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The Potential Protective Effect Of Arabic Gum *(Acaciasenegal)* On Rats Infected With Gastric Ulcer Causedby Ethyl Alcohol

HebaEzz El-Din Youssef and FatmaTalatSaif
Nutrition and Food Sciences Department, Faculty of Home Economics
Minufiya University, Shibin El-Kom, Egypt

Abstract

Arabic gum is used throughout the world for various purposes including food additive and pharmaceutical excipient. Arabic gum was showed to be used internally for the treatment of inflammation of the intestinal mucosa. This study aimed to evaluate the potential protective effect of arabic gum on rats infected with gastric ulcer rats caused by ethyl alcohol.Thirty male albinorats were randomly divided into five groups (6 rats each), the first and second groups fed standard diet, the third, fourth and fifth groups fed standard diet containing 2.5, 5, 7.5% arabic gum (AG) powder respectively. At the end of experimental period (30^{th} day),the rats were fasted for 24 h with free access to water. The rats of second, third, fourth and fifth groups were given a single orally dose of ethyl alcohol 95% at 10 ml/kg body weight while, the negative control group gave a single orally dose of saline (0.9%, w/v).After two hour later and under anesthesia by diethyl ether, abdominal wall was opened and gastric juice collected and centrifuged for studying of gastric secretion parameters. The results showed that groups which treatment with AG was significantly decreased in ulcer score, ulcer index and increase in preventive index compared with the positive control group. Supplemented rats diet with 7.5% of AG was more effective to protect the stomach of ulcer than supplemented with 2.5% and 5%. Arabic gum was used to replace part of the whole wheat flour (2.5%, 5% and 7.5%) in standard bread. Sensory evaluation showed that all replacement of AG in bread was showed acceptable by the panelists.The study concluded that arabic gum had a protective activity against peptic ulcer in adult rats which induced by ethyl alcohol.

Key words: Arabic gum– peptic ulcer –gastric juice – bread.

Introduction

Peptic ulcer is the most common disease of gastrointestinal tract **(Kulshreshtha*et al.*, 2017).**There are many types of ulcer such as mouth ulcer, esophagus ulcer, peptic ulcer, and genital ulcer **(Debjit*et al.*, 2010)**.The two most common types of peptic ulcer are called "gastric ulcer" and "duodenal ulcer." The name refers to the site of ulceration **(Yuan *et al.*, 2006)**. Factors responsible for peptic ulcers includes eating too muchspicy and fatty food, stress, drinking alcohol and coffee **(Anwar and Sadeeqa, 2018).**It is believed that peptic ulcers develop due to an imbalance between aggressive factors (Helicobacter pylori, non-steroidal anti-inflammatory drugs, gastric acid) and protective factors (mucin, bicarbonate and prostaglandins) leading to an interruption in the mucosal integrity **(Kumar, 2019**).Symptoms of peptic ulcer include epigastric pain, heartburn, nocturnal pain, nausea, postprandial pain, weight loss due to decreased appetite. If symptoms remain untreated, it may lead to complications like gastrointestinal bleeding, perforations, penetration, narrowing and obstruction **(Rashid *et al.*, 2016)**

Arabic gum (AG) is one of these natural compounds and is composed of water-soluble dietary fibers that are produced from the dried gummy exudates of the stems and branches of *Acacia senegal* and *Acaciaseyal***(Hammad*et al.*,2019**).It is widely used throughout the world especially in the Arabian countries, where it is chewed or its powder is added to juices to make them viscous. it is used commercially as a stabilizer, thickening agent and emulsifier, mainly in the food and pharmaceutical industries (**Verbeken*et al.*, 2003).**Gum arabic is reported to possess antioxidant **(Gado and Aldahmash, 2013),** renal protective **(Ali *et al.*, 2015)** and anti-diabetic effects**(Nasir*et al.*,** 2010). Furthermore, gum arabic is reported to prevent development of indomethacin induced gastric ulcers in rats **(Gohar and Zaki, 2014).**Therefore, the present study aims todetermine the protective effect of arabic gum on gastric ulcers in rats.

Materials and Methods

Materials

Arabic gum*(AcaciaSenegal)*was purchased from the local market.Ethyl alcohol (95%) and all other chemicals were obtained from El-Gomhoreya Company, Cairo, Egypt.

