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GASTROPROTECTIVE EFFECTS OF GINGER AND POMEGRANATE IN RATS

BY

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ABSTRACT

The antiulcerogenic effect of ethanolic extract of ginger rhizome (350 and 700 mg/kg BW) and methanolic extract of pomegranate fruit rind (250 and 500 mg/kg BW) in comparable to ranitidine (50 mg/kg BW) was investigated against gastritis gastric ulcerations induced by aspirin in rats. The extracts minimized gastric ulcerations at a dose-dependant manner. When the in-vivo antioxidant levels were evaluated, the administration of aspirin caused a significant decrease in the level of glutathione (GSH), and an increase in the lipid peroxidation malonaldehyde (MDA) level. While, the administration of all doses of the two studied extracts reversed the trend, inducing a significant increase in GSH and a reduction in MDA level in stomach tissues. In addition, the histopathological examination of the ulcerated animal's stomach showed severe histological changes as compared to treated groups. These results suggested that the ginger rhizome and pomegranate fruit rind extracts have gastroprotective effect which can be attributed to their enhancing effects on antioxidant defense systems, which may support the potent antiulcer properties.

Key words: Aspirin-induced ulcer; Gastroprotective; Ginger; Pomegranate; Rats.

INTRODUCTION

Peptic ulcer is one of the major gastro-intestinal disorders, which occur due to an imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors (Hoogerwerf and Pasricha, 2006). Consequently, reduction of gastric acid production as well as re-inforcement of gastric mucosal production has been the major approaches for therapy of peptic ulcer disease. As a result, peptic ulcer therapy has undergone many strides over the past few years and a number of drugs are now available for treatment, although these drugs have brought about remarkable changes in ulcer therapy, the efficacy of these drugs is still debatable. Reports on clinical evaluation of these drugs show that there are incidences of relapses and adverse effects and danger of drug interactions during ulcer therapy. Hence, in recent years, a widespread search has been launched to identify new antiulcer-drugs from natural sources, which safety, quality, decrease the incidence of relapse and efficacy (Goel and Bhattacharya, 1991; Anonymous, 1999).

Literature survey revealed that ginger, the rhizome of *Zingiber officinale*) Zingiberaceae) is a perennial herb with an aromatic pungent taste. The rhizomes of ginger are used as a spice in food and beverages (Iwu, 1993). Some of potential clinical benefits are: anti *Helicobacter pylori* (Mahady *et al.*, 2003); antioxidant (Sharma and Gupta, 1998); anti-inflammatory (Grzanna *et al.*, 2005); anti-tumor (Surn *et al.*, 1999); carminative to enhance digestion; inhibited experimental gastric ulcers in rats and protecting gastric mucosa against alcohol, non-steroidal, anti-inflammatory drugs and hydrochloric acid (Serthe *et al.*, 1992). Organic solvent extract of ginger rhizomes has also been shown to cause significant inhibition of skin tumor (Katiyar *et al.*, 1996).

Pomegranate (*Punica granatum L.*) is native to the Mediterranean region and has been used extensively in the folk medicine of many countries. In India, it is used in the form of juice, concentrate, canned beverage, wine, jam, and jelly (Adsule and Patil, 1995). Pomegranate peel had the highest antioxidant activity among the peels and is a very rich source of natural antioxidants such as ellagic acid derivatives (Gil *et al.*, 2000; Yasuko *et al.*, 2002; Guo *et al.*, 2003; Yunfeng *et al.*, 2006). Therefore, the plant is used in folklore medicine for the treatment of various diseases, such as ulcer, hepatic damage, snake bite, etc. The rind of the fruit is antihelminthic, useful in diarrhea, dysentery and ulcer (Ajaikumar *et al.*, 2005; Navindra *et al.*, 2006). In recent years several pomegranate-containing products have been widely marketed for health benefits around the world, including the United States (Cerda *et al.*, 2003a).

On the basis of these common uses of these natural sources of antioxidants in traditional folk medicine and their above reported activities in the literature, we evaluated the Gastroprotective and anti-ulcerogenic effects of the rhizome extract of ginger and the rind extract of pomegranate in rats.

