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Evaluation of antiulcer activity of indole-3-carbinol and/or omeprazole on aspirin-induced gastric ulcer in rats

Nashwa A El-Shinnawy[1], Samira A Abd-Elmageid[1] and Eda MA Alshailabi[2]

Abstract

The present work is an attempt to elucidate the antiulcer activity of indole-3-carbinol (I3C), which is one of the anticarcinogenic phytochemicals found in the vegetables of *Cruciferae* family such as broccoli and cauliflower, alone or in combination with omeprazole (OMP), a proton pump inhibitor, to diminish the effects of induced acute gastric ulcer by aspirin (ASA) in male albino rats. A total of 48 adult male albino rats were used in the present study. Animals were divided into eight experimental groups (six animals each group). They were given different experimental inductions of ASA at a dose of 500 mg/kg/body weight, OMP at a dose of 20 mg/kg/body weight and I3C at a dose of 20 mg/kg/body weight either alone or in combination with each other orally for a duration of 7 days. Inner stomach features, ulcer index, pH activity, body weight, stomach weight, hematological investigations, serum total protein albumin and reduced glutathione activity were investigated in addition to the histological, histochemical and immunohistochemical stain of cyclooxygenase-2 to the stomach tissue of normal control, ulcerated and treated ulcerated rats. The results of this study revealed that oral administration of ASA to rats produced the expected characteristic mucosal lesions. OMP accelerated ulcer healing but the administration of I3C either alone or in combination with OMP to ASA-ulcerated rats produced a profound protection to the gastric mucosa from injury induced by ASA. Our results suggested that administration of antiulcer natural substances such as I3C in combination with the perused treatment such as OMP is a very important initiative in the development of new strategies in ulcer healing.

Keywords

Aspirin, cyclooxygenase-2, gastric ulcer, indole-3-carbinol, omeprazole

Introduction

Currently, varieties of nonsteroidal anti-inflammatory drugs (NSAIDs) are being used for treating inflammatory diseases (Nagesh and Gokul, 2011). Aspirin (ASA), known as acetylsalicylic acid, is a salicylate drug, often used as an analgesic to relieve minor aches and pains, as an antipyretic to reduce fever and as an anti-inflammatory medication. Despite the cardiovascular benefits of ASA, a potential gastrointestinal harm has been noted. The main undesirable side effects of ASA are gastrointestinal irritation, ulcers and stomach bleeding (Choi et al., 2010).

Peptic ulcer occurs due to imbalance between aggressive (acid and pepsin) and defensive (mucus gastric mucosal barrier) factors of gastric mucosa. The treatment of peptic ulcer is directed against either reduction of aggressive factors or enhancement of mucosal defense of stomach and duodenum with cytoprotective agents (Jaikumar et al., 2010).

Hypersecretion of gastric acid due to the use of NSAID is a pathological condition, which occurs due to uncontrolled secretion of hydrochloric acid from

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the parietal cells of the gastric mucosa. Luminal acid interferes with the process of restitution, resulting in the conversion of superficial injury to deeper mucosal lesion and inactivates the acid-labile growth factors important for the maintenance of mucosal integrity (Wallace and Muscara, 2001). The modern approach to control gastric ulceration is to inhibit gastric acid secretion, to promote gastro protection, block apoptosis and stimulate epithelial cell proliferation for effective healing (Sharma et al., 2011).

Furthermore, the pharmacological activity of NSAIDs is related to the suppression of prostaglandin biosynthesis popularly known as cyclo-oxygenase-2 (COX-2). Prostaglandins synthesized by the gastric mucosa are one of the defensive factors known to inhibit the secretion of gastric acid and stimulate the secretion of mucus and bicarbonate (Bhandari et al., 2008).

Omeprazole (OMP) is an effective agent in the treatment of peptic ulcer disease. The effects of OMP against gastric mucosal injuries have been thought to depend on its inhibitory action on gastric acid secretion (Kobayashi et al., 2002). However, clinical evaluation of this drug have shown relapse in the long run (Szabo and Vincze, 2000), side effects and drug interactions (Abdul-Aziz, 2011). This has been rational for the development of innovative drug that reduces the offensive factors and is proved to be safe, clinically effective, having better patient tolerance, relatively less expensive and globally competitive. Plant extracts, however, are some of the most attractive sources of new drugs and have been shown to produce promising results in the treatment of ulcers (Sunilson et al., 2008).

