

Antiulcer protective activity of gum Arabic (*Acacia Senegal*) in adult rats

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ABSTRACT

Arabic Gum (AG) is an edible, and has a complex chemical composition. In folk medicine, AG has been reported to be used internally for the treatment of inflammation of the intestinal mucosa, and externally to cover inflamed surfaces. It has been claimed to act as an antioxidant. The objective of this study were assessed the protective activity of Arabic gum on against peptic ulcer which induced by ethyl alcohol in adult male rats and its application in bakery product. Forty rats were randomly divided into five groups (n=8 for each), the first and second groups fed standard diet, the third, fourth and fifth groups fed standard diet containing 2.5, 5, 10% Arabic gum powder (AGP) respectively. At the last day of experimental period (28th day), the rats were fasted for 24 h with free access to water. The rats of second, third, fourth and fifth groups were received a single orally dose of ethyl alcohol 90% at 10 ml/kg body weight. After one hour later and under anesthesia by diethyl ether, abdominal wall was opened, the pylorus identified, stomachs ligated from esophageal opening, removed, opened at greater curvature, gastric juice collected and centrifuged for studying of gastric secretion parameters. Also, AGP was used to replace part of the whole wheat flour (0%, 2.5%, 5% and 10%) in standard bread. Appearance, taste, flavor, texture, , color and overall acceptability were evaluated in bread. The results showed that groups which treatment with AGP was significantly decreased in ulcer score, ulcer index and increase in preventive index compared with the positive control group. Supplemented rats diet with 10% of AGP was more effective to protect the stomach of ulcer. Moreover sensory evaluation showed that all replacement of GAP in bread was showed acceptable by the panelists. It conclude that Arabic gum had a protective activity against peptic ulcer in adult rats which induced by ethyl alcohol.

Key words: Arabic gum powder – peptic ulcer – ethyl alcohol ulcer score – gastric juice - bread

INTRODUCTION

Ulcers are most common on the skin of the lower extremities and in the gastrointestinal tract, although they may be encountered at almost any site. There are many types of ulcer such as mouth ulcer, esophagus ulcer, peptic ulcer, and genital ulcer (**Debjit et al., 2010**).

Peptic ulcer is a major health hazard both in terms of morbidity and mortality. The two most common types of peptic ulcer are called “gastric ulcer” and “duodenal ulcer.” The name refers to the site of ulceration. Many factors, such as non-steroidal anti-inflammatory drugs, helicobacter pylori, alcohol abuse, bile salts and acidifying salts are reported to change the gastro-duodenal mucosal defense with subsequent development of peptic ulcer. Also, stress, cigarette

smoking and spices are known to be contributing causative factors for peptic ulcer development (**Yuan et al., 2006**). Symptoms of peptic ulcer include nausea, vomiting and weight loss (**Vyawahare et al., 2009**). In some cases, peptic ulcer can be life threatening with symptoms like bloody stool, severe abdominal pain, and cramps along with vomiting blood. The World Health Organization (WHO) has listed more than 21,000 plants, which are used for many medicinal purposes around the world (**Kathe, 2005**).

Arabic Gum (AG) is defined as the natural secretion from stems and branches of Hashab tree (Acacia Senegal) (**JECFA, 1990**). Arabic Gum is a branched chain complex polysaccharide, either neutral or slightly acidic, found as a mixed calcium,

magnesium, and potassium salt of a poly saccharide acid (Ali et al., 2009). It can be considered as an arabinogalactan containing less than 5% glycoprotein (Codipilly et al., 2006).

AG is used widely as an additive in food materials e.g. confectionery, ice-cream industries and bakery products. It is classified as an edible coating and it is used to increase stability and shelf-life and to enhance microbial safety of fruits (Roony, 2005). Gum arabic 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, AG is reported to possess antioxidant (Trommer and Neubert, 2005) and Gado

and Aldahmash, 2013) renal protective (Ali et al., 2015 and Nasir, 2013)) and antidiabetic effects (Nasir et al., 2010). Therefore, the present investigation aims to evaluate the anti-ulcerogenic protective activity of gum arabic in adult male rats and its application in food.