MATERIALS AND METHODS

1. Material:

The dried rhizomes of ginger (*Zingiber officinale* Roscoe rhizome, Zingiberaceae) and Pomegranate fruits (*Punica granatum*, Punicaceae) were purchased from the local market in Cairo, Egypt. While, Commercial kits used for determining malonaldehyde (MDA) and total glutathione (GSH) were obtained from Biodiagnostic Co. Dokki, Egypt. Ranitidine and aspirin were obtained from El-Gomhoreya Co., Cairo, Egypt.

2. Methods

2.1. Preparation of ginger rhizomes extract:

The rhizomes were cut into small pieces, air-dried then ground into powder. Cold extraction was carried out at room temperature of this powder using ethanol 70 % for 72 hours. The extract was then shacked, filtered and evaporated in a rotatory evaporator under reduced pressure to remove the remained alcohol. The gastroprotective tests were carried out using the dry extract, Braun and Cohen (2007).

2.2. Preparation of pomegranate rinds extract:

The air-dried powdered rinds were extracted with 70% methanol by stirring at room temperature for 24 h. The extract was filtered, concentrated and evaporated to dryness. The dried extract was suspended in distilled water and used for further studies, Ajaikumar *et al.* (2005).

2.3. Animals Experimental design:

Male Wistar rats (180–200 g) were obtained from Organization of Biological Products and Vaccines (Helwan Farm, Cairo, Egypt). The animals were maintained on a standard pelleted diet and water *ad libitum* and were left 48 hours for acclimization to animal room conditions. The food was with drawn 24 hours before the experiment but animal was allowed to have free access to water. The animals were randomly divided into seven groups with six rats in each group. Group I was served as normal control without any treatment, and all other animals were administration orally with aspirin (400 mg/kg BW). Groups II was kept as ulcerogenic control; groups III, IV animals were administrated ginger extract (350, 700 mg/kg BW). Groups V, VI and VII were treated orally with pomegranate extract (250, 500 mg/kg BW) and ranitidine as standard drug (50 mg/kg BW), respectively, 1 h prior to the administration of aspirin. The animals were sacrificed after 4 h of the administration of aspirin (Parmar, and Desai, 1993).

2.4. Assessment of gastric mucosal damage (Ulcer Index, UI):

The stomachs were removed and opened along the greater curvature to determine ulcer index. The curative percentage was calculated by the formula: [(UI control – UI treated) / UI control] x 100 according to Takagi *et al.* (1969).

2.5. Biochemical investigation of stomach tissues:

The total glutathione (GSH) and malonaldehyde (MDA) in stomach tissues were determined by the following methods. The tissues lipid peroxidation was estimated by measuring the thiobarbituric acid reactive substances (TBARS), the last product in lipid peroxidation pathway, were measured at 534 nm according to Ohkawa, *et al.* (1979), GSH was estimated by its reaction with 5,5-dithiobis 2-nitrobenzoic acid (DTNB) to produce a yellow colored complex with absorption at 405 nm according to Beutler *et al.* (1963).

2.6. Histopathology:

Autopsy samples were taken from the stomach of the sacrificed rats and fixed in 10% formalin saline solution for ten hours at least. The obtained tissue sections were collected on the glass slides and stained by hematoxylin and eosin stain for histopathological examination by the light microscope (Banchroft *et al.*, 1996).

2.7. Statistical analysis:

All the obtained data were subjected to statistical analysis according to the procedure reported by Snedecor and Cochran (1980) and the Statistical Analysis System Program (SAS, 1996) using Student t-test and factorial analysis.

RESULTS AND DISCUSSION

1. Effects on lipid peroxidation:

Increased lipid peroxidation is generally believed to be an important underlying cause of the initiation of oxidative stress related various tissue injury, cell death, and further progression of many acute and chronic diseases (Halliwell and Gutteridge, 1999).

As shown in Table (1), gastric MDA levels increased significantly in the nontreated rats after aspirin induced stress. Treatment with ginger rhizomes and pomegranate rinds extracts as well as ranitidine significantly decreased the MDA levels. Oral administration of ginger rhizomes and pomegranate rinds extracts showed a better effect than ranitidine.

The significant decrease in lipid peroxidation (MDA) indicated an increase in antioxidant defense. The decrease in MDA levels in these tissues is suggestive of reduced tissue damage due to failure of the formation of excessive free radicals. This revealed potent gastroprotective for ginger rhizomes and pomegranate extracts in comparable with the standard drug ranitidine as free radical scavengers.