One of the most important anticarcinogenic phytochemicals contained in cruciferous vegetables of the *Brassica* genus, such as cabbage and broccoli, is indole-3-carbinol (I3C). It is an enzymatic breakdown product of indole glucosiniolates and sulfur-containing compounds contained in cruciferous vegetables (Anderton et al., 2004). Considerable evidence shows that I3C inhibits experimentally induced tumorigenesis at different sites in the colon, lung, skin, liver, cervix and mammary gland in mouse and rat models (Melkamu et al., 2010). Therefore, the aim of the present work is to study the effects of I3C as a new safer cytoprotective compound found in cabbage and broccoli, which protects the gastric mucosa from ulcer. In addition, the efficacy of combined treatments of OMP and I3C to normalize the acidity of stomach in ulcerated rats is examined.

Materials and methods

Experimental animals and treatment

The present study was conducted using 48 adult male albino rats (*Rattus norvegicus*) weighing 150 $\pm$ 10 g. The animals were housed in the vivarium of the Animal house of Medical Research and Bilharizia center, Faculty of Medicine, Ain Shams University. Animals housed under standard laboratory conditions with a 12:12 light/dark cycle and a temperature of 23–25°C. Rats were fed standard laboratory diet and water *ad libitum* with fresh daily supplies. All procedures have been performed in accordance with national animal welfare legislation of Faculty of Medicine, Ain Shams University. They were allowed for 10 days in the pre-experimental period to adapt to the laboratory conditions. The animals were divided into eight experimental groups of six animals in each group as follows: normal control group, OMP group (received OMP at a dose of 20 mg/kg/body weight), I3C group (received I3C at a dose of 20 mg/kg/body weight), OMP + I3C group, ASA group (received ASA at a dose of 500 mg/kg/body weight), ASA + I3C group, ASA + OMP group and ASA + I3C + OMP group. All chemicals were administered to rats by stomach tube. Dissection was done after an experimental period of 7 days.

Experimental chemicals and drugs

ASA, also known as acetyl salicylic acid (Bayer AG, Germany), was given to animals by stomach tube at a dose of 500 mg/kg /body weight after a fasting duration of 24 h according to Sarkar and Guha (2008) for 7 consecutive days for acute ulcer induction.

OMP(C17H19N3O3S) (European Egyptian Pharmaceutical Industries, Cairo Egypt) was given to animals at a dose of 20 mg/kg/body weight according to Suleyman et al. (2001) by stomach tube for 7 consecutive days.

I3C was purchased from Sigma-Aldrich Chemical Company USA (Cairo, Egypt). Animals were given I3C at a dose of 20 mg/kg/body weight (Crowell et al., 2006) orally by stomach tube for 7 consecutive days.

Measurement of gastric acid and pH

After dissection, stomachs of the rats were legated from its two ends; the pylorus and lower esophagus were also ligated and injected with 2 ml distilled water. A small incision was made for each forestomach and

then the stomach contents were expelled (Hsu et al., 2009). Gastric contents were collected in tubes and then measured. Gastric juice was centrifuged at 3500 r/min for 15 min and the supernatant was used for determining total acidity. Overall, 1 ml of the supernatant liquid was pipetted out and diluted to 10 ml with distilled water. The solution was titrated against 0.01 N sodium hydroxide (NaOH) using phenolphthalein reagent as an indicator to the endpoint when the solution turned to pink color. The volume of NaOH required was denoted to calculate the total acidity. The total acidity and pH of the supernatant were calculated according to Vinothapooshan and Sundar (2010) and Sadler and Murphy (2010).

Determination of ulcer index, ulcer score and percentage of ulceration

Each stomach was opened along the greater curvature and washed with distilled water and examined using magnifying lens to determine the ulcer index according to Parmar and Desai (1993). Evaluation of the percentage and degree of ulceration was expressed in terms of ulcer score according to Al-dalain et al. (2008) and Mabrouk et al. (2009).

Body weight, stomach weight, relative stomach weight and general stomach features

Rats were individually weighted by means of a Meopta sensitive balance. Whole weights were recorded to the nearest milligram to determine weekly changes. After dissection, each stomach was removed, weighted and recorded to the nearest milligram to determine weekly changes. The relative stomach weight of each animal was calculated. Small incisions were made in the forestomachs of all the experimental groups and then the opened stomachs were photographed for the demonstration of the inner features.