Materials and Methods

Materials:

Arabic Gum (*Acacia Senegal*) was purchased from Hraz – Cario – Egypt. Ethyl alcohol (90%), Folin–Ciocalteu phenol reagent, gallic acid were purchased from Sigma–Aldrich Inc. (St. Louis, MO, USA). All other chemicals were obtained from El - Gomhoreya Company, Cairo, Egypt.

Preparation of Arabic gum

Arabic Gum were milled by (Moulinex miller, France) to be a fine powder.

Determination of chemical analysis of gum Arabic:

Arabic Gum were milled by (Moulinex miller, France) to be a fine powder. Sample of the prepared gum arabic was taken for estimating its chemical composition (moisture, protein, fat, ash, total sugars, some sugars and polysaccharide, using the methods of **A.O.A.C (2012)**. Total phenolic content expressed as gallic acid equivalent (GAE) was determined by the Folin–Ciocalteu micro-method according to **Saeedeh and Asna, (2007)**. Total flavonoids content expressed as quercetin equivalent (QE) was determined by the method of **Ordon et al., (2006)**.

Animals:

Forty adult male Sprague Dawley rats, with an average body weight 195-200 g were purchased from the Veterinary Medicine Institute, Cairo, Egypt. Under normal laboratory conditions, with 12-hours

light-dark cycle at $25 \pm 1^\circ\text{C}$, rats were housed in cylindrical wire cages with wire bottoms. The diet was introduced to rats in special food cups to avoid scattering of food. Also, water was provided to the rats by glass tube projection through the wire cage. Food and water provided and checked daily. Rats were fed standard diet according to AIN-93 guidelines (**Reeves et al., 1993**). All animals received care in compliance with the Egyptian rules for animal protection.

Experimental groups:

Rats were randomly divided into five groups (n=8 for each), the first and second groups fed standard diet. The third, fourth and fifth groups fed standard diet containing 2.5, 5, 10% of Arabic gum powder (AGP) respectively. At the last day of experimental period (28th day), the rats were fasted for 24 h with free access to

water. The rats of second, third, fourth and fifth groups were received a single orally dose of ethyl alcohol at 10 ml/kg body weight (**Huang et al., 2014**) to induce gastric ulceration for 2 h. And the first group (a negative control group) received a single orally dose of saline (0.9%, w/v).

Collection of gastric secretion and determine ulcer index:

After administration of ethyl alcohol to animals at two hour later **and** under anesthesia, Abdominal wall was opened, the pylorus identified, stomachs ligated from esophageal opening and removed, opened at greater curvature, gastric juice collected and centrifuged for studying of gastric secretion parameters including volume in (ml), titratable acidity, Meq/L, **titratable acid output MEq/h**. Stomach examined for ulceration. Evaluation of

degree of ulceration was expressed in terms of ulcer score which is calculated by dividing the total number of ulcers in each group by number of rats in that group (**Robert et al., 1968**). Ulcer index (U.I) was calculated by multiplying ulcer score x 100 (**Radwan et al., 2003**), the ulceration (%) was calculated by dividing the number of animals with ulcer by the total number of animals and multiplying by hundred (**Ohara et al., 1992**) and the preventive index was calculated according to the method of **Hano et al. (1976)**.

Determination of titratable acidity and pH value of gastric secretion

0.2 ml of centrifuged gastric juice was titrated using phenol red as an indicator with end point at 7.0 pH against 0.01 NaOH . Titratable acidity was calculated in Meq/L. **Total titratable acid output**

Meq/L amount of NaOH that neutralize 100mg of gastric juice (**Deverport, 1972**), pH value were determine according to (**Debnath et al., 1974**).

Histopathology examinations of the stomach:

A histopathology examination of the stomach was determined according to the method described by **Banchroft et al., (1996)**.

