ± 0.02              |
| (G-) Control Negative                    | 9,462.88 bc ± 333.96| 3.86 b ± 0.14              |
| G1 (feed GA 500mg/kg weight of rat)      | 6,601.81 h ± 232.99 | 2.76 f ± 0.10              |
| G2 (feed GA 1000mg/kg weight of rat)     | 7,999.63 fg ± 776.91| 3.35 d ± 0.33              |
| G3 (feed GA 1500mg/kg weight of rat)     | 9,133.26 cd ± 274.26| 3.73 bc ± 0.11             |
| G4 (feed M.o. 800mg/kg weight of rat)    | 8,238.02 ef ± 574.41| 3.36 d ± 0.23              |
| G5 (feed M.o. 1000mg/kg weight of rat)   | 9,720.00 ab ± 383.41| 4.37 a ± 0.17              |
| G6 (feed M.o. 1200mg/kg weight of rat)   | 10,132.00 a ± 130.27| 4.56 a ± 0.06              |
| F                                        | 122.421             | 212.008                    |
| Sig.                                     | 0.000               | 0.000                      |

Values with the same letters by column indicate no significant difference (p<0.05) and vice versa.

2- Effect of gum Arabic and *Moringa oleifera* extracts on pH and volume of gastric juice.

It was obvious from data in Table (3) that pH of gastric juice of rats in groups G1, G2 and G3 fed gum arabic extract was significantly increased when compared with pH value in positive control group (G+) with the highest value in G3. While it had significant decrease in G1, G2 and G3 when compared with negative control group (G−). Aqueous extract of *Moringa oleifera* at the investigated doses caused significant increase in pH of gastric juice of rats in groups G4, G5 and G6 as compared with positive control group (G+), while pH value had significant decrease in G4 and G5 and insignificant increase in G6 when compared with pH in gastric juice of the negative control group (G−).

The volume of gastric juice in rats treated with gum Arabic doses was significantly decreased in rats of groups G1, G2 and G3 when compared with negative and positive control groups (G- and G+, respectively) as indicated from Table (3). On the other hand, it was clear that volume of gastric juice in stomach of rats treated with *M. oleifera* extract increased in G5 and G6 and decreased in G4 when compared with positive control group (G+), while it was significantly decreased in G4 and increased in G5 and G6 when compared with negative control group (G−). The highest significant increase in volume of gastric juice was found in G6 as shown in Table (3).
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Table 3: Effect of gum arabic and *Moringa oleifera* extracts on pH and gastric juice volume in stomach of investigated rats.

| Treat                                        | pH       | Volume (cm$^3$) |
|----------------------------------------------|----------|----------------|
|                                              | Mean ± SD| Mean ± SD      |
| (G)$^+$ Control Positive                     | 1.69 f ± 0.08 | 1.34 c ± 0.04 |
| (G)$^-$ Control Negative                     | 2.80 a ± 0.10 | 1.26 d ± 0.04 |
| G1 (feed GA 500mg/kg weight of rat)          | 2.07 e ± 0.07 | 0.99 g ± 0.03 |
| G2 (feed GA 1000mg/kg weight of rat)         | 2.51 c ± 0.24 | 1.20 de ± 0.12 |
| G3 (feed GA 1500mg/kg weight of rat)         | 2.70 ab ± 0.08 | 1.21 de ± 0.04 |
| G4 (feed M.o. 800mg/kg weight of rat)        | 2.44 c ± 0.17 | 1.09 f ± 0.08 |
| G5 (feed M.o. 1000mg/kg weight of rat)       | 2.72 a ± 0.11 | 2.43 b ± 0.10 |
| G6 (feed M.o. 1200mg/kg weight of rat)       | 2.84 a ± 0.04 | 2.53 a ± 0.03 |
| F                                            | 41.841   | 401.133        |
| Sig.                                         | 0.000    | 0.000          |

Values with the same letters by column indicate no significant difference (p<0.05) and vice versa.