Hematological investigation

Blood samples were drawn from the heart on EDTA tubes. The levels of total red blood cells (RBCs), hematocrit (HCT), hemoglobin (Hb), white blood cells (WBCs), platelets (PLTs), lymphocyte count and monocyte count were counted using a hemocytometer.

Determination of serum total protein, albumin level and tissue reduced glutathione (GSH) activity

Blood samples were drawn from the heart of the rats and allowed to coagulate at room temperature and then centrifuged at 3000 r/min for 15 min. Clear non-hemolysed serum was used for the determination of serum total protein, according to Doumas (1975), and albumin, according to Doumas et al. (1971), by colorimetric method. A known weight of the stomach was homogenized in a suitable aliquot of ice-cold phosphate-buffered saline solution (pH 7.4) to make 10% homogenate (w/v). The homogenate was centrifuged at 4000 r/min for 15 min and the supernatant was separated and used to measure stomach tissue GSH activity according to Villegas et al. (2000) using spectrophotometer (Humalyzer, Junior, Semi-automated Bench top Chemistry Photometer).

Histological, histochemical and immunohistochemical methods

For histological, histochemical and immunohistochemical examination, other parts of rats' stomachs were placed in 10% buffered formalin, dehydrated, cleared with xylene and infiltrated (at 60°C) and embedded with paraffin wax. Paraffin blocks were cut at 5 μm and affixed to slides and then stained.

Sections were stained with hematoxylin and eosin for general histological examination (Bancroft and Cook, 1994). Other sections were stained with bromophenol blue for the demonstration of sites of total protein content (Mazai et al., 1953). According to Mowry (1956), sections were stained with combined alcian blue periodic Schiff technique to demonstrate the presence of mucins and clearly distinguish between acid and neutral mucins.

From 10% formalin-fixed paraffin-embedded samples, 4-μm-thin sections were prepared and stained with COX-2 and then observed for the demonstration of particular immunohistochemical features according to Niijima et al. (2002). The number of positive cells in the sections with COX-2 was counted under microscope magnified 400× (Sun et al., 2005).

Immunoreactivity was evaluated in a blinded fashion by two independent observers using a grading system where no staining was regarded as negative (−ve), but when they were between 1% and 10%, the staining was considered positive (+). Furthermore,

when stained cells were recorded between 11% and 30%, the staining was considered positive (++) and (+++) for 31% or greater (Niijima et al., 2002).

Statistical analysis

Results were expressed as the mean ± SE. Statistical analysis was performed using the statistical package for social science (SPSS) version 17 statistical software. The significance of the difference between means of the control and all treated rats (a) and between the treated ulcerated rats and the ASA-ulcerated rats alone (b) was analyzed using one-way analysis of variance. The percentage change was taken as the difference between the experimental groups and control value, divided by the control value, calculated in percentage or the difference between the experimental groups and ASA, divided by ASA value × 100.

Results

General features of stomach

The administration of ASA for 7 days had resulted in spot ulcer and severe red coloration in the dissected stomach of rats, whereas this color fainted but did not disappear by the concomitant treatment of OMP to the ASA-ulcerated rats. On the other hand, all ulcerated animals treated with either I3C or I3C + OMP showed no ulcers or hemorrhagic streaks throughout the experimental period (Figure 1).

Ulcer index, percentage of ulceration and ulcer score

Table 1 represents ulcer index, ulcer score and percentage of ulceration in normal control group and ASA groups at 7 days. No ulcers were detected in the stomach of normal control rats. An increase in the mean values of ulcer index, ulcer score and percentage of ulceration in rats' stomachs was noted after 7 days of ASA administration.

Total acidity and pH levels

Data recorded for total acidity and pH levels were presented in Table 2. Normal control animals signed more or less constant varying levels of total acidity and pH throughout the experimental period. ASA rats alone showed a significant ($p < 0.001$) increase in total acidity than normal control rats at 7 days. The percentage of increase was 112.61%. More pronounced significant ($p < 0.001$) decline in total acidity was established in ASA + OMP, ASA + I3C and ASA + OMP + I3C groups after 7 days of treatment compared with the ASA groups. Furthermore, significant ($p \leq 0.05$) decrease in the mean pH value occurred in the ASA group (1.13 ± 0.45) compared with normal control rats (2.93 ± 0.66) at 7 days, whereas significant ($p \leq 0.05$) increase in the mean values in ASA + OMP, ASA + I3C and ASA + OMP + I3C groups was noted (2.83 ± 0.27, 2.67 ± 0.19 and 3.73 ± 0.19, respectively) at 7 days compared with ASA group (1.13 ± 0.45).