Preparation of bread and sensory evaluation

Bread was prepared by mixing 100 g of wheat flour (82% extraction), 0.5 g of active dry yeast, 1.5 g of sodium chloride, 75–80 ml of water by hand for about 6 min to form the needed dough. AGP was used to replace part of the whole wheat flour (0, 2.5, 5 and 10 %) in a standard .Bread recipe. The dough was left to ferment for 1 h at 30°C and was then divided into 125 g pieces. The pieces were

arranged on a wooden board that had been sprinkled with a fine layer of bran and were left to ferment for about 45 min at the same temperature. The pieces of fermented dough were flattened to be about 20-cm in diameter. The flattened loaves were proofed at 30–35°C for 15 min and then were baked at 400–500°C for 1–2 min. The loaves were allowed to cool at room temperature for 2 h before for sensory evaluation (**Eissa et al., 2007**). Samples of bread were subjected to organoleptic tests (by fifteen judges) according to **Watts et al., (1989)**. Judging rang for appearance, taste, flavor, texture, compressibility, color and overall acceptability was as follow, Excellent (9-10), Very good (8 -7), Good (5-6), Fair (3-4), Poor (1-2) and very poor (0-1).

Statistical Analysis

Results were expressed as the mean \pm SD. Data for

multiple variable comparisons were analyzed by one-way analysis of variance (ANOVA). For the comparison of significance between groups, Duncan's test was used as a post hoc test according to the statistical package program (**Artimage and Berry, 1987**).

RESULTS & DISCUSSION

Data in Table (1) showed proximate the major chemical constituents of gum Arabic powder. The AGP had 4.75% ash, 2.26% crude protein and 0.69% total fat. **Idris et al., (1998)** reported that gum arabic comprised of 3 1.5 –2.6% protein and 12.5 –16.0% moisture. The chemical composition of AG can vary with its source, the age of the trees from which it was obtained, climatic condition and soil environment (**Al-Assaf et al., 2005**). In the same table, gum arabic contain 73.57% fiber, 6.88 % total sugar,

0.18 glucose, 1.61 galactose, 2.66 mannose and 71.06 polysaccharide. (**Badreldin et al., 2008; Abdul-Hadi et al., 2010**) reported that Arabic gum is a branched-chain, complex polysaccharide, either neutral or slightly acidic.

Also total phenolic compounds and flavonoids of AGP were 60.89 mg gallic acid / 100 mg and 18.25 mg catechin / 100 gm respectively. These results were higher than the results obtained by **El Sheikh, (2014)** who reported that gum arabic contain 10 mg / 100 gm phenolic compounds. **Abdulrahman and AL-Yahya, (2016)** reported that flavonoids and carbohydrates were found to be present in arabic gum.

Data in Table (2) showed the effect of gum arabic on ulcer score, ulcer index, % ulceration and preventive index in normal rats and rats with stomach ulcer. No ulcer score, ulcer

index and % ulceration in rats which received saline solution (normal group).

On the contrary, positive control group which received ethyl alcohol alone produced bleeding indicating severe gastric damage and an increase in ulcer score, ulcer index and % ulceration than other treated group. **Ko and Cho, (2000)** reported that alcohol had been shown to affect the mucosal barrier and histology. These ulcerogenic effects play a crucial role in altering gastric mucosal defense mechanisms.

The gastric lesion produced by ethanol induced gastric ulcers is due to stasis in gastric blood flow that leads to the development of the hemorrhage and necrosis. All these events lead to cell death and exfoliation in the surface epithelium (**Brzowski et al.,1998**). Rats which feeding AGP were effective to reduce the ulcer score, ulcer index, %

ulceration and the 10% AGP was more effective. These results were similar to the results obtained by **Abdulrahman and AL-Yahya (2016)**, who found that gum arabic at both tested doses orally produce significant reduction in the ulcer index.

Also, **Helala et al., (2015)** showed that pretreatment with AG significantly decreased the gastric lesions. And both ulcer severity and score were significantly decreased compared with the untreated group. Arabic Gum has been reported to reduce the development of indomethacin induced gastric ulcers (**Gohar and Zaki, 2014**).

In the same table positive control group which received ethyl alcohol alone produced reduction in preventive index, while an increase in preventive index was observed in groups which treated with Arabic

gum and the rats which administration 10% AG produced a higher increase in preventive index which was 83.3%.

In the same table positive control group produced reduction in preventive index. However an increase in preventive index was observed in groups treated with Arabic gum, and the rats supplemented with 10% AG produced a higher increase in preventive index which was 83.3%. Arabic Gum is a known antioxidant and this would have contributed to its antiulcer action (**Goodrum et al., (2000)**).