Preparation of arabic gum

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

Determination of chemical analysis of gum arabic

Moisture, crude protein, crude fat, total ash and crude fiber contents were determined indried gum arabic according to **AOAC method (2012).** The total carbohydrate content was calculated by difference. 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).**

Experimental groups

Thirty six adult male rats Sprague Dawley weighting (150±5 g) were used in this study. The animals were housed individually in well aerated cages under hygienic laboratory condition and fed standard diet according to AIN-93 guidelines **(Reeves *et al.*, 1993)** for 7 days as an adaptation period. Rats were randomly divided into five groups (6 rats each), the first and second groups fed standard diet. The third, fourth and fifth groups fed standard diet containing 2.5, 5 and 7.5% of arabic gum powder (GA) respectively. At the last day of experimental period (30^{th} 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. The negativecontrol 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 two hour laterand under anesthesia by diethyl ether. 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 0.01NaOH.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:

Histopathology examinations of the stomach was determine 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. AG was used to replace part of the whole wheat flour (2.5, 5 and 7.5 %) in a standard bread recipe. Sensory evaluations of balady bread performed using 10 panelists of staff members of Nutrition and Food Science Department, Menoufia University. Panelists were selected on the basis of their interest and availability. Sensory quality properties were evaluated using a 9 point hedonic rating scale with 1 for dislike extremely to 9 for like extremely for each property. Flat bread was evaluated for appearance, crust color, crumb colors, taste, aroma 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) (**Attia-Afaf, 1986).**

Statistical Analysis:

The results recorded as the mean ± SD. The experimental data were subjected to an analysis of variance (ANOVA) for a completely randomized design using a statistical analysis system **(Artimageand Berry, 1987)**. Duncan's multiple range tests were used to determine the differences among means at the level of 5%.

Results and Discussion

Chemical composition, total phenolics and total flavonoids of dried arbic gum were presented in Table (1). Data showed that the arbic gum (GA) contained 2.2, 12.34,2.5, 0.58,73.07 and 8.72 % for protein, moisture,ash, fat,fiber and carbohydrates respectivelly. These results are similar with those reported by**Musa*et al.*, (2018) and Kheder, (2017)**who found thatarabic gumhad protein (2.3-2.7%), moisture (12-15%), fat (0.69%),ash (2.4- 4.51%) and fiber (73.57%). In the same table**,** gum arabic hadtotal phenolic compounds (61.45 mg gallic acid/100 mg) and flavonoids (25.29 mg catechin/100 gm). These results were higher than the results obtained by **El Sheikh, (2014)** who reported that gum arabic contain 10 mg/100 gm total phenolic compounds.

Data presented in Table (2) illustrated the effect of arabic gum on volume, pH, terrible acidity and total acid output in gastric juice of negative control and gastric ulcer groups. Ethanol is known to rapidly penetrate the gastric mucosa causing damage to the plasma membrane that causes increased membrane permeability to sodium and water **(AL-Yahya and Asad, 2016)**. The negative group was significantly lower ($P\leq 0.05$) in pH and higher ($P\leq 0.05$) in volum gastric juice, tetrable acidity and total acid output compared to the positive group. Oral injection to rats with ethanol in positive group led to decreased pH and increase volume gasric juice, acidity tetrable and total acid output is directly caused by a peptic ulcer **(ENO *et al.*, 2004).** Feedingrats on diets suplemented with 2.5 and 5% did not differ on their effect on tetrable acidity and total acid output**.** Also there was no signifcant ($p>0.05$) differences in pH and total acid output between positive rats and rats fed ondiets suplemented with 2.5.The group which treated with 7.5% AG showed more effective in reducing ($P\leq 0.05$) the volume of gastric juice and increasing pH, acidity and acid tetrable total output than groups treated with **2.5 and 5%**. Furthermore feeding rats on the diet treated with 7.5% AG for 30 days increasing the protective of mucosa membrane lining of stomach which led to reach the levels of volum gastric juice, pH and tetrable acidity to the level of negative control rat. These results are agreement with**kheder, (2017)** who reported that arabic gum improved significantly in reducing the volume of gastric juice acidity and acid tetrable total output and increasing pH. Morover, **Cipriani *et al*, (2009)** reported 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.

