

Gastroprotective Effect of *Cordia Myxa L.* Fruit Extract against Indomethacin-Induced Gastric Ulceration in Rats

Inas, Z.A. Abdallah^{*a}, Hala, A.H. Khattab^a and Gehan, H. Heeba^b

^a Nutrition and Food Science Department, Faculty of Home Economics, Helwan University, Egypt

^b Pharmacology and Toxicology Department, Faculty of Pharmacy, Minia University, Egypt

^{*}izaa2011@hotmail.com

Abstract: Gastric ulcer is one of the most serious diseases in the world. Although there are many drugs used for the treatment of gastric ulcer, most of these produce several adverse reactions. This study investigated the protective effects of Assyrian plum (*Cordia myxa L.*) fruit extract (CME) against indomethacin-induced gastric ulcer in rats. Gastric ulceration was induced by a single intraperitoneal injection of indomethacin (30 mg/kg⁻¹ b.wt.). CME was administered orally at a dose of 125 mg/kg b.wt. and ranitidine (RAN), a reference drug, at a dose of 50 mg/kg b.wt. two weeks prior to indomethacin injection. Pretreatment with CME produced significant reduction in gastric mucosal lesions (U.I.), malondialdehyde (MDA), and serum tumor necrosis factor (TNF α) associated with significant increase in gastric juice mucin content and gastric mucosal catalase (CAT), nitric oxide (NO), and prostaglandin E₂ (PGE₂) levels. A similar increase in mucin content, NO and PGE₂ was not observed with RAN although it generated a preventive index of 75.9%. RAN significantly increased pH value and decreased pepsin activity, and gastric juice free and total acidity. Histological studies of stomach mucosa confirmed these results. Stomach of rats administrated with RAN showed leukocytic infiltration in submucosal layer. Meanwhile, stomach of rats administrated CME either alone or with RAN showed no histopathological changes. CME can protect indomethacin-induced gastric ulceration due to its antioxidative and mucin enhancing properties. The protection afforded by co-administration of CME and RAN was found to be better than that of RAN alone. Results of the present study suggest that RAN should be used together with CME for better gastroprotective effect as well as to reduce H₂ antagonists drugs adverse effects.

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1. Introduction:

Gastric ulcer is a major health hazard in terms of both morbidity and mortality (Chaturvedi et al., 2007). Untreated gastric ulcer is capable of inducing upper gastrointestinal bleeding (Tortora and Grabowski, 2003). The etiology of gastroduodenal ulcers is influenced by various aggressive and defensive factors such as acid-pepsin secretion, parietal cell, mucosal barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents (prostaglandins and epidermic growth factors) (Repetto and Llesuy, 2002). According to Malysenko et al. (2005) and Kim (2008), some other factors, such as inadequate dietary habits, cigarette smoking, excessive ingestion of non-steroidal anti-inflammatory agents, stress, hereditary predisposition and infection by *Helicobacter pylori*, may be responsible for the development of peptic ulcer. Several pharmaceutical products have been employed for the treatment of gastroduodenal ulcer and peptic diseases, resulting in decreasing mortality and morbidity rates, but they are not completely effective and produce many adverse effects (Rates, 2001).

Ulcer therapy has progressed from vagotomy to anticholinergic drugs, histamine H₂ receptor antagonists, antacids and to proton pump inhibitors (Wallace and Granger, 1996). A widely used drug associated with rare idiosyncratic hepatotoxicity is the histamine H₂ receptor antagonist ranitidine (RAN) (Bourdet et al., 2005). It is available over the counter for oral administration or by

prescription for parenteral administration for treatment of gastric ulcers, hypersecretory diseases, and gastroesophageal reflux disease. Idiosyncratic RAN hepatotoxicity occurs in few people taking the drug (Fisher and Le Couteur, 2001). Most liver reactions are mild and reversible; however, extensive liver damage have occurred in individuals undergoing RAN therapy (Cherqui et al., 1989 and Ribeiro et al., 2000).

In recent years, there has also been growing interest in alternative therapies and the use of natural products, especially those derived from plants (Rates, 2001 and Schmeda-Hirschmann and Yesilada, 2005). Plant extracts are some of the most attractive sources of new drugs and have been shown to produce promising results for the treatment of gastric ulcer (Alkofahi and Atta, 1999 and Schmeda-Hirschmann and Yesilada, 2005).

Cordia myxa fruit (family: *Boraginaceae*), is popularly used for the treatment of chest and urinary infections, and as an anthelmintic, diuretic, astringent, demulcent and expectorant agent (Alami and Macksad, 1974). Moreover, it has been reported that leaf extracts of certain species of *Cordia* such as *C. myxa*, *C. francisci*, and *C. serratifolia* have significant analgesic, anti-inflammatory, and antiarthritic activities in rats (Ficarra et al., 1995). The anti-inflammatory properties of the *C. myxa* fruit preparation in the treatment of experimental colitis have been demonstrated by Al Awadi et al. (2001). However, there are few data about its pharmacological effects on gastrointestinal system as

well as about its possible toxic properties and chemical composition. This promoted us to investigate the effect of *C. myxa* fruit extract (CME) on indomethacin induced gastric ulcer in rats as well as to evaluate its acute toxicity and qualitative phytochemical profile. RAN was taken as a reference drug with which the antiulcer potential of CME was compared, and the combined gastroprotective effect of CME with RAN was also evaluated.