2. Effect on GSH concentrations:

Non-steroidal anti-inflammatory drugs, such as aspirin induced cytotoxicity is associated with reduced antioxidant levels. Glutathione acts as an antioxidant both intracellularly and extracellularly in conjuction with various enzymatic processes that reduce hydrogen peroxide and hydroperoxides (Allison *et al.*, 1992).

Changes in GSH content of different groups has been shown in Table (1), rats administered with aspirin alone were found significantly lowered glutathione (GSH) level. But oral administration of ginger rhizomes and pomegranate rinds extracts significantly improved the level of glutathione in comparable with the standard drug ranitidine, which revealed the potent gastroprotective activity of the extracts.

3. Effect on gastric mucosal damage (ulcer index):

The treatments of rats with aspirin produced extensive gastric lesions mainly confined to glandular portion of the stomach. The ginger rhizomes and pomegranate rinds extracts produced a dose-dependent inhibition of gastric ulceration ranging in comparable with the standard drug ranitidine (Table 2 and Fig 1). The protection against ulcerogenesis, as manifested in significant reduction in ulcer index confirmed that the ginger rhizomes and pomegranate rinds extract has gastroprotective properties against aspirin induced gastric inflammation and ulceration in rats.

Table (1): Effect of ginger rhizomes, pomegranate rinds extracts and ranitidine on tissue MDA and GSH in normal and aspirin-treated rats

Groups		Dose (mg/kg BW)	MDA (nmol/g)	GSH (nmol/g)
Group I	(Normal control)	—	12.18 ± 0.12	3.56 ± 0.18
Group II	(Aspirin)	400	15.49 ± 0.07*	2.45 ± 0.31*
Group III	Ginger	350	5.41 ± 0.03†	3.33 ± 0.06†
Group IV	Ginger	700	4.88 ± 1.02†	3.92 ± 0.05†
Group V	Pomegranate	250	7.21 ± 0.28†	3.19 ± 0.37†
Group VI	Pomegranate	500	6.51 ± 3.08†	3.57 ± 0.53†
Group VII	Ranitidine	50	12.06 ± 0.04†	3.12 ± 0.98†

Values are mean ± S.D., n=6; * $P \leq 0.05$ vs. normal control; † $P \leq 0.05$ vs. Aspirin control.

Table (2): Effect of ginger rhizomes, pomegranate rinds extracts and ranitidine on gastric lesion surface induced by aspirin

Treatment	Dose (mg/kg)	Ulcer index	Curative %
Normal control	—	—	100
Aspirin control	400	4.82 ± 0.63	0
Ginger	350	0.88 ± 0.61*	81.74
Ginger	700	0.65 ± 0.56*	86.51
Pomegranate	250	1.02 ± 0.26*	78.84
Pomegranate	500	0.92 ± 0.21*	80.91
Ranitidine	50	2.01 ± 0.40*	58.3

Values are mean ± S.D., n=6; * $P \leq 0.05$ vs. normal control.

4. Histopathological examination:

Data in Figures (2) to (8) reveal the histopathological examination of semi-thin sections of stomach rat stained with hematoxylin and eosin.

The normal control group 1 observed no histopathological alteration in mucosa and lamina propria (Figure 2). Treatment with aspirin (group 2) caused sever haemorrhages and ulceration and necrosis in the mucosal covering epithelium associated with massive number of inflammatory cells infiltration and oedema with congested blood vessels in the lamina proptria (Figure 3). These changes is consistent with studies of Ivey (1988) who reported that the non-steroidal anti-inflammatory drugs, such as aspirin are commonly used as pain killers inhibit gastric peroxidase and increase mucosal H_2O_2 and OH^- levels to cause oxidative mucosal damage.

Pretreatment of ginger rhizome extract at doses of 350 and 700 mg/kg (Group 3 and 4), observed focal desquamation in the lining mucosal epithelium associated with inflammatory cells in the lamina propria associated with oedema in the lamina propria (Figures 4 and 5). While, pretreatment with pomegranate rinds extract at doses of 250 and 500 mg/kg BW and aspirin for group 5 and 6 showed focal desquamation of the lining mucosal epithelium in focal manner associated with oedema and few inflammatory cells infiltration and congested blood vessels in the lamina propria (Figure 6 and 7).

Pretreatment of drug ranitidine at 50 mg/kg BW for Group 7 showed desquamation in the lining epithelium and diffuse inflammatory cells infiltration in the mucosal layer associated with inflammatory oedema in the lamina propria (Figure 8).