Moreover, Arabic gum contain an arabinogalactan, has been reported to possess antiulcer effect in rats (**Goodrum et al., (2000)**). It is known to reduce development of ethanol induced gastric ulcers in rats (**Cipriani et al., 2006**).

Data presented in Table (3) illustrated the

effect of Arabic gum on volume, pH, tetrable acidity and total acid output of gastric juice of normal rats and rats with stomach ulcer. Negative control group was significantly lower ($P \leq 0.05$) in volume of gastric juice and higher ($P \leq 0.05$) in pH, tetrable acidity and total acid output of gastric juice when compared to positive control group and AGP groups. However rats which treated with ethyl alcohol alone were produce an increase in volume of gastric juice and reduction in pH, tetrable acidity and total acid output of gastric juice. High gastric acidity is known to be a factor in the etiology of peptic ulcer (**ENO et al., 2004**).

In current table, AGP 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

with 10% AGP showed more effective in improvement. These results are agreement with by some researchers **Cipriani et al., (2009)** and **Tanaka et al., (2010)** who showed that arabinogalactan which is found in gum arabic significantly inhibited induced gastric lesions in rats.

The potential activity of AG to act as a direct cytoprotective agent has been explained by **Cipriani et al., (2009)** who have stated that many mechanisms suggested for antiulcer 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 or scavenging radicals.

Sensory evaluation of breads prepared with AGP portions are shown in Table (4) and picture (1). No significant ($p > 0.05$) difference was observed

appearance, flavor, texture and color between bread prepared with 2.5, 5, and 10% of AGP and control bread. However the bread prepared with 10% of AGP had lower ($P \leq 0.05$) taste, compressibility and overall acceptability than bread prepared with 0, 2.5 and 5% of GAP.

Arabic Gum is also useful in the baking industry because of its viscous and adhesive properties (**ITC, 2008**). **KL khalifa et al., (2007)** indicated that acceptable bakery products e.g. bread and pizza could be obtained using Arabic gum. Arabic Gum is used in a range of bakery products (**FAO, 1995**).

Microscopically, stomach of rat from negative control group (normal rats) revealed the normal histological gastric structure (Figs.1). In contrary, stomach of rats from positive control group (group which treated with ethyl alcohol

alone) showed severe histopathological changes described by focal necrosis of gastric mucosa, haemorrhage, submucosal oedema associated with inflammatory cells infiltration (Figs.2). These results were agree with **Ko and Cho (2000)**, who reported that chronic active gastritis is associated with chronic alcohol ingestion. Examined sections from 2.5% AGP group revealed focal necrosis of gastric mucosa and slight submucosal oedema (Figs.3). However, stomach of rats from group 5% AGP group revealed no histopathological changes except slight submucosal oedema (Fig.4)., whereas, other sections from this group revealed no histopathological changes (Fig. 5). These agreed with the result by **Galati et al., (2001)** and **Ghildyal et al (2010)** who showed gastro protective properties in several studies when

polysaccharide was administered to rats before experimentally induced gastric ulcer.

Antiulcer effects of polysaccharides lie in their ability to bind to the mucosal surface and to function as protective coating pepsin and protecting the mucosa by increasing mucus synthesis or scavenging radicals (**Cipriani et al., 2009**).

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Table (1): Major chemical constituents of Arabic gum

Parameters	Gum arabic
Moisture (g/100 ml)	12.57 ± 0.91
fat (g/100 ml)	0.69 ± 0.17
protein (g/100 ml)	2.26 ± 0.29
ash (g/100 ml)	4.51 ± 0.79
Fiber (g/100 ml)	73.57 ± 4.33
Total sugar (g/100g)	6.88 ± 0.65
Glucose (g/100g)	0.178± 0.00
Glactose (g/100g)	1.61±3.33
Mannose (g/100g)	2.66± 0.19
Polysuccaride (g/100g)	71.06± 3.28
Total phenolics (mg gallic/100 gm)	60.89 ± 5.13
Total Flavonoids (mg catechin/100 gm)	25.67± 2.42

Each value in the table is the mean ± standard deviation of three replicates.