2. Materials and Methods:

2.1. Drugs and chemicals:

Indomethacin was obtained from Sigma Chemical Co. (St. Louis, MO, USA), and was suspended in 1% aqueous solution of Tween 80. Ranitidine was kindly provided by GlaxoSmithKline, Egypt. Thiobarbituric acid, 1,1,3,3-tetramethoxy-propane, trichloroacetic acid, ethanol absolute and diethyl ether were obtained from Sigma-Aldrich (USA). All drug solutions and suspensions were freshly prepared. Casein (>85% protein) was obtained from Misr Scientific Co. Dokki, Giza, Egypt. Cellulose and D-L methionine were purchased from Morgan Co. Cairo, Egypt. Minerals and vitamins constituent and sucrose were obtained from El-Gomhoriya Pharm. and Chem. Ind. Co. Cairo, Egypt. Corn oil was obtained from the local market. Corn starch was obtained from Starch and Glucose Co. Helwan, Cairo, Egypt.

2.2. Plant material:

Ripe Assyrian plum (*C. myxa* L.) fruits were collected from the farm of Medicinal and Aromatic Plants Research branch, Al Kanater Al Khayria, Horticultural Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt under the supervision of **Prof. Dr. Said G. I. Soliman**, Professor of Medicinal and Aromatic Plants and branch manager.

2.3. Preparation of extract:

The *C. myxa* fruits were cleaned carefully and washed several times with running tap water. The ethanolic extract was prepared by soaking 500 g of *C. myxa* fruits in 1 liter of a solvent composed of 700 ml ethanol 95% and 300 ml distilled water, with daily shaking for 2 days and kept in a refrigerator. The infusion was filtered by a piece of double layer gauze and fresh solvent was then added to the plant materials. The combined filtrates were evaporated using a rotary evaporated apparatus (Switzerland) attached with vacuum pump then centrifuged at 3000 rpm for 10 min (Muralidharan and Srikanth, 2009). 100 gm of plant contain 43 gm husks and 40 gm seeds and 17 gm extract

2.4. Phytochemical screening:

The crude *C. myxa* fruit extract (CME) was analyzed for glycosides, flavonoids, sterols, saponins, terpenoids, alkaloids, tannins, phenolic acids, gums and mucilage using standard procedures of analysis (Evans, 2002 and Harborne, 2007).

2.5. Determination of acute toxicity of CME:

Acute toxicity of CME was performed as described by Souza Brito (1995). The male mice were

divided into four groups of ten animals each. A group received saline (10 mL/kg) by gavage and kept as normal control. A single dose of CME was administered orally to group 2, 3 and 4 at doses of 50, 500, and 5000 mg/kg b.wt., respectively. The mortality, measured body weight and behavioral screening were recorded daily during 14 days after the extract administration.

2.6. Experimental Animals:

Forty male albino wistar rats weighing 190–200 g were obtained from the animal house of Faculty of Agriculture, Minia University, El-Minia, Egypt. They were used after acclimatization for a period of 1 week to animal house conditions and had free access to food and water. Basal diet was formulated to contain 14% casein, 10% sucrose, 5% corn oil, 5% fiber (cellulose), 3.5% mineral mixture, 1% vitamin mixture, 0.25% choline chloride, 0.3 % D-L methionine, and 60.95% corn starch (Reeves et al., 1993). Protocol was approved by the Local Animal Care Committee at Minia University (Egypt), and all the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals. The experiment was conducted at Faculty of Pharmacy, Minia University, Egypt.

2.7. Experimental design:

Rats were fasted for 24 h prior to the experiment in mesh-bottomed cages to minimize coprophagia but allowed free access to water except for the last hour before the experiment. All experiments were performed during the same time of the day to avoid diurnal variations of putative regulators of gastric functions. The animals were randomly classified into 5 groups (8 rats per each): (1) Control group; in which animals were left freely wandering in their cages for 3 h after receiving a single i.p. injection of 1% aqueous solution of Tween 80 (vehicle of indomethacin). (2) IND group; in which gastric ulceration was induced by i.p. injection of a single dose of 30 mg/kg⁻¹ b. wt. indomethacin (IND). (3) IND + RAN group; in which animals pretreated with 50 mg/kg b. wt. RAN orally, two weeks before indomethacin administration. (4) IND + CME group; in which animals pretreated with 125 mg/kg b.wt. CME orally two weeks before indomethacin administration. (5) IND + CME + RAN group; in which animals concurrently pretreated with CME and RAN orally two weeks before indomethacin administration. The doses of CME and RAN used in this study were chosen according to Ficarra et al. (1995) and Prakash et al. (2007), respectively. Gastric ulceration was induced by intraperitoneal administration of indomethacin (30 mg/kg⁻¹ b.wt., suspended in 1% aqueous solution of Tween 80) immediately after pyloric ligation (Khatab et al., 2001).

2.8. Pyloric ligation:

Pyloric ligation was carried out in each animal before indomethacin administration to collect gastric juice under light ether anesthesia, a mid-line abdominal incision was performed; the pyloric portion of the stomach was gently mobilized and carefully ligated with

a silk ligature around the pyloric sphincter taking care not to interfere with gastric blood supply. The abdominal incision was sutured and the animals were allowed to recover from anesthesia (Alumets et al., 1982).

2.9. Assessment of gastric mucosal lesions:

The animals were killed with an ether overdose three h after indomethacin administration. Each stomach was removed and opened along the greater curvature, and the gastric juice was collected. The stomachs were washed with ice-cold saline and examined for macroscopical mucosal lesions by an observer unaware of the treatment protocol. The gastric mucosal lesions were expressed in terms of ulcer index (U.I.) according to Peskar et al. (2002) which depends on the calculation of a lesion index by using of a 0-3 scoring system based on the severity of each lesion. The severity factor was defined according to the length of the lesions. Severity factor 0 = no lesions; 1 = lesions < 1 mm length ; 2 = lesions 2-4 mm length and 3 = lesions > 4 mm length. The lesions score for each rat was calculated as the number of lesions in the rat multiplied by their respective severity factor. The U.I. for each group was taken as the mean lesion score of all the rats in that group. The preventive index (P.I.) of a given drug was calculated by the equation of Hano et al. (1976).

$$\text{P.I.} = \frac{\text{U.I. of IND group} - \text{U.I. of pretreated group}}{\text{U.I. of IND group}} \times 100$$

2.10. Analysis of gastric juice:

Gastric juice collected from each animal was centrifuged at 3000 rpm for 10 min to remove any solid debris and the volume of the supernatant was measured. The supernatant was then assayed for the pH (Moore, 1968), pepsin activity (Sanyal et al., 1971) and mucin concentration (Winzler, 1955). Free and total acid outputs were calculated by multiplying gastric juice volume by the measured free and total acid concentrations, respectively (Hara et al., 1991 and Feldman, 1998).