**Histopathological results**

Microscopically, stomach of rats from control negative group revealed the normal histological structure of gastric layers (Fig. A). Stomach of rats from control positive group revealed mucosal inflammatory cells infiltration, submucosal oedema, congestion and inflammatory cells infiltration (Figs. B). Some examined sections from group 1 showed congestion of mucosal blood vessels and submucosal oedema (Fig. C). Stomach of rat from group 2 showing submucosal oedema, congestion and few inflammatory cells infiltration. (Fig. D). However, stomach of rats from group 3 showed slight submucosal oedema (Fig. E). Moreover, stomach of rat from group 4 showing congestion of submucosal blood vessels and submucosal oedema. (Fig. F). Stomach of rat from group 5 showing slight submucosal oedema (Fig. G). Stomach of rat from group 6 showing no histopathological changes. (Fig. H).

On the other hand, Stomach of rat from group 7 showing focal necrosis of the mucosa, mucosal haemorrhage and submucosal inflammatory cells infiltration (Fig. J). Sections from stomach of rat from group 8 showing focal necrosis of the mucosa associated with inflammatory cells infiltration and submucosal oedema. (Fig. K). Stomach of rat from group 9 showing focal necrosis of the mucosa, and submucosal inflammatory cells infiltration. (Fig. L).
Figs. A-L. T.S. of stomach of rat. Stained Hx-E, X 100

**Fig. (A):** Control Negative group showing the normal histological structure of gastric layers.

**Fig. (B):** Control Positive group showing congestion of mucosal blood vessels (small arrow) and submucosal oedema with inflammatory cells infiltration (large arrow).

**Fig. (C):** Group 1AG (500mg/kg) showing congestion of mucosal blood vessels (small arrow) and submucosal oedema (large arrow).

**Fig. (D):** Group 2AG (1000 mg/kg) showing submucosal oedema (small arrow), congestion (large arrow) and few inflammatory cells infiltration (arrow head).

**Fig. (E):** Group 3 AG (1500mg/kg) showing slight submucosal oedema (arrow).

**Fig. (F):** Group 4M (800mg/kg) showing congestion of submucosal blood vessels (small arrow) and submucosal oedema (large arrow).

**Fig. (G):** Group 5M (1000mg/kg) showing slight submucosal oedema (arrow).

**Fig. (H):** Group 6M (1200mg/kg) showing no histopathological changes.

**Fig. (J):** Group 7 M&AG (500mg/kg) showing focal necrosis of the mucosa (small arrow), mucosal haemorrhage (large arrow) and submucosal inflammatory cells infiltration (arrow head).

**Fig. (K):** Group 8 M&AG (1000mg/kg) showing focal necrosis of the mucosa associated with inflammatory cells infiltration (small arrow) and submucosal oedema (large arrow).

**Fig. (L):** Group 9 M & AG (1500mg/kg) showing focal necrosis of the mucosa (small arrow), and submucosal inflammatory cells infiltration (large arrow).
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**Discussion**

Our finding showed that gum arabic aqueous extracts at the different tested doses (500, 1000, 1500 mg/kg) and moringa oleifera aqueous extracts at the different tested doses (800, 1000, 1200 mg/kg) had increased and improvement in WBC and lymphocytes when compared with those of positive control group (Co+). It has a therapeutic effect on the peptic ulcers when using gum arabic dose 1000 mg/kg and moringa oleifera at dose of 800 mg/kg. The rat’s stomachs showed signs of improvement in peptic ulcers in most sections with use moringa oleifera at dose of 800, 1200 mg/kg. results are similar that obtained in animal models, in another recent study, two doses of gum arabic (500, 1000 mg/kg) were found to exhibit antiulcer potentiality against acute indomethacin-associated ulceration in rats (AL-Yahya and Asad, 2016). In the same approach, treatment of rats with aspirin followed by gum Arabic (1 g/kg/day) for 21 days revealed remarkable preservation of gastric mucosa (Nasif, et al., 2011). The role of gum arabic in preventing gastric injury may be explained in terms of the antioxidant capacity of its constituents in protecting the cells from the injurious effect of ROS (AL-Yahya and Asad, 2016).