The histopathological changes as observed in pretreated groups with ginger rhizome, pomegranate rinds extracts and ranitidine indicated that as compared with that induced with aspirin alone indicated marked protective effects of these substances. Also, compare the histopathological observation in the pretreated groups indicated that the histopathological changes were less in ginger and pomegranate groups as compared with ranitidine group (Table 3).

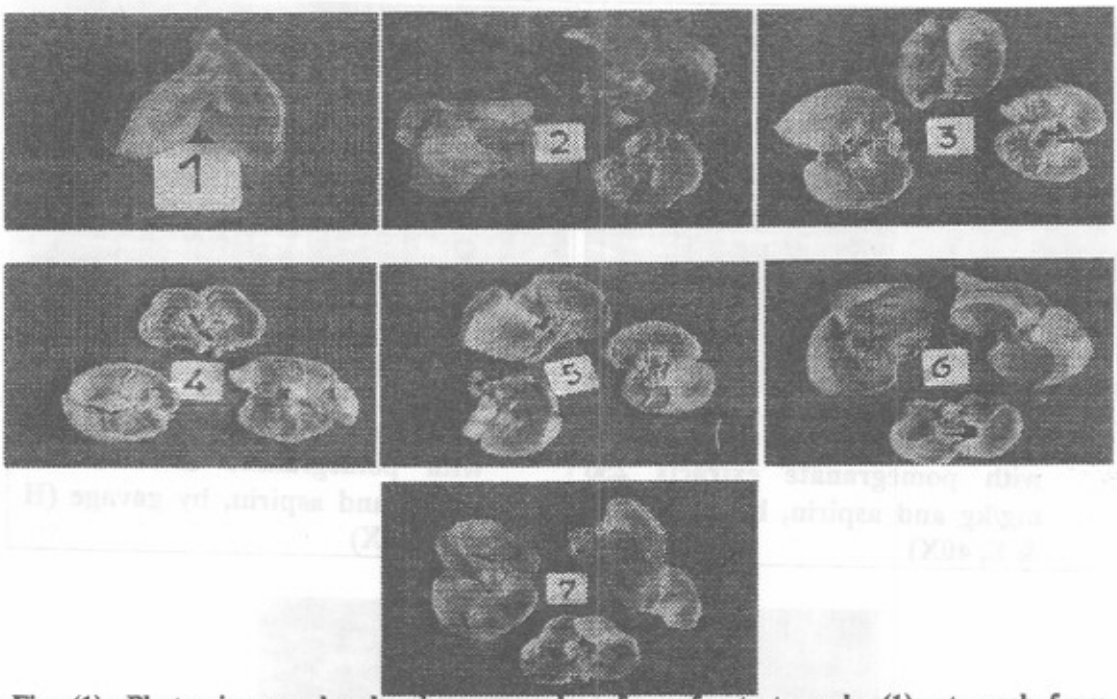

Fig. (1): Photomicrographs showing mucosal surface of rat stomach; (1): stomach from normal control. (2): stomach from aspirin control. (3&4): stomach from rat treated with ginger rhizome extract 350, 700 mg/kg + aspirin. (5&6): stomach from rat treated with pomegranate rinds extract 250, 500 mg/kg + aspirin. (7): stomach from rat treated with ranitidine 50 mg/kg + aspirin.

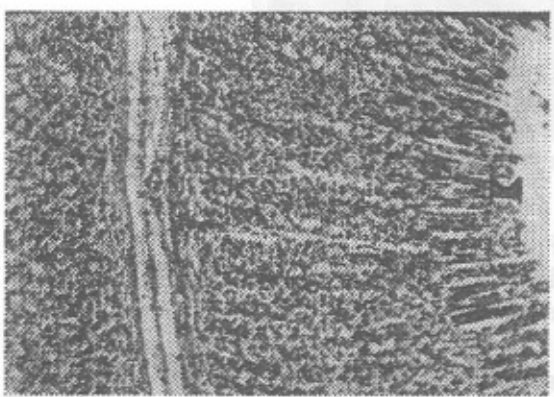

Fig. (2): The stomach wall of a normal control rat showing its normal appearance (H & E, 40X)