2.11. Biochemical analysis of gastric mucosa.

Gastric mucosal malondialdehyde (MDA) level was measured by the method of Mihara and Uchiyama (1978). Nitric oxide (NO) content was determined as total nitrites/nitrates, the stable degradation products of NO (Sastry et al., 2002). Catalase activity was estimated based on the method of Aebi (1984). Prostaglandin E₂ (PGE₂) assay was performed with PGE₂ enzyme immunoassay kit (R&D Systems, Inc., MN, USA) according to supplier's instructions. Serum tumor necrosis factor (TNF- α) was determined by enzyme-linked immunosorbent assay (ELISA) using rat TNF- α assay kit (Biosource, USA) as previously described by Su et al. (2002).

2.12. Histopathological studies:

The stomach from all groups were removed rapidly, opened along the greater curvature, and thoroughly rinsed with ice-cold saline. After recording

the ulcers produced in the stomach, a longitudinal section of the gastric tissue was taken from the anterior part of the stomach and fixed in a 10% formalin solution. After 24 h of fixation followed by embedding in a paraffin block, it was cut into sections of 5 micron onto a glass slide and stained with hematoxylin-eosin for histological assessment of the gastric mucosa according to Bancroft et al. (1996).

2.13. Statistical analysis:

Data are expressed as Mean \pm S.E.M., with a value of $p < 0.05$ considered statistically significant. Statistical evaluation was performed by ANOVA followed by the Student's t-test. All analysis was made with the statistical software Microcal Origin (Version 6, Microcal Software Inc., Northampton, USA).

3. Results:

3.1. Phytochemical screening:

The Preliminary Phytochemical screening carried out on *C. myxa* fruit extract revealed the presence of phytoconstituents such as glycosides, flavonoids, sterols, saponins, terpenoids, alkaloids, phenolic acids, gums and mucilage (Table 1).

Table (1): Preliminary phytochemicals screening of *C. myxa* fruit extract .

Phytochemical Tests	Results
Test for glycosides	+
Test for flavonoids	+
Test for sterols	+
Test for saponins	+
Test for terpenoids	+
Test for alkaloids	+
Test for tannins	-
Test for phenolic acids	+
Test for gums and mucilage	+

+ : presence of the constituents.

- : Absence of the constituents.

3.2. Acute toxicity results:

The extract did not produce any toxic symptoms of mortality up to the dose level of 5000 mg/kg body weight in the treated animals, and hence it was considered safe for further pharmacological screening.

3.3. Effect of CME on indomethacin induced gastric lesions:

From Table (2), indomethacin administration caused a remarkably high ulcer index (21.6 \pm 2.01) when compared to control group. Pretreatment with RAN or CME offered significant protection against indomethacin-induced gastric ulcer in the experimental rats. CME reduced ulcer index to 7.5 \pm 0.44 showing 65.3% prevention whereas RAN reduced ulcer index to 5.2 \pm 0.31 showing 75.9% prevention. Pretreatment of rats with both RAN and CME produced higher gastroprotective effect as compared to RAN alone, they decreased the ulcer index to 2.1 \pm 0.15 providing 90.3 % prevention against gastric mucosal injury.

Table (2): Effect of *C. myxa* extract (CME), ranitidine (RAN) pretreatment, and their combination on ulcer index and preventive index in indomethacin (IND)-induced gastric ulcer in rats.

Groups	Ulcer index	Preventive index
Control	1.1 ± 0.01	-
IND	21.6 ± 2.01 ^a	-
IND + RAN	5.2 ± 0.31 ^{a,b}	75.9 %
IND + CME	7.5 ± 0.44 ^{a,b}	65.3 %
IND + CME + RAN	2.1 ± 0.15 ^{a,b,c}	90.3 %

Data represent the mean ± S.E.M. of observations from 8 rats.

^a Significantly different from control group at P < 0.05.

^b Significantly different from IND group at P < 0.05.

^c Significantly different from IND+RAN group at P < 0.05.

3.4. Effect of CME on the gastric juice analysis:

Table (3) and (4) indicate the effect of CME on the gastric juice analysis. Table (3) shows that indomethacin administration caused significant decrease in pH value associated with significant increase in gastric juice free and total acidity. Pretreatment with CME produced insignificant changes in pH value and free and total acidity as compared to indomethacin group. Pretreatment with RAN either alone or with CME produced significant increase in pH value and significant decrease in free and total acidity when compared to indomethacin group. Co-administration of RAN and CME showed more potent efficacy in reduction of free and total acid output.

Table (4) shows that indomethacin administration caused significant increase in gastric juice pepsin activity associated with significant reduction in gastric juice mucin content. Pretreatment with CME produced insignificant change in pepsin activity and significant increase in mucin content as compared with indomethacin group. Pretreatment with RAN either alone or with CME significantly decreased the gastric juice pepsin activity as compared to indomethacin group. Meanwhile, pretreatment with RAN did not produce any significant change in the mucin content, co-administration of RAN and CME significantly increased mucin content as compared to indomethacin group and RAN pretreated group.

Table (3): Effect of *C. myxa* extract (CME), ranitidine (RAN) pretreatment, and their combination on pH, free and total acid output of the gastric juice in indomethacin (IND)-induced gastric ulcer in rats.