Table (3) indicated the effect of arabic gum on ulcer score, ulcer index, ulceration **(%)** and preventive index. The results showed that the negative group did not get ulcer score, ulcer index and ulceration (%) becouse it gave saline solution only. However positive group receiving ethyl alcohol had the higest ulcer score, ulcer index and ulceration (%) while, preventive index had opposite trend compared with gastric ulcer groups treated with gum arabic**. Ko and Cho, (2000)** reported that alcohol had been shown to affect the the mucosa of the stomach wall. Pretreatments rat diets with 2.5, 5 and 7.5% of GA led to decrease the ulcer score, ulcer index and ulceration (%) and increase preventive index. These results had the same trend reported by **Kheder, (2017**) who reported that feeding rat on diets suplemented with differents concentration of gum arabic **(**2.5, 5 and 10%)resulted inimprovement ulcer score, ulcer index and ulceration (%) and preventive index**.** Also**Abdulrahman and AL-Yahya(2016)** found that gum arabic at both tested doses orally (500 and 1000 mg/kg BW) was effective in reducing ulcer index. In the same table the highest reduction in the ulcer index ulcer index and ulceration (%) was obsereved in gastric ulcer group treated with 7.5% of gum arabic. These improvevement may be due to its high amount of total flavonoids and total phenolics compounds in diet suplemented with 7.5% of gum arabic than 2.5 and 5%. **Romano *et al.*, (2013)** indicate that flavonoidspossess good antiulcer effect due to their antioxidant effect.Moreover, gum arabiccontain a arabinogalactan, has been reported to possess antiulcer effect in rats (**Goodrum*et al.*, (2000)**.

Data in table (4) indicated the effect of arabic gum on antioxidants status of negative control and gastric ulcer groups. The levels of catalase (CAT), glutatione transfears (GST) and superoxide dismutase (SOD) in positive control were significantly decreased after oral injection of ethyl alcohol ($p \leq 0.05$), while MDA had opposite trend. The decrease in the levels of CAT, GST, SOD and increase in MDA may be due to the cause of the oxidative stress resulting from exposure to ethyl alcohol on the stomach.These results are in agreement with **Mi YUN *et al.*, (2017)** who reported ethylalcohol affected the mucosal

barrier and histology**.** Catalase, GST and SOD were significantly increased ($p \leq 0.05$) by feeding rats on diets replaced with 2.5, 5, 7.5% of arabic gum copared with positive control. These results are in agreement with **Salma, (2018)** who showed that arabinogalactan which is found in arabic gum significantly inhibited induced gastric lesions in rats. Furthermore, arabic gum is a known antioxidant and this would have contributed to its antiulcer action **(Goodrum *et al.*, 2000)**. The MDA levels were significantly reduced ($p \leq o,o5$) by 28.6, 40.5 and 67. 6% in rats fed on 2.5, 5 and 7.5% respectively. Moreover,feeding rats on the diet treated with 7.5% AG for 30 days led to return the GST and MDA to the level of negative control rat.

Sensory evaluation of baldy breadsprepared by replacing different levels of arabic gum are shown in Table (5). No significant ($p>0.05$) differences were observed in appearance, taste, flavor, texture, color and overall acceptability between bread prepared with 2.5, 5, and 7.5 % of GA and control bread. However the bread prepared with 7.5% of AG had lower ($P\leq 0.05$) compressibility than bread prepared with 2.5 and 5% of AG. These results are agreement with **kheder, (2017)** who reported that the bread prepared with high replacement (10%) of AG had lower ($P\leq 0.05$) compressibility than bread prepared with low concentration (2.5 and 5%) of AG. Also 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 gum arabic.Moreover **KL khalifa *et al.*, (2007)** indicated that acceptable bakery products e.g. bread and pizza could be obtained using gum arabic. Gum arabic is used in a range of bakery products **(FAO, 1995)**.

The effect of arabic gum on histological examination are showed in Fig. (1). Microscopically, stomach of rats from negative control rats revealed no histopathological changes with the normal histological structure of gastric layers (mucosa, submucosa and musculosa)**.**In contrary, stomach of rats from positive control revealedfocal necrosis and ulceration of gastric mucosa associated with submucosaloedema and inflammatory cells infiltration and haemorrhage. 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% AG group revealed focal necrosis of gastric mucosa and

slight submucosaloedema. Examined sections from 5% showed congestion of mucosal blood vessels and submucosal blood vessel as well as submucosaloedema .Meanwhile, stomach of rats from 7.5% revealed no histopathological changes .These agreed with the result by **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 aprotective coating pepsin and protecting the mucosa by increasing mucus synthesis or scavenging radicals (**Ciprianiet *al.*, 2009)**.