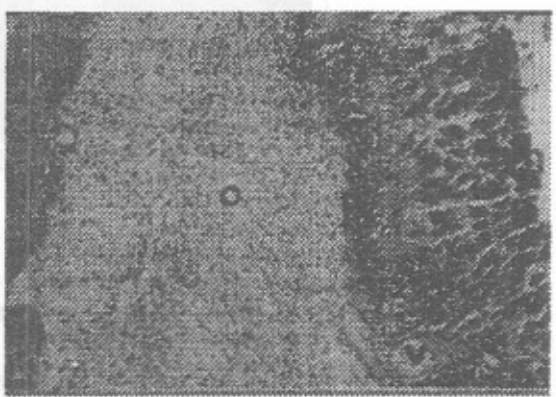

Fig. (3): The stomach wall of rat treated with aspirin, by gavage (H & E, 40X)

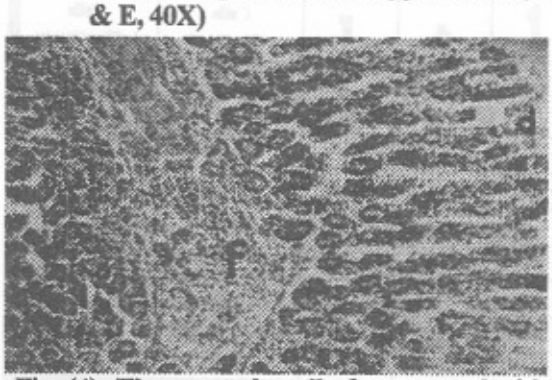

Fig. (4): The stomach wall of rat treated with ginger extracts 350 mg/kg and aspirin, by gavage (H & E, 40X)

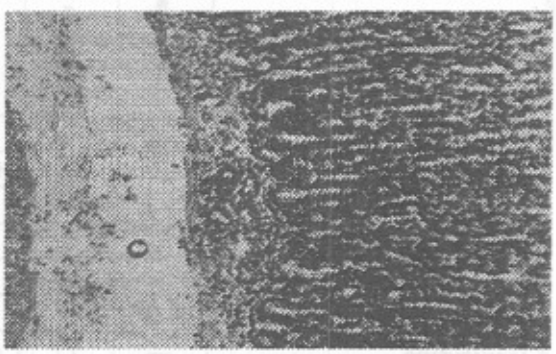

Fig. (5): The stomach wall of rat treated with ginger extracts 700 mg/kg and aspirin, by gavage (H & E, 40X)

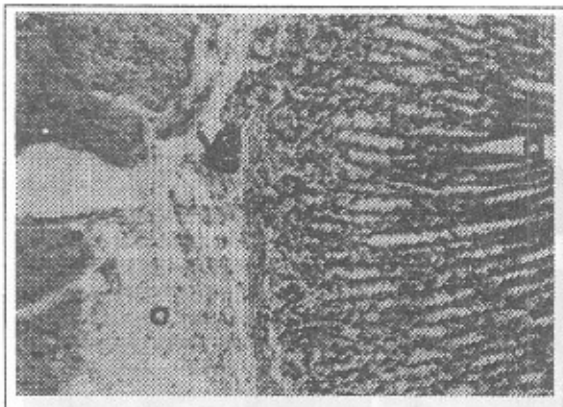

Fig. (6): The stomach wall of rat treated with pomegranate extracts 250 mg/kg and aspirin, by gavage (H & E, 40X)

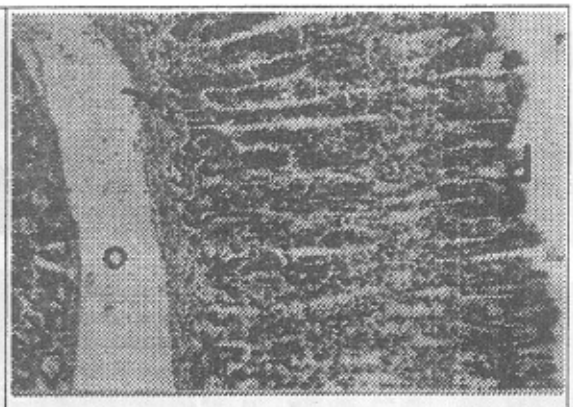

Fig. (7): The stomach wall of rat treated with pomegranate extracts 500 mg/kg and aspirin, by gavage (H & E, 40X)