Groups	pH	Gastric juice	
		Free acid output (μEq/3hours)	Total acid output (μEq/3hours)
Control	2.88 ± 0.12	35.5 ± 3.1	49.3 ± 2.4
IND	1.62 ± 0.06 ^a	129.8 ± 12.1 ^a	152.6 ± 11.9 ^a
IND + RAN	2.67 ± 0.09 ^b	43.4 ± 4.2 ^b	70.5 ± 6.1 ^{a,b}
IND + CME	1.83 ± 0.15 ^{a,c}	110.5 ± 9.5 ^{a,c}	138.6 ± 10.2 ^{a,c}
IND + CME + RAN	2.71 ± 0.24 ^b	41.6 ± 3.8 ^b	61.3 ± 5.1 ^b

Data represent the mean ± S.E.M. of observations from 8 rats.

^a Significantly different from control group at P < 0.05.

^b Significantly different from IND group at P < 0.05.

^c Significantly different from IND+RAN group at P < 0.05.

Table (4): Effect of *C. myxa* extract (CME), ranitidine (RAN) pretreatment, and their combination on pepsin activity and mucin content of the gastric juice in indomethacin (IND)-induced gastric ulcer in rats.

Groups	Gastric juice	
	Pepsin activity (μg/ml tyrosine)	Mucin content (mg % hexose)
Control	189 ± 8.2	168.1 ± 11.4
IND	236 ± 18.9 ^a	93.7 ± 7.5 ^a
IND + RAN	184 ± 16.1 ^b	101.2 ± 9.3 ^a
IND + CME	219 ± 20.5 ^{a,c}	141.4 ± 13.2 ^{a,b,c}
IND + CME + RAN	180 ± 13.8 ^b	144.1 ± 12.6 ^{a,b,c}

Data represent the mean ± S.E.M. of observations from 8 rats.

^a Significantly different from control group at P < 0.05.

^b Significantly different from IND group at P < 0.05.

^c Significantly different from IND+RAN group at P < 0.05.

3.5. Effect of CME on the gastric mucosal lipid peroxides (MDA):

As shown in Fig. (1), administration of indomethacin significantly elevated the gastric mucosal MDA concentration to about two folds the value observed for the control group, reaching 113.8 ± 11.4 nmol/g wet tissue as compared to 54.5 ± 3.12 nmol/g wet tissue for control group. Interestingly, all the pretreatments which used produced significant reduction in gastric mucosal MDA concentration as compared to indomethacin group. CME pretreatment reduced the gastric mucosal MDA concentration to 57.3 ± 4.05 nmol/g wet tissue. While, RAN pretreatment reduced the gastric mucosal MDA concentration to 65.4 ± 3.07 nmol/g wet tissue, co-administration of RAN and CME significantly augmented the decrease of gastric mucosal MDA concentration to 41.6 ± 3.18 nmol/g wet tissue.

3.6. Effect of CME on the gastric mucosal catalase (CAT) activity:

As shown in Fig.(2), administration of indomethacin significantly reduced the gastric mucosal catalase activity, reaching 3.46 ± 0.31 H₂O₂/g tissue/min. as compared to 5.65 ± 0.42 H₂O₂/g tissue/min. for control group. All the pretreatments which used induced significant increase in gastric mucosal catalase activity as compared to indomethacin group. CME pretreatment increased the gastric mucosal catalase activity to 7.51 ± 0.62 H₂O₂/g tissue/min. While, RAN pretreatment increased the gastric mucosal catalase activity to 6.21 ± 0.38 H₂O₂/g tissue/min., co-administration of RAN and CME significantly increased gastric mucosal catalase activity to 8.77 ± 0.55 H₂O₂/g tissue/min.

3.7. Effect of CME on the gastric mucosal nitrites/nitrates content:

In indomethacin group, gastric mucosal nitrites/nitrates content was significantly reduced from 325 ± 15.1 to 193 ± 11.5 nmol/g wet tissue. Pretreatment with RAN failed to alter significantly the gastric mucosal nitrites/nitrates content (207 ± 18.5 nmol/g wet tissue) when compared to indomethacin group. Meanwhile, pretreatment of CME significantly increased gastric mucosal nitrites/nitrates content to 298 ± 12.4 nmol/g wet tissue vs. indomethacin group. More increase in the gastric mucosal nitrites/nitrates content (334 ± 28.6 nmol/g wet tissue) was observed when CME co-administered with RAN (Fig. 3).

3.8. Effect of CME on the gastric mucosal prostaglandin E₂ (PGE₂) level:

The synthesis of mucosal PGE₂ was markedly suppressed by indomethacin compared to that in the normal rats (Fig. 4). However, the mucosal synthesis of PGE₂ in the CME- pretreated rats increased significantly compared to that of indomethacin group. The effect of pretreatment with CME either alone or with RAN was significantly better than that of RAN alone, which increased the PGE₂ level marginally.

3.9. Effect of CME on serum level of proinflammatory cytokine (TNF α):

Serum level of pro-inflammatory cytokine (TNF α) in ulcerated rats upon administration of CME is presented in Fig.(5). Compared with control group, the serum level of TNF- α was significantly increased in indomethacin group. Interestingly, all the pretreatments used had a significant suppression effect on serum level of TNF- α when compared to indomethacin group. The effect of pretreatment with both RAN and CME was significantly better than that of RAN alone.