Table (2): Effect of gum Arabic on ulcer score, ulcer index, % ulceration and preventive index of normal rats and rats with stomach ulcer

Parameters	negative control group	Ethyl alcohol groups			
		Positive control group	2.5% GAP	5% GAP	10% GAP
Ulcer score	--	9.5	5.97	2.33	0.83
Ulcer index	--	950	597	233	83
% Ulceration	--	83.3	66.6	33.4	16.7
Preventive index	--	16.7	33.4	66.6	83.3

Table (3): Effect of gum Arabic on volume, pH, tetrable acidity and total acid output in gastric juice of normal rats and rats with stomach ulcer

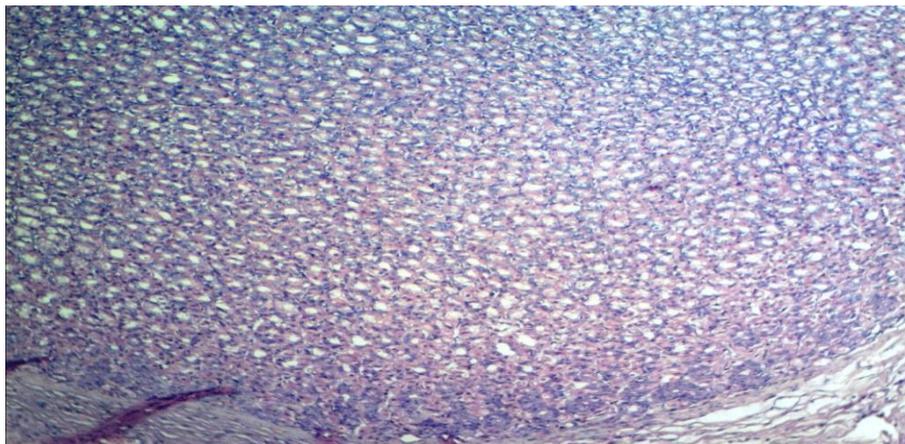
Parameters	negative control group	Ethyl alcohol groups			
		Positive control group	2.5% GAP	5% GAP	10% GAP
Volume of gastric juice (ml)	2.27 ^d ± 0.19	5.70 ^a ± 0.58	3.91 ^b ± 0.60	3.36 ^c ± 0.29	2.9 ^c ± 0.36
pH	3.20 ^a ± 0.16	1.30 ^e ± 0.08	1.97 ^d ± 0.94	2.49 ^c ± 0.18	2.97 ^b ± 0.07
Tetrable acidity (Meq/L)	9.12 ^e ± 0.90	15.59 ^a ± 1.25	12.87 ^b ± 0.65	11.55 ^c ± 0.26	10.25 ^d ± 0.65
Total acid output (Meq/1h)	182.4 ^e ± 14.05	311.9 ^a ± 24.97	257.5 ^b ± 13.07	231.1 ^c ± 5.21	205.1 ^d ± 12.98

Data are expressed as mean ± SD. Values within a row having different superscripts are significantly different (p ≤ 0.05); where the small letters indicate significant among dietary treatment groups as indicated by one-way ANOVA followed by Duncan's multiple range test (a > b > c > d > e)

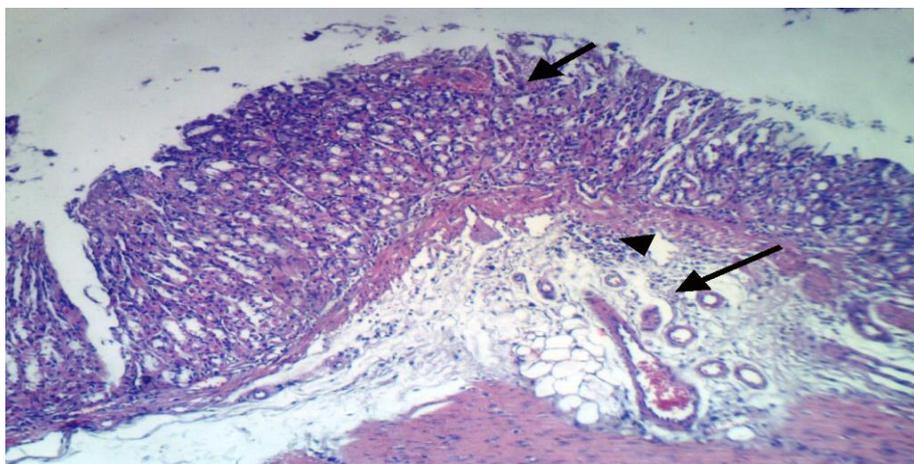
Table (4): Sensory evaluation of breads prepared with GAP portions.