Ari-egoro, et al. (2019) showed that moringa oleifera at 37.5 g, 56.0 g and 75.0 g caused increase in the level of lymphocyte count in all the test groups. This observation showed the function of differential white blood cell (Lymphocyte) to fight against invading microorganisms. This study suggests that the consumption of moringa oleifera leaves at small quantity seems better. Lymphocytes are produced naturally by the body system in response to any foreign substances in the body. In order to avoid induction of lymphocytes, minimum quantities which are sufficient to perform the nutritional and/or therapeutic function by which it is used for must be taken into consideration during consumption.

Also, none of the doses tested at moringa oleifera (800, 1000, 1200 mg/kg) produced any gross apparent effect on general motor activity, muscular weakness, fecal output, feeding behavior etc., not cause any mortality in rats. These results are in agreement with those of Vinay et al. (2012), study found that 50% ethanolic leaves extract of moringa oleifera up to 2000 mg/kg did not cause any mortality in rats. None of the doses tested produced any gross apparent effect on general motor activity, muscular weakness, fecal output, feeding behaviour etc. during the period of observation.

Gum arabic and moringa oleifera showed improvement in reduction volume of gastric juice and increase pH, tetrable acidity and total acid output of gastric juice of rats and the group which treated gum arabic showed more effective in improvement. These results are in agreement with those obtained by Abeer, (2017) who showed that rats fed gum arabic powder (GAP) were effective to reducing the ulcer score, ulcer index, % ulceration and the 10% (GAP) was more effective. Also, Abdulrahman and Mohammed (2016): found that gum arabic at both tested doses orally produce significant reduction in the ulcer index; Also, Devaraj et al. (2007): who found that Healing of indomethacin-induced gastric ulcers the acetone and methanol leaf extracts of moringa oleifera showed a significant reduction in ulcer index and a significant increase in mucus content when compared with that of control. Stress-induced gastric ulcers all the three leaf extracts of moringa oleifera showed a significant reduction in
ulcer index when compared with those of control. Again, the acetone extract of the leaves was most potent in reducing ulcer index.

Conclusion
The present findings concluded that water extracts of gum arabic and moringa oleifera had treatment effect on peptic ulcer. Moringa oleifera extracts were more superior to gum arabic in its treatment and improvement of the stomach. The ratios used for gum arabic and moringa oleifera to treat and improve stomach ulcers did not result in any negative effects.

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دراسة مقارنة على فعالية مستخلصات الصمغ العربي والمورينجا على القرحة الهضمية في فئران التجربة

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المستخلص

الهدف من هذه الدراسة هو تقييم تأثير استخدام مستخلصات الصمغ العربي والمورينجا أوريغرا على قرحة المعدة. تم تقسيم 50 فأر إلى 8 مجموعات (6/كل مجموعة) ووضع واحدة على النظام الغذائي الأساسي فقط كمجموعة ضابطة. تم إعطاء 4 فأر في مجموعة G1 (G+) مع نظام غذائي غني بالدهون، وتم تأكيد الأصابات من القرحة بالفحص، وتم وضع مجموعة G2 (G-) على النظام الغذائي الأساسي فقط، وتم وضع مجموعة G3 على النظام الغذائي G6، G5، G4 على النظام الغذائي الأساسي + مستخلص المورينجا أوريغرا (G0). في نهاية التجربة (6 أسابيع) تم صيام الفئران لمدة 12 ساعة قبل الذبح وجمع عينات الدم للحصول على مصل الدم وفحص المعدة وجمع عصير المعدة وتحليل PH وتحديد الأسباب الناجمة. ولاحظت أن المجموعة 6 تم تذبحها في الثالث，则 تلاحظ الزيادة في الخلايا الليفية وحجم عصير المعدة. تمت تحليل عصير المعدة 6 ثم 3 تم تذبحها في المجموعة 6 مع المجموعة G4 بالمقارنة مع المجموعة PH بفضل مركبات Co-3،4 و 6،8،8 بينما أظهرت الأقسام الأخرى والجموعة 5 اكتساب الأوعية الدموية المخاطية وودمة تحت الخلايا الالتهابية وكتلة الدافئة تحت المخاطية في المجموعة 7.