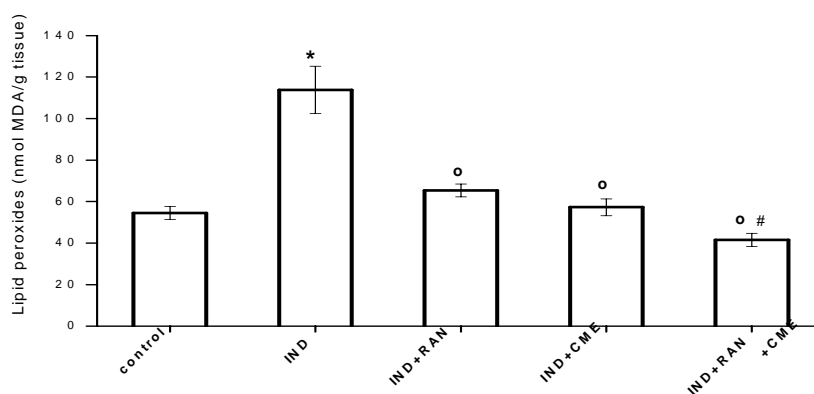


Figure 1: Effect of *C. myxa* extract (CME), ranitidine (RAN) pretreatment, and their combination on gastric mucosal lipid peroxides in indomethacin (IND)-induced gastric ulcer in rats. Data represent the mean \pm S.E.M. of observations from 8 rats.

* Significantly different from control group at $P < 0.05$.

° Significantly different from IND group at $P < 0.05$.

Significantly different from IND+RAN group at $P < 0.05$.

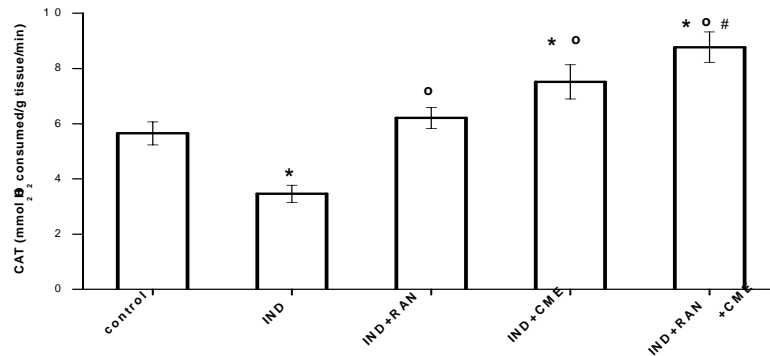


Figure 2: Effect of *C. myxa* extract (CME), ranitidine (RAN) pretreatment, and their combination on gastric mucosal catalase activity in indomethacin (IND)-induced gastric ulcer in rats. Data represent the mean \pm S.E.M. of observations from 8 rats.

* Significantly different from control group at $P < 0.05$.

° Significantly different from IND group at $P < 0.05$.

Significantly different from IND+RAN group at $P < 0.05$.

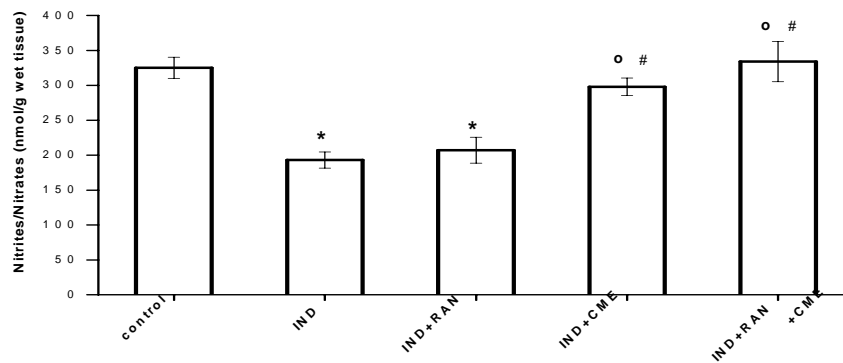


Figure 3: Effect of *C. myxa* extract (CME), ranitidine (RAN) pretreatment, and their combination on gastric mucosal nitrites/nitrates in indomethacin(IND)-induced gastric ulcer in rats. Data represent the mean \pm S.E.M. of observations from 8 rats.

* Significantly different from control group at $P < 0.05$.

° Significantly different from IND group at $P < 0.05$.

Significantly different from IND+RAN group at $P < 0.05$.

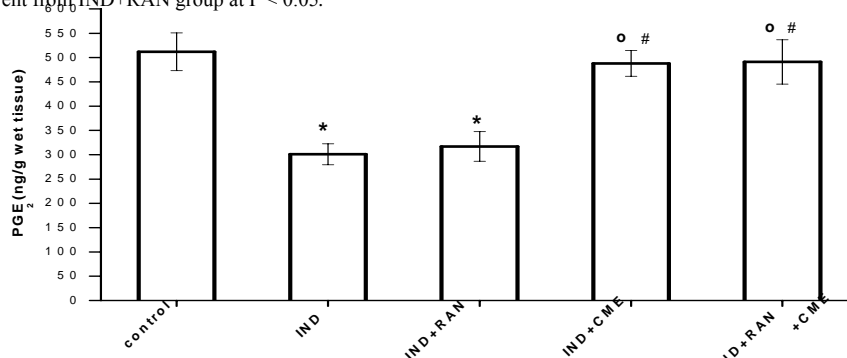


Figure 4: Effect of *C. myxa* extract (CME), ranitidine (RAN) pretreatment, and their combination on gastric mucosal prostaglandin E₂ in indomethacin (IND)-induced gastric ulcer in rats. Data represent the mean \pm S.E.M. of observations from 8 rats.

* Significantly different from control group at $P < 0.05$.

° Significantly different from IND group at $P < 0.05$.

Significantly different from IND+RAN group at $P < 0.05$.