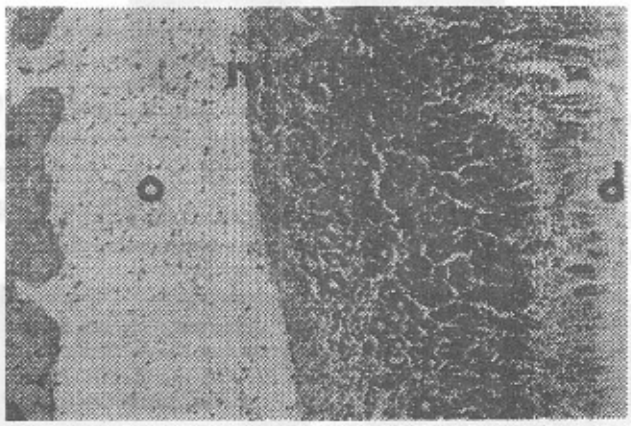

Fig. (8): The stomach wall of rat treated with ranitidine 50 mg/kg and aspirin, by gavage (H & E, 40X

M, mucosal layer; **L**, lamina propria; **O**, oedema; **F**, inflammatory cells infiltration; **H**, haemorrhagic ulceration; **HH**, mucosal lening epithelium; **V**, dilated blood vessels; **D**, desquamation of lining epithelium & few inflammatory cells infiltration are appointed by **arrow**.

Table (3): Effect of ginger rhizomes, pomegranate rinds extracts and ranitidine on aspirin induced histopathological changes in rat stomach

Treatment	Congestion	Oedema	Haemorrhage	Necrosis	Sloughing	Cells infiltration in mucosal epithelium	Cells infiltration in lamina propria
Normal control	—	—	—	—	—	—	—
Aspirin control	++	++++	++++	++++	++++	++++	++++
Ginger 350 mg/kg	—	+	—	—	+	—	++
Ginger 700 mg/kg	—	+	—		—	+	—
Pomegranate 250 mg/kg	+	+	—	—	+	—	+
Pomegranate 500 mg/kg	—	+	—	—	+	+	—
Ranitidine	—	++	—	—	+	++	++

-: normal; +: little effect; ++: appreciable effect; ++++: very severe effect.

In conclusion, all the used doses of ginger rhizome and pomegranate rinds extracts showed a significant gastroprotective effect in the aspirin-induced ulcer model. The gastroprotective effect of ginger and pomegranate can be attributed to their reducing effect against oxidative damage and their inhibitory effects on neutrophil infiltration in stomach rat tissues. The underlying mechanism of the action of ginger and pomegranate can be centered on the cytoprotective effect by improv-ement of gastric mucosal function by elevating GSH content, alleviation of inflammation by decreasing the level of MDA. In view of these, the ginger rhizome and pomegranate rinds used in the current study provide a better alternative as antiulcer agents.

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التأثير الواقى للزنجبيل والرمان للقرح وإلتهاب المعدة فى الفئران

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إستهدف هذا البحث دراسة تأثير كل من المستخلص الإيثانولى لريزومات الزنجبيل بتركيزات (٣٥٠ ، ٧٠٠ مج / كجم من وزن الجسم) والمستخلص الميثانولى لقشور ثمار الرمان بتركيزات (٢٥٠ ، ٥٠٠ مج / كجم من وزن الجسم) مقارنة بعقار الرانيتيدين (٥٠ مج / كجم من وزن الجسم) فى علاج الفئران المصابة بقرحة المعدة نتيجة المعاملة بالإسبرين. ولقد أظهرت المستخلصات المستخدمة تثبيط لقرحة المعدة طبقاً للتركيز المستخدم. وقد وجد إنخفاض معنوى فى مستوى الجلوتاثيون وزيادة فى مستوى المالونالدهيد فى الفئران المصابة مقارنة بالمجاميع المعاملة بالمستخلصات محل الدراسة. كما أظهرت الدراسات الهيستوباثولوجية للمعدة تغييرات خطيرة فى المجموعة المصابة مقارنة بالمجموعات المعاملة. وتدل هذه النتائج على التأثير الواقى لكل من مستخلصات ريزومات الزنجبيل وقشور ثمار الرمان للجهاز الهضمى والذى يعزى إلى زيادة كفاءة نشاط نظام مضادات الأكسدة الموجودة بها فى الحماية من حدوث قرحة المعدة