Table (1):Chemical composition, total phenolics and total flavonoids of driedarabic gum

Parameters	Arabic gum
Protein(g/100g)	2.20±0.02
Moisture(g/100g)	12.34±0.5
Ash(g/100g)	2.50±0.02
Fat(g/100g)	0.58±0.01
Fiber(g/100g)	73.10±0.5
Carbohydrates(g/100g)	8.72±0.5
Total phenolic(mg gallic/100 gm)	**61.05±**0.16
Total flavonoids(mg catechin/100 gm)	**25.29±**0.36

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

Table (2): Effect of arabic gum on volume, pH, terrible acidity and total acid output in gastric juice of negative controland gastric ulcer groups

Parameters	Negative control group	Gastric ulcer groups				LSD
		Positive	A G (2.5%)	A G (5%)	A G (7.5%)	
Volume of gastric juice (ml)	2.47^{d} ±0.17	5.38^{a} ±0.27	3.7^{b}±0.22	3.15^{c}±0.13	2.6^{d}±0.26	0.13
PH	3.40^{a} ±0.14	1.22^{c} ±0.17	1.55^{c}±0.37	2.25^{b}±0.40	3.02^{a}±0.30	0.53
Tetrable acidity (Meq/L)	9.2^{c}±0.83	14^{a}±0.13	12^{b}±0.53	11^{b}±0.33	10^{d}±0.43	0.76
Total acid output(Meq/1h)	163^{c}±2.2	266^{a}±3.3	271^{a}±2.2	244^{ab}±3.5	219^{b}±3	33.5

Values are expressed as means ± SD; means in the same raw with different letter are significantly different ($P \leq 0.05$).A.G: arabic gum

Table (3): Effect of arabic gum on ulcer score, ulcer index, % ulceration and preventive index of negative controland gastric ulcer groups

Parameters	Negative control group	Gastric ulcer groups			
		Positive	A G (2.5%)	A G (5%)	A G (7.5%)
Ulcer score	--	9.5	5.8	4.75	1.5
Ulcer index	--	950	580	475	150
Ulceration (%)	--	85.3	70.6	33.4	12.7
Preventive index	--	14.7	29.4	66.6	87.3

A G: Arabic gum

Table (4): Effect of arabic gum on antioxidants status of negative control and gastric ulcer groups

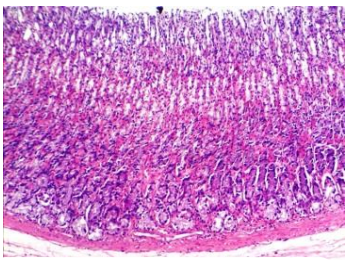
Parameters	Negative control group	Gastric ulcer groups				LSD
		Positive	A G (2.5%)	A G (5%)	A G (7.5%)	
CAT	40.5^{a}±0.24	13.6^{e}±0.75	21.4^{d}±1.34	27.0^{c}±1.5	35.0^{b}±1.6	1.6
GST	30.0^{a}±2.0	15.0^{d}±1.5	22.3^{c}±1.2	27.0^{b}±1.2	29.0^{a}±0.61	1.8
SOD	37.8^{a}±0.57	14.6^{e}±1.8	22.0^{c}±1.6	24.8^{d}±0.21	31.0^{b}±2.9	2.6
MDA	11.0^{d}±0.49	37.0^{a}±2.6	26.4^{b}±0.86	22.0^{c}±1.1	12.0^{d}±1.26	2.2

Values are expressed as means ± SD; means in the same raw with different letter are significantly different ($P \leq 0.05$).A.G: arabic gum, CAT: catalase, GST: glutathione transferase, SOD: superoxide dismutase, MDA: malondialdehyde**.**

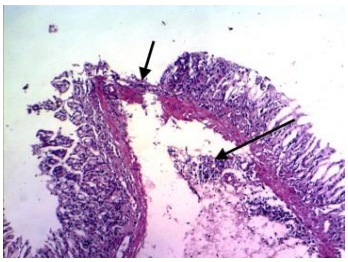
Table (5): Sensory evaluation of flatbreadsprepared by replacing different levels of arabic gum

Bread / Parameters	A G replacer levels (%)				LSD
	0%	2.5%	5%	7.5%	
Appearance	8.1^{a}±0.57	8.2^{a}±0.63	8.2^{a}±0.42	8.0^{a}±0.47	0.48
Taste	8.1^{a}±0.87	8.1^{a}±0.47	8.3^{a}±0.67	8.4^{a}±0.52	0.59
Flavor	8.0^{a}±0.67	8.1^{a}±0.67	8.3^{a}±0.48	8.5^{a}±0.53	0.54
Texture	8.2^{a}±0.32	7.8^{a}±0.79	7.8^{a}±0.63	7.7^{a}±0.48	0.54
Compressibility	7.7^{ab}±0.67	8.1^{a}±0.32	8.4^{a}±0.52	7.5^{b}±0.63	0.53
Color	8.0^{a}±0.67	8.1^{a}±0.74	8.4^{a}±0.52	8.5^{a}±0.71	0.60
Overall acceptability	8.1^{a}±0.74	8.2^{a}±0.42	8.5^{a}±0.53	8.3^{a}±0.48	0.50