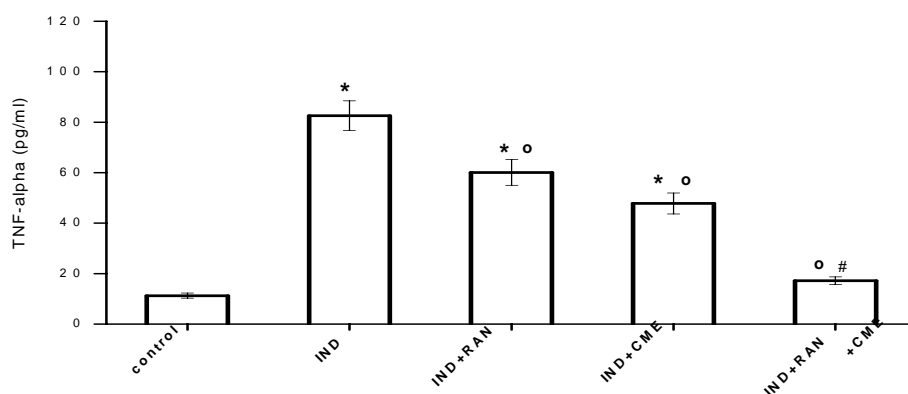


Figure 5: Effect of *C. myxa* extract (CME), ranitidine (RAN) pretreatment, and their combination on serum TNF- α in indomethacin (IND)-induced gastric ulcer in rats. Data represent the mean \pm S.E.M. of observations from 8 rats.

* Significantly different from control group at $P < 0.05$.

o Significantly different from IND group at $P < 0.05$.

Significantly different from IND+RAN group at $P < 0.05$.

3.10. Histopathological results:

Figure (6) shows that stomach of rats from control group (A) revealed normal gastric mucosa. On the other hand, stomach of rats from IND group (B) showed necrosis of lamina epithelialis, exposed muscularis mucosa, congestion of blood vessels associated with massive leukocyte cells infiltration in

lamina propria. However, examined stomach of rats concurrently pretreated with RAN + IND (C) showed leukocyte cells infiltration in submucosal layer. Meanwhile, stomach of rats concurrently pretreated with CME + IND (D) or CME+ RAN + IND (E) revealed no histological changes.

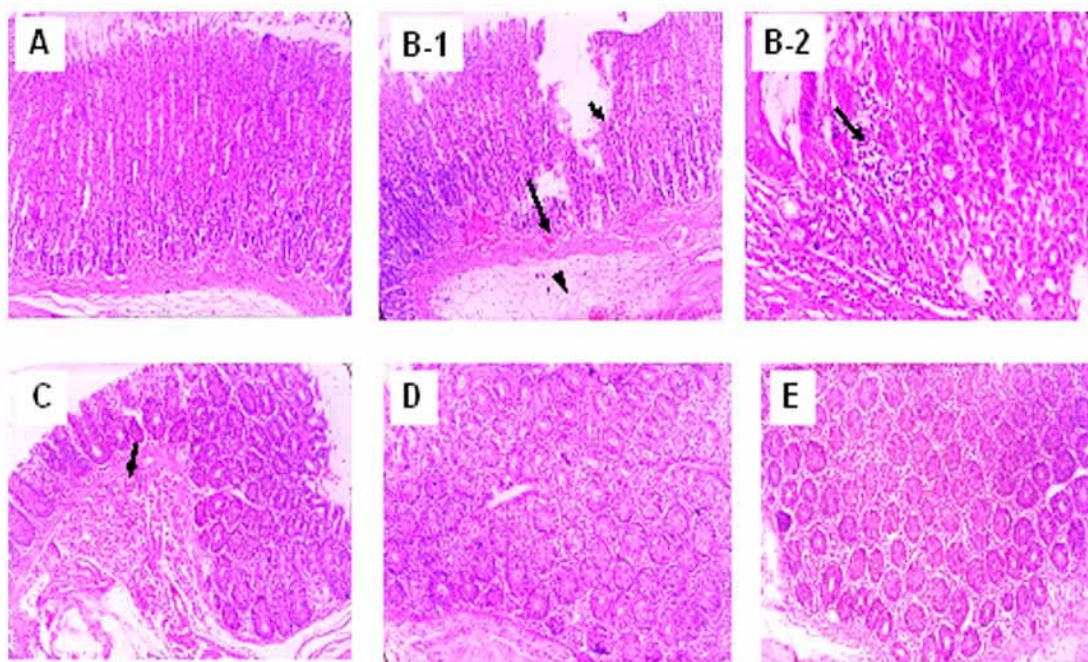


Figure 6: Light micrograph of rat stomach (A) control (B) treated with IND showing: (B-1) necrosis of lamina epithelialis (short arrow), exposed muscularis mucosa and congestion of blood vessels (long arrow), associated with submucosal edema (arrow head) (H&E X100), (B-2) massive leukocyte cells infiltration in lamina propria (H&E X200). (C) pretreated with RAN + IND showing leukocyte cells infiltration in submucosal layer (H&E X100). (D) pretreated with CME + IND and (E) pretreated with CME + RAN + IND showing no histopathological changes (H&E X100).

4. Discussion:

The use of nonsteroidal anti-inflammatory drugs (NSAIDs) is considered to be the major risk factor in gastric ulcers. The mechanisms suggested for the gastric damage caused by NSAIDs are inhibition of prostaglandin synthesis and inhibition of epithelial cell proliferation in the ulcer margin, which is critical for the reepithelization of the ulcer crater (Levi et al., 1990). There has been a considerable interest in finding natural antioxidants from plant materials to replace synthetic ones for effective management of therapeutic drug toxicity such as peptic ulcer (Pratt, 1992).

The phytochemical studies performed in the present study demonstrated that CME present carbohydrates, glycosides, flavonoids, sterols, saponins, terpenoids, alkaloids, phenols, gums and mucilage. Among these secondary compounds, saponins, terpenoids and flavonoids are referred as antiulcer compounds (Lewis and Hanson, 1991). This phytochemical composition of CME could explain the antiulcer activity produced by fruit extract which was detected in our study. Moreover, several plants containing high amounts of saponins have been shown to possess antiulcer activity in several experimental bioassays (Yesilada and Takaishi, 1999 and Morikawa et al., 2006) probably acting as an activator of mucus membrane protective factors (Saito et al., 1977). Triterpenoids are a widespread class of secondary compounds with several pharmacological activities, including anti-inflammatory effect in rat paw edema model (Jung et al., 2005) and antiulcer activities (Arrieta et al., 2003). Additionally, the gastroprotective effect of flavonoids has been previously reported (Reyes et al., 1996). Free radical scavenging ability of flavonoids has been reported to protect the gastrointestinal tract from ulcerative and erosion lesion (Borelli and Izzo, 2000).