Changes in body and stomach weight

The initial body weight, final body weight, stomach weight and relative stomach weight in rats following 7 days of ASA administration are presented in Table 3. In relation to the control animals, a significant ($p \leq 0.001$) increase in the final body weight was denoted in the ASA, group. On the other hand, the increase in the stomach weight of the rats in ASA group began to be attenuated by different treatments to record a percentage of decrease in 29.19%, 23.78% and 24.86% in ASA + OMP, ASA + I3C and ASA + OMP + I3C groups, respectively, compared with the ASA group.

Total RBCs, HCT and Hb levels

Table 4 represents the results of total RBCs, HCT and Hb levels in normal and ASA-ulcerated rats. Table 4 also shows a decrease in the mean values of RBC and Hb with an increase in HCT% in ASA group compared with the normal control animals.

Total WBCs, lymphocyte, monocyte and PLTs count

From the inspection of the data recorded in Table 5, a pronounced decrease in the percentage of monocytes and PLTs count with a slight increase in WBCs and lymphocytes was detected in the ASA-treated rats. Moreover, these results began to be attenuated in the I3C and OMP + I3C groups with a percentage of increase in 319.85% and 149.63% in the PLT levels of ASA + I3C and ASA + OMP + I3C groups, respectively, compared with ASA-administered animals.