Bread Parameters	GAP portions			
	0%	2.5%	5%	10%
Appearance	9.12 ^a ± 0.35	9.25 ^a ± 0.37	9.06 ^a ± 0.49	9.06 ^a ± 0.32
Taste	9.37 ^a ± 0.35	9.13 ^a ± 0.23	9.25 ^a ± 0.25	7.93 ^b ± 0.39
Flavor	9.43 ^a ± 0.41	9.37 ^a ± 0.44	9.31 ^a ± 0.37	9.25 ^a ± 0.27
Texture	9.63 ^a ± 0.44	9.75 ^a ± 0.27	9.38 ^a ± 0.35	9.38 ^a ± 0.35
compressibility	9.62 ^a ± 0.44	9.63 ^a ± 0.35	9.43 ^a ± 0.41	8.06 ^b ± 0.49
Color	9.38 ^a ± 0.44	9.18 ^a ± 0.25	9.25 ^a ± 0.26	9.25 ^a ± 0.27
Overall acceptability	9.25 ^a ± 0.46	9.25 ^a ± 0.26	9.18 ^a ± 0.25	8.13 ^b ± 0.52

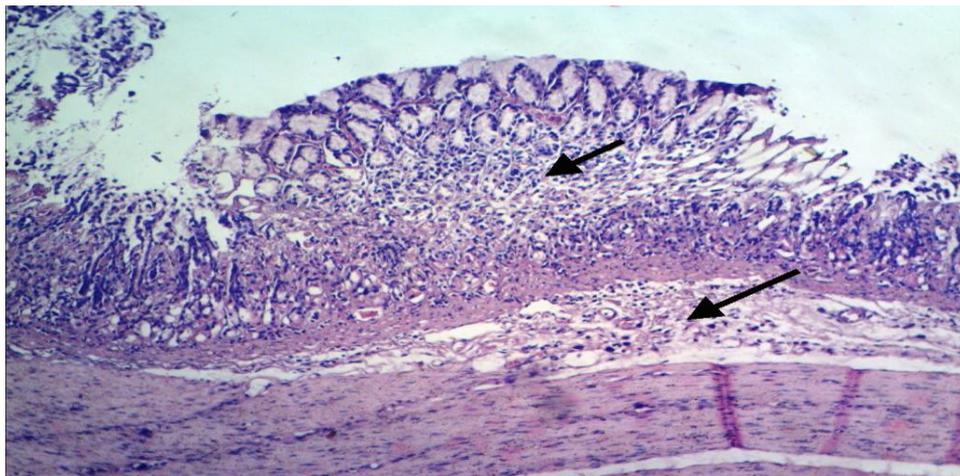
Data are expressed as mean ± SD. Values within a row having different superscripts are significantly different ($p \leq 0.05$); where the small letters indicate significant among dietary treatment groups as indicated by one-way ANOVA followed by Duncan's multiple range test ($a > b > c > d > e$)



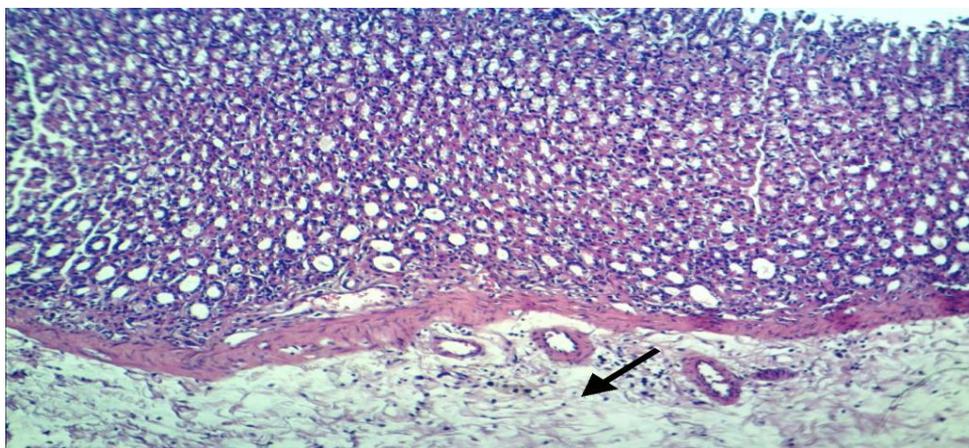
Picture (1): Stomach of rat from negative control group showing the normal histological layers (H & E X 100)