The volume of acid present in gastric secretion which encompasses HCl, pepsinogen, mucus, bicarbonates, intrinsic factor and protein reflects acid volume. Exposure of unprotected lumen of the stomach to accumulating acid could facilitate ulceration (Olsen, 1988). Another major aggressive factor responsible for ulcers is the content of acid present in gastric juice. Over secretion of histamine contributes to increased secretion of gastric juice (Grossman, 1978). When the concentration of hydrogen ions in gastric juice decreases, it is reflective of high pH. The genesis of ulcer and gastric damage is facilitated by hydrogen ions which serve as another aggressive factor (Lüllmann et al., 2000).

In the present study, indomethacin injection, a representative of NSAIDs family, caused a remarkably significant increase in ulcer index, gastric

juice free and total acidity and pepsin activity. The ulceration induced by indomethacin is attributed mainly to various processes, including generation of reactive oxygen species, initiation of lipid peroxidation, infiltration of leukocytes, induction of apoptosis, and inhibition of prostaglandin synthesis (Bech et al., 2000). Decreased prostaglandin level impairs almost all aspects of gastroprotection and increases acid secretions which, in turn, aggravate the ulcer (Miller, 1983).

Oral administration of RAN significantly reduced ulcer index, gastric juice free and total acidity and pepsin activity. However, the drug has not produced any significant quantitative change in the mucin content. Gastric acid decimation by RAN is attributed to its ability to antagonize the binding of histamine to the H₂ receptor on the parietal cells (Banji et al., 2010). RAN can therefore counter the effect of indomethacin on acid secretion. Oral administration of CME produced significant decrease in ulcerative index, with insignificant change in free and total gastric acid values and pepsin activity. Therefore, this result reinforced the absence of antisecretory activity of CME and possible strengthening of gastroprotective factors such as antioxidant elements in this extract.

Gastric mucus (mucin) is an important protective factor for the gastric mucosa and consists of a viscous, elastic, adherent and transparent gel formed by 95% water and 5% glycoproteins that cover the entire gastrointestinal mucosa. Moreover, mucus is capable of acting as an antioxidant, and thus can reduce mucosal damage mediated by oxygen free radicals (Repetto and Llesuy, 2002). The protective properties of the mucus barrier depend not only on the gel structure but also on the amount or thickness of the layer covering the mucosal surface (Penissi and Piezzi, 1999).

In this study, the decreased mucin secretion in the indomethacin-administered rats indicated reduced ability of the mucosal membrane to protect the mucosa from physical damage and back diffusion of hydrogen ions. Mucosal damage can be easily produced by the generation of exogenous and endogenous active oxygen and free radicals (Naito et al., 1995). An increase in mucus production usually assists the healing process by protecting the ulcer crater against irritant stomach secretions (HCl and pepsin) thereby enhancing the rate of the local healing process. Treatment with CME protected the gastric mucosa from damage by increasing the mucin content significantly. Apparently, the free radicals scavenging property of CME might contribute in protecting the oxidative damage to gastric mucosa. In addition, the content of mucilage in CME might

carpet the gastroduodenal lining, thereby abrogating the impact of ulcerogens such as indomethacin.

Indomethacin is known to induce the reactive oxygen metabolites in animal models, which may contribute to mucosal injury (Chattopadhyay et al., 2006). These free radicals also damage the cellular antioxidant enzymes such as CAT, SOD and others, acting as the first line of cellular defense against oxidative injury. This might lead to aggravated tissue damage during stomach ulceration (El-Missiry et al., 2001). Our experimental results are in line with these previous data. Indomethacin- induced stomach ulceration was accompanied with a severe oxidative stress in gastric tissue causing damage to key biomolecules such as lipids. This was apparent from the stimulated lipid oxidation leading to increased accumulation of MDA as well as reduction in the gastric activity of CAT.

As shown in the present results, RAN treatment significantly reverted the indomethacin-induced changes in MDA and CAT. This significant reduction in MDA levels along with significant increase in CAT level suggest decreased lipid peroxidation and antioxidant activity of RAN. Ranitidine, an antisecretory drug, has often been reported to possess antioxidant and immunosuppressive actions, which may be responsible for its antiulcerogenic activity (Lapenna et al., 1994 and Ardestani et al., 2004). CME provided a marked suppression of oxidative damage due to its excellent radical scavenging capacity, It brought MDA level closer to normal levels, than observed in RAN with concomitant increase in CAT level.

Nitric oxide (NO) is an endogenous defensive factor for gastric cells and exhibits gastroprotective properties against different types of aggressive agents (Samini et al., 2002). It is involved in the maintenance of mucosal integrity through the regulation of mucus and alkaline secretion, gastric motility and microcirculation (Tsukimi and Okabe, 2001). NO is known to modulate acid levels, gastric mucus secretion, and blood flow in gastric tissues (Martín et al., 2001). NO has also been reported to prevent membrane lipid peroxidation (Hogg and Kalyanaraman, 1999), it may protect against NSAID damage by promotion of prostaglandin synthesis (Salvemini et al., 1993).

In the present study, indomethacin significantly reduced gastric mucosal NO level compared to control group. This finding was in accordance with Lanas et al. (2000) and Cadirci et al. (2007) who reported a decrease in NO level in stomach tissue damaged by indomethacin. Tripp and Tepperman (1995) also reported a decrease in NO biosynthesis, as a result of decreased nitric oxide synthase (NOS) activity that was associated with an increase in the

extent of damage. Treatment with CME significantly increased mucosal NO level when compared to indomethacin treated rats. The possible mechanism of the increased NO action of CME may be due to its flavonoids content. Matsuda et al. (2003) reported that flavonoids are the major secondary metabolites class with several descriptions of antiulcer, antioxidant and gastroprotective properties.