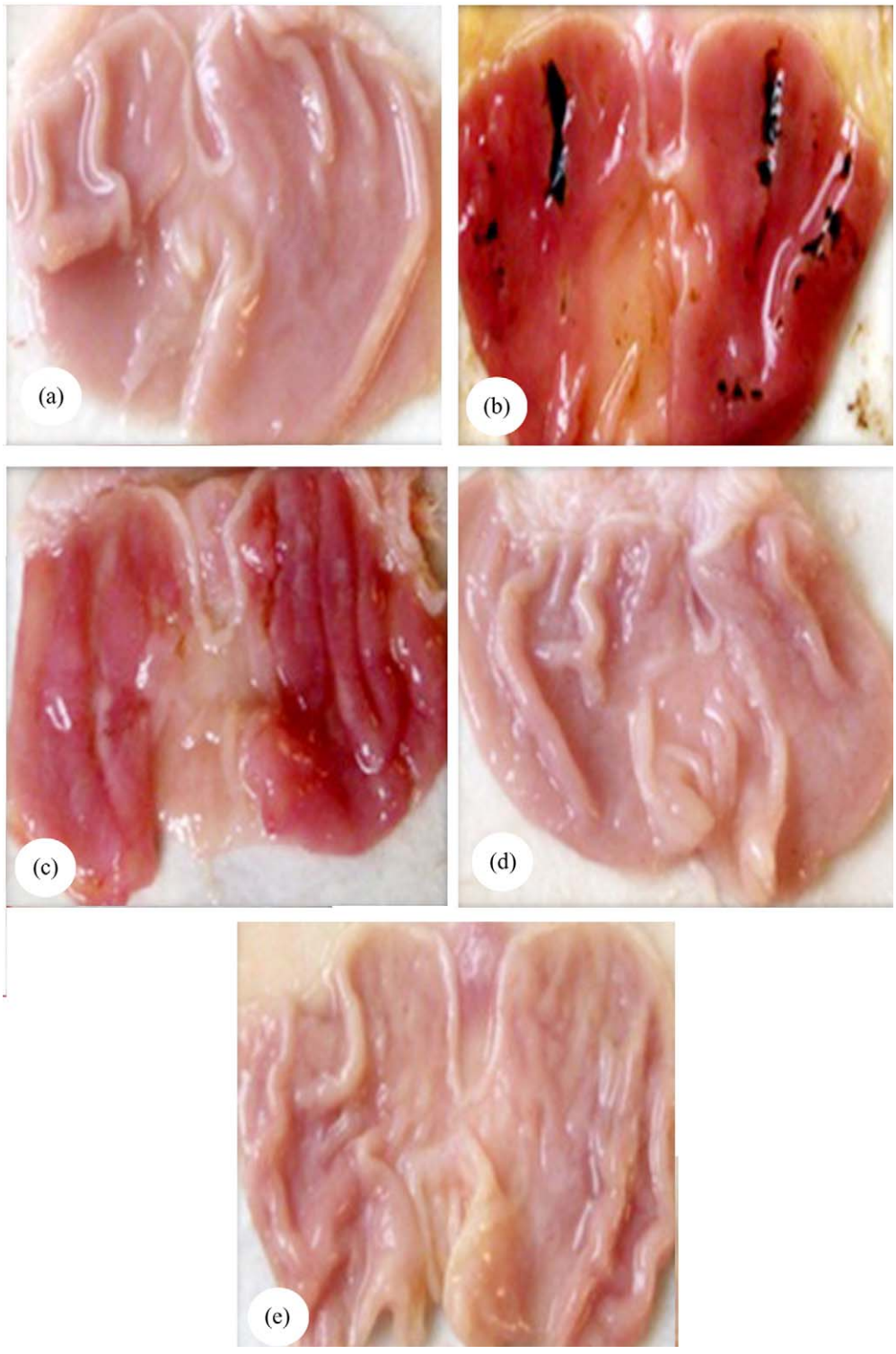

Figure 1. General features of opened excised stomach of normal control stomach tissue (photomicrograph (a)), ulcerated ASA rats (photomicrograph (b)), OMP-treated tissue (photomicrograph (c)), I3C-treated tissue (photomicrograph (d)) and OMP + I3C-treated tissue (photomicrograph (e)). ASA: aspirin; I3C: indole-3-carbinol; OMP: omeprazole.

Table 1. Ulcer index, percentage ulceration and ulcer score of normal control and ASA group at 7 days[a]

	Normal control	ASA
Ulcer index	0.00	2.40 ± 0.87
Percentage of ulceration	0.00	71.28 ± 0.14
Ulcer score	0.00	1.67 ± 0.71

ASA: aspirin.
[a]Values are mean ± SE when compared with the control group.

Serum total protein, albumin levels and stomach GSH activity

Table 6 shows that there is a significant increase in the serum total protein and albumin levels in the ASA-treated rats at 7 days compared with normal control rats. Detecting stomach glutathione levels revealed that rats treated with ASA showed a decline in the mean value of stomach GSH to reach 0.18 ± 0.08 mg/g tissue compared with 0.41 ± 0.03 mg/g tissue in normal control animals. Following different treatments to the ulcerated rats, a significant ($p < 0.001$) increase was recorded in ASA + OMP, ASA + I3C and ASA + OMP + I3C groups.

Figure 2 (a) to (c) demonstrates that normal control, I3C and OMP groups alone showed normal gastric mucosal tissue with no ulcer. Histological examination of gastric mucosal tissue of ASA group (Figure 2(d)) showed sharply defined ulcer crater at the site of exposure to ASA almost reaching the submucosal layer with hemorrhage, discontinuity of lining epithelium and a deep edema covering the total glandular epithelium. In addition, damaged disrupted mucosal epithelium with leukocytic infiltration was observed. On the contrary, I3C treatment to ulcerated ASA rats alone (Figure 2(f)) illustrated retaining of normal mucosa, absence of ulcer crater, clearance of necrosis and maintenance of mucosal layers along with normal glands. Nevertheless, mild hyperplasia in epithelial lining of the mucosal gastric tissue and mild edematous submucosa with some leukocytic infiltration were still recognized in OMP-treated ulcerated rats (Figure 2(e)) and OMP + I3C-treated groups (Figure 2(g)). OMP-treated ulcerated rats also showed discontinuation of the epithelial lining with diffuse lymphocytic infiltration extending into submucosa, where dilated congested blood capillaries were present, while all layers of fundus were looking healthy. The dilation of gastric epithelial cells, loss of mucosal architecture and tendency of exfoliation of gastric pits were not observed in all ASA-treated animals. However, few inflammatory cells were predominant in the terminal fundus gland regions.

Histochemical results

Stomachs of normal control animals demonstrated normal distribution of mucin content in the gastric tissue throughout the whole experimental period, which was showed by combined alcian blue periodic Schiff technique (Figure 3).

A strong reactivity was displayed by the peptic and oxyntic cells of the control animals in the bromophenol blue stain. Their contents of protein were located in a mildly reactive ground cytoplasm. Their nuclei exhibited a strong reactivity with bromophenol blue (Figure 4).