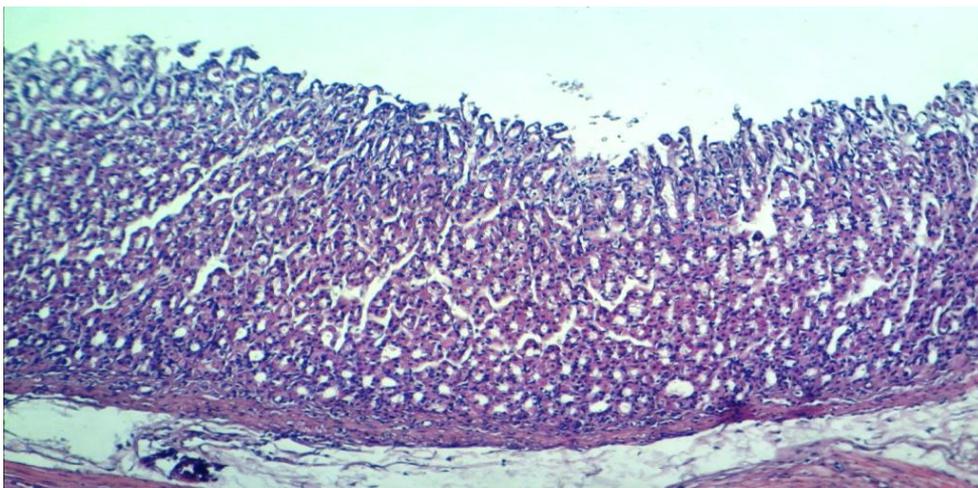
Picture (2): Stomach of rat from positive control group showing focal necrosis of gastric mucosa (small arrow), submucosal oedema (large arrow) associated with inflammatory cells infiltration (arrow head) (H & E X 100).



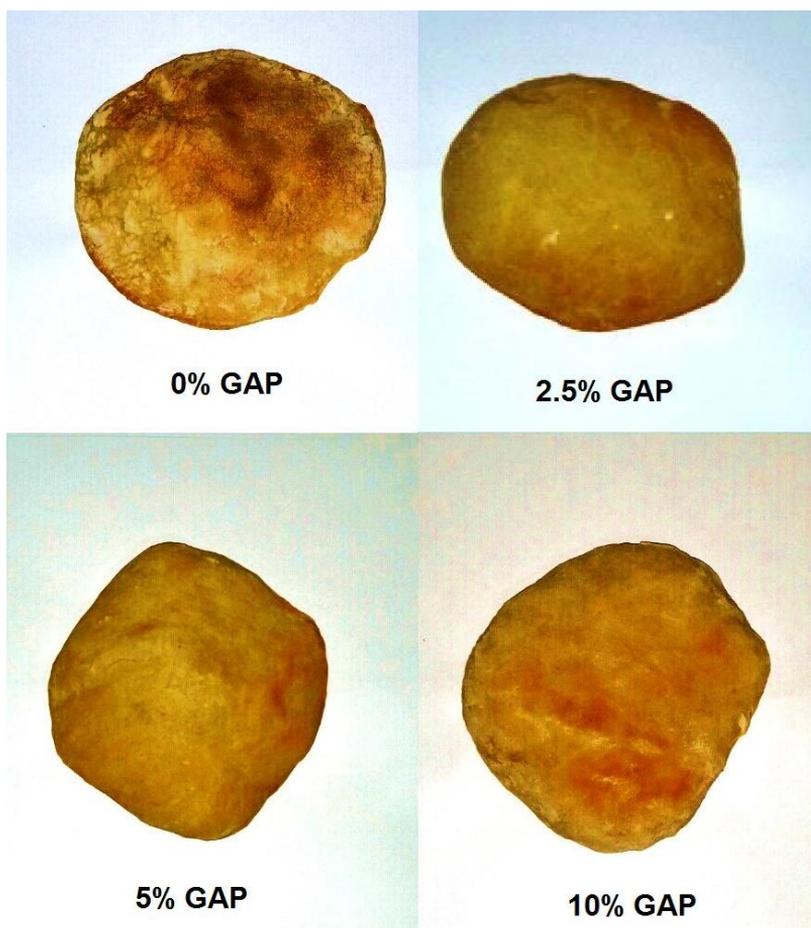
Picture (3): Stomach of rat from 2.5% AGP group showing focal necrosis of gastric mucosa (small arrow) and slight submucosal oedema (large arrow) (H & E X 100).