Prostaglandin, a key molecule that stimulates the complex array of ulcer healing mechanism, gets synthesized in the mucosal cells by cyclooxygenase (COX) enzymes. It stimulates the secretion of bicarbonate and mucus, maintains mucosal blood flow and regulates mucosal turn over and repair (Hayllar and Bjarnason, 1995 and Hiruma-Lima et al., 2006). Suppression of prostaglandins synthesis by indomethacin results in increased susceptibility of stomach to mucosal injury and gastroduodenal ulceration. Indomethacin causes ulcer mostly on the glandular (mucosal) part of the stomach (Nwafor et al., 1996) by inhibiting prostaglandin synthesis through the inhibition of the cyclooxygenase enzymes (Rainsford, 1987). Prostaglandin and bicarbonate secretion and gastric blood flow have been shown to be reduced in animals by indomethacin treatment (Selling et al., 1987). Our experimental results were in line with these previous data, indomethacin significantly reduced gastric mucosal prostaglandin E₂ (PGE₂) level compared to control. Treatment with CME significantly increased PGE₂ level when compared to indomethacin treated rats. This finding was explained by Borrelli and Izzo (2000) who reported that flavonoids may protect the gastric mucosa from damage by increasing the mucosal prostaglandin content and by inhibiting histamine secretion from mast cells by inhibition of histidine decarboxylase.

Tumor necrosis factor (TNF- α) is a pro-inflammatory cytokine secreted by macrophages increasingly during ulcerative stress (Hamaguchi et al., 2001), it is a potent stimulator of neutrophil infiltration into gastric mucosa (Wei et al., 2003) and inducible nitric oxide expression (Calatayud et al., 2001). Overproduction of TNF- α increases the risk of gastric ulcer and cancer (Mitsushige et al., 2007). The inhibition of TNF- α and neutrophil infiltration will ultimately inhibit tissue destruction by reactive oxygen species (Kwiecien et al., 2002). In this study indomethacin significantly increased serum TNF- α as compared to control group. This finding was coincided with the finding of Appleyard et al. (1996) who reported that indomethacin up-regulated the synthesis of pro-inflammatory molecules like TNF- α contributing to mucosal injury. Moreover, Swarnakar et al. (2005) found that indomethacin-induced serum TNF- α and mucosal TBARS up-regulation at the

ulcer site seemed mostly to be responsible for ulcerogenesis.

Polymorphonuclear migration is an early and critical event in the pathogenesis of gastric mucosal injury caused by indomethacin. TNF- α was previously reported to be a proinflammatory cytokine that causes polymorphonuclear neutrophil migration, through up-regulating the expression of adhesion molecules in both neutrophil and endothelial cells (Santucci et al., 1995). Probably, prostaglandins inhibition by NSAIDs is responsible for the TNF- α rise, as these class of drugs markedly reduce prostaglandin synthesis, which were known to be potent inhibitors of TNF- α release from both macrophages (Kunkel et al., 1986) and mast cells (Hogaboam et al., 1993).

On the other hand, the up-regulatory action of indomethacin to serum TNF- α is possibly responsible for the decrease in mucosal NO. This action was in agreement with reported results of Bauer et al. (1997) who recorded that TNF- α is a potent inhibitor to constitutive NO, which mediated a protective effect in the stomach, mostly through modulation of cytokine production. CME produced significant suppression of TNF- α production and this may be attributed to the anti-inflammatory activity of CME. The anti-inflammatory effect of CME was previously proved by a reduction in myeloperoxidase activity in experimentally induced colitis in rats (Al-Awadi et al., 2001). It is interesting to find that in our study RAN showed a significant decrease in serum TNF- α . In accordance with our results, Odabasoglu et al. (2008) reported that ranitidine showed a decreasing effect on the myeloperoxidase activity. Van Zyl et al. (1993) reported also that ranitidine and other H₂ antagonists in clinical use have potent inhibitory effect on myeloperoxidase catalyzed reactions.

Histopathological studies on the gastric mucosa revealed that indomethacin administration induced a mucosal ulceration, associated with significant increase in lipid peroxidation. This was manifested as lamina epithelial necrosis, blood vessels congestion, and leukocytic infiltration. This effect on mucosal oxidative stress and histological derangement was in accordance with the reports of (Valcheva-Kuzmanova et al. 2007 and El-Moselhy et al. 2009). CME had protective effect against indomethacin-induced inflammatory infiltration and congestion at the ulcer sites. It prevented gastric mucosal lesions through its flavonoid content. Flavonoids could scavenge free radicals, inhibit lipid peroxidation, and increase prostaglandins and mucosal content of the gastric mucosa; showing cytoprotective effects (Alanko et al., 1999).

In conclusion, CME can protect indometacin-induced gastric ulceration due to its antioxidative,

anti-inflammatory and mucin enhancing properties. The mechanism of its gastroprotective activity may be attributed to reduction in gastric mucosal lipid peroxidation (MDA), and serum TNF α along with elevation in gastric juice mucin content and gastric mucosal CAT, NO, and PGE₂ levels. The presence of phytoconstituents in this medicinal plant, particularly flavonoids and mucilage, might be responsible for these pharmacological actions. The protection afforded by co-treatment of CME and ranitidine was found to be better than that of CME alone and ranitidine alone. Consequently, CME could be used together with RAN for the treatment of gastric ulcer.

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Corresponding Author:

Dr. Inas Zeidan Abdou Abdallah, Nutrition and Food Science Department, Faculty of Home Economics, Helwan University, Egypt.
E. mail: izaa2011@hotmail.com

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