Histochemical staining of gastric sections from animals treated with ASA for the identification of the mucin content represented by combined alcian blue periodic Schiff technique (Figure 3) and total protein content (Figure 4) manifested a diminution in mucosal cells total content. The stain ability of the cytoplasm and nuclei of the oxyntic, peptic and mucous neck cells was greatly reduced and the protein granules in most cells were highly reduced. In such case, a weak feeble stainability with both stains was quite clear in the constituent cells, whereas proteinic content began to regain affinity to stainability. The cytoplasm and nuclei of peptic, parietal mucous neck cells and surface mucous cells had restored a greater part of their reactivity with bromophenol blue or with combined alcian blue periodic Schiff by the I3C, OMP and the combined treatments of I3C and OMP. It is obvious from the stomach sections that I3C treatment alone to ASA-ulcerated rats has more pronounced effects on the gastric tissue mucin content than OMP treatment alone.

Immunohistochemical results

The gastric tissues obtained in the ASA model of gastric ulcer were used for immunohistochemical localization of COX-2 antibodies. The analysis of histological slices (Figure 5) showed a great number of proliferation cells in the stomach of animals treated with I3C, OMP and OMP + I3C. Thus, the results indicate that this protein participated in the healing of the gastric ulcer treated with I3C, OMP and OMP + I3C. Table 7 shows that the most pronounced expression of COX-2 stain was in I3C-treated ulcerated rats.

Table 2. Total acidic activity and pH level of control and experimental groups at 7 days[a]

		Non-ulcerated groups				Ulcerated groups			
		NC	OMP	I3C	OMP + I3C	ASA	ASA + OMP	ASA + I3C	ASA + I3C + OMP
Total acidic activity (mEq/L)	Mean ± SE	18.00 ± 1.15	19.33 ± 0.33	18.80 ± 0.61	20.00 ± 0.58	38.27[b] ± 4.95	21.33[c] ± 0.8	21.00[c] ± 1.00	20.33[c] ± 0.33
pH	Mean ± SE	2.93 ± 0.66	4.10 ± 0.86	4.40 ± 0.06	3.37 ± 0.43	1.13[d] ± 0.45	2.83[e] ± 0.27	2.67[f] ± 0.19	3.73[c] ± 0.19

ASA: aspirin; I3C: indole-3-carbinol; NC: normal controls; OMP: omeprazole.
[a]Values are expressed as mean ± SE.
[b]$p < 0.001$ compared with the control group.
[c]$p < 0.001$ compared with the ASA group.
[d]$p < 0.01$ compared with the control group.
[e]$p < 0.01$ compared with the ASA group.
[f]$p < 0.05$ compared with the ASA group.

Table 3. Initial body weight, final body weight, stomach weight and relative stomach weight of control and experimental groups at 7 days[a]

		Non-ulcerated groups				Ulcerated groups			
		NC	OMP	I3C	OMP + I3C	ASA	ASA + OMP	ASA + I3C	ASA + I3C + OMP
Initial body weight (g)	Mean ± SE	156.97 ± 9.17	164.00 ± 5.81	165.33 ± 10.73	154.97 ± 1.73	168.00 ± 4.80	171.44 ± 5.50	157.00 ± 5.77	174.66 ± 3.47
Final body weight (g)	Mean ± SE	159.00 ± 3.79	167.00 ± 8.73	172.40 ± 5.94	159.40 ± 3.73	209[b] ± 16.59	185.00[c] ± 0.58	162.00[d] ± 6.43	183.00[ce] ± 8.67
Stomach weight (g)	Mean ± SE	1.48 ± 0.16	1.61 ± 0.06	1.56 ± 0.08	1.67 ± 0.28	1.85 ± 0.12	1.31[f] ± 0.09	1.41[e] ± 0.04	1.39[e] ± 0.08
Relative stomach weight	Mean ± SE	0.85 ± 0.01	1.02 ± 0.03	0.99 ± 0.12	1.12 ± 0.19	0.90 ± 0.13	0.76 ± 0.01	0.87 ± 0.05	0.80 ± 0.01

ASA: aspirin; I3C: indole-3-carbinol; NC: normal controls; OMP: omeprazole.
[a]Values are expressed as mean ± SE.
[b]$p < 0.001$ compared with the control group.
[c]$p < 0.05$ compared with the control group.
[d]$p < 0.001$ compared with the ASA group.
[e]$p < 0.05$ compared with the ASA group.
[f]$p < 0.01$ compared with the ASA group.