Picture (4): Stomach of rat from 10 % AGP group showing slight submucosal oedema (arrow) (H & E X 100)



Picture (5): Stomach of rat from 10 % AGP group showing no histopathological changes (H & E X 100)



Pic. (1): Flatbread supplemented with different portions with AGP.

التأثير الوقائي المضاد للقرحة للصمغ العربي في الفئران البالغة

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الملخص العربي

الصمغ العربي يحتوي على تركيبة كيميائية معقدة، وقد ذكر في الطب الشعبي استخدامه داخل الجسم لعلاج التهاب الغشاء المخاطي في الأمعاء، وخارجيا لتغطية الأسطح الملتهبة. وقد ادعى أنه يعمل كمضاد للأكسدة. هدفت هذه الدراسة إلى تقييم النشاط الوقائي للصمغ العربي ضد القرحة الهضمية التي يسببها الكحول الإيثيلي لدى الفئران البالغة الذكور وتطبيقها في منتجات المخابز. تم تقسيم أربعين فئران عشوائيا إلى خمس مجموعات (ن = 8 لكل منهما)، الأولى (مجموعة الضابطة السالبة) والمجموعة الثانية (المجموعة الضابطة الموجبة) تغذت على الوجبة الرئيسية. أما المجموعات الثالثة والرابعة والخامسة تغذت على الوجبة الأساسية و 2.5، 5، 10٪ من مسحوق الصمغ العربي على التوالي. في اليوم الأخير من الفترة التجريبية (اليوم 28)، تم تصويم الفئران لمدة 24 ساعة مع تناول الماء. وقد اعطيت الفئران من المجموعات الثانية والثالثة والرابعة والخامسة جرعة واحدة عن طريق الفم من الكحول الإيثيلي 90٪ (10 مل / كغم من وزن الجسم) و بعد ساعة واحدة وتحت التخدير بواسطة الأثير ثنائي إيثيل. تم فتح جدار البطن، وتحديد البواب، والمعدة من فتحة المريء وإزالتها، وفتحت من الانحناء الأكبر، تم تجميع عصير المعدة وعمل طرد مركزي له لدراسة المؤشرات الخاصة به. أيضا تم استخدام الصمغ العربي ليحل محل جزء من دقيق القمح الكامل (0٪، 2.5٪، 5٪ و 10٪) في الخبز القياسي. تم تقييم المظهر والطعم والنكهة والملمس والانضغاطية واللون وقبولها بشكل عام في الخبز. وأظهرت النتائج أن المجموعات التي تناولت الصمغ العربي البودر انخفضت بشكل ملحوظ في درجة القرحة، ومؤشر القرحة وزيادة في المؤشر الوقائي مقارنة مع المجموعة الضابطة الموجبة. ولكن المجموعة التي تغذت على 10٪ من بودرة الصمغ العربي كانت أكثر فعالية لحماية المعدة من القرحة. كما أظهر التقييم الحسي للخبز ان جميع نسب الصمغ العربي المضافة للخبز اعطت قبولاً من قبل المحكمين و خلصت الدراسة الى ان الصمغ العربي يمتلك نشاط وقائي مضاد للقرحة المحدثة بواسطة الكحول الايثيلي عند الفئران البالغة .

الكلمات المفتاحية : بودرة الصمغ العربي- قرحة المعدة - الكحول الإيثيلي - العصير المعدى - الخبز - معامل القرحة .