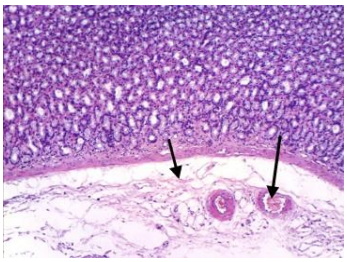
Values are expressed as means ± SD; means in the same row with different letter are significantly different ($P \leq 0.05$).A.G: arabic gum



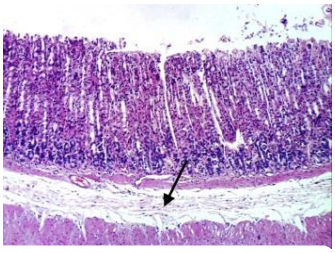
Negative control



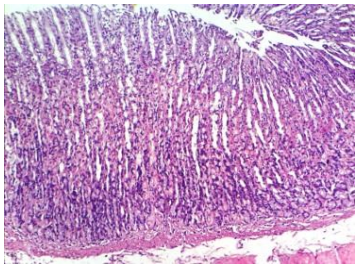
Positive control



Group2 (2.5% arabic gum)



Group 3(5% arabic gum)



Group 4(7.5% arabic gum)

Fig (1): Effect of arabic gum on histological examination of stomach tissue of rats

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التأثير الوقائي المحتمل للصمغ العربي على الفئران المصابة بقرحة المعدة الناجم عن الكحول الإيثيلي

هبه عز الدين يوسف ،فاطمة طلعت سيف
قسم التغذية وعلوم الأطعمة - كلية الاقتصاد المنزلي- جامعة المنوفية-شبين الكوم- مصر

الملخص العربي:

يستخدم الصمغ العربي في جميع أنحاء العالم لأغراض مختلفة بما في ذلك المواد المضافة والأدويةوقد تبين أن الصمغ العربييستخدم داخليا لعلاج التهاب الغشاء المخاطي في الأمعاء. لذا هدفت هذه الدراسة إلى تقييم التأثير الوقائي المحتمل للصمغ العربي على الفئران المصابة بقرحة المعدة الناجمة عن الكحول الإيثيلي.تم تقسيم ثلاثونفأر من ذكور الفئران البيضاء البالغة عشوائيا إلى خمس مجموعات (6 فئران لكل منهم) المجموعتان الأولى والثانية تغذتان على الوجبة الغذائية القياسيةوالمجموعة الثالثة والرابعة والخامسة تغذوا على الوجبة القياسية المحتوية على 2.5 ، 5 و 7.5 ٪ مسحوق الصمغ العربي على التوالي. في نهاية فترة التجربة (اليوم الثلاثون) تمتصويم الفئران لمدة 24 ساعة مع السماح لهم بشرب الماء. أعطيت فئران المجموعة الثانية والثالثة والرابعة والخامسة جرعة واحدة عن طريق الفم من الكحول الإيثيلي(95 ٪) 10 مل / كجم من وزن الجسم في حين أعطت المجموعة الضابطة السالبة جرعة واحدة عن طريق الفم من المحلول الملحي (0.9 ٪ ، وزن / حجم). بعد ساعتين وتحت تأثيرالتخدير بواسطةالإيثيل إيثر ، تم فتح جدار البطن ، وتم تحديد البواب ، وتم ربط المعدة من فتحة المريء ، وإزالتها ، وفتحها في انحناء أكبر ، وتم جمع عصير المعدة والطرد المركزي لدراسة معلمات إفراز المعدة. أظهرت النتائج أن المجموعات التي عوملت بالصمغ العربي قد انخفضت بشكل ملحوظ في درجة القرحة ، مؤشر القرحة وزيادة في مؤشر الوقاية مقارنة مع المجموعة الضابطة الموجبة. تغذية الفئران علي وجبة غذائيةمدعمة بالصمغ العربي بنسبة 7.5 ٪ كان أكثر فعالية لحماية المعدة من القرحة مقارنة بالمدعمة بـ 2,5 و5 %. تم استخدام الصمغ العربي لاستبدال جزء من دقيق القمح الكامل (2.5 ٪ ، 5 ٪ و 7.5 ٪) في الخبز القياسي. أظهر التقييم الحسي أن كل الاستبدلات بالصمغ العربي في الخبز كان مقبولاً من قبل المحكمين. خلصت الدراسة إلى أن الصمغ العربي كان له نشاط وقائي ضد قرحة المعددة لدى الفئران البالغة والتي سببها الكحول الإيثيلي.

الكلمات الافتتاحية: الصمغ العربي - قرحة المعدة - عصير المعدة - الخبز