Table 4. RBCs, hemoglobin and hematocrit of control and experimental groups at 7 days[a]

		Non-ulcerated groups				Ulcerated groups			
		NC	OMP	NC	OMP	ASA	ASA + OMP	ASA + I3C	ASA + I3C + OMP
RBCs (10^6/mm^3)	Mean ± SE	6.68 ± 0.45	6.61 ± 0.35	6.52 ± 0.47	7.10 ± 0.17	5.88 ± 0.22	5.77 ± 0.55	6.64 ± 0.08	5.37[b] ± 0.52
Hb (g/dl)	Mean ± SE	14.40 ± 1.36	13.95 ± 0.03	13.85 ± 0.84	14.40 ± 0.29	13.95 ± 0.03	14.07 ± 0.29	14.35 ± 0.38	13.40 ± 0.35
HCT (%)	Mean ± SE	44.33 ± 3.39	45.63 ± 1.89	45.73 ± 3.09	43.00 ± 1.82	47.93 ± 0.97	41.70[c] ± 0.58	42.20[c] ± 0.87	38.55[bc] ± 0.26

ASA: aspirin; I3C: indole-3-carbinol; Hb: hemoglobin; HCT: hematocrit; RBCs: red blood cells; NC: normal controls; OMP: omeprazole.
[a]Values are expressed as mean ± SE.
[b]$p < 0.05$ compared with the control group.
[c]$p < 0.05$ compared with the ASA group.

Table 5. WBCs, lymphocytes, monocytes and blood PLTs of control and experimental groups at 7 days[a]

		Non-ulcerated groups				Ulcerated groups			
		NC	OMP	NC	OMP	ASA	ASA + OMP	ASA + I3C	ASA+I3C+OMP
WBCs (10^3/mm$^{3)}$	Mean ± SE	17.19 ± 0.17	18.77 ± 1.11	19.60 ± 0.78	18.87 ± 3.43	20.53 ± 1.75	16.25 ± 0.20	17.60 ± 0.64	16.50 ± 0.23
Lymphocytes (%)	Mean ± SE	80.77 ± 0.65	75.43 ± 3.78	79.17 ± 2.13	76.33 ± 2.49	82.23 ± 1.23	68.83[bc] ± 1.17	75.83[d] ± 0.60	68.50[bc] ± 0.29
Monocytes (%)	Mean ± SE	12.70 ± 1.37	15.17 ± 0.49	13.87 ± 2.31	14.10 ± 1.21	7.47[c] ± 0.52	10.33 ± 0.88	10.57 ± 0.32	12.50[d] ± 0.08
Blood PLTs (10^3/mm^3)	Mean ± SE	570.00 ± 35.22	579.00 ± 31.79	551.50 ± 45.89	494.00 ± 34.64	136.00[e] ± 33.02	177.50[e] ± 1.44	571.67[c] ± 71.32	339.50[bc] ± 0.20

ASA: aspirin; I3C: indole-3-carbinol; NC: normal controls; OMP: omeprazole; PLTs: platelets; WBCs: white blood cells.
[a]Values are expressed as mean ± SE.
[b]$p < 0.001$ compared with the control group.
[c]$p < 0.001$ compared with the ASA group.
[d]$p < 0.01$ compared with the ASA group.
[e]$p < 0.01$ compared with the control group.

Table 6. Serum total protein, serum albumin and tissue reduced glutathione activity of control and experimental groups at 7 days[a]

		Non-ulcerated groups				Ulcerated groups			
		NC	OMP	NC	OMP	ASA	ASA + OMP	ASA + I3C	ASA + I3C + OMP
Total protein (g/dl)	Mean ± SE	7.55 ± 0.02	9.81[b] ± 0.03	10.35[c] ± 0.36	8.51 ± 1.16	9.63[b] ± 0.82	9.00 ± 0.79	10.57[c] ± 0.42	10.06[c] ± 0.60
Albumin (g/dl)	Mean ± SE	2.76 ± 0.31	4.57[d] ± 0.03	4.05[d] ± 0.24	3.82[c] ± 0.37	4.16[d] ± 0.24	2.92[e] ± 0.09	3.29[f] ± 0.26	3.11[f] ± 0.27
GSH (mg/g tissue)	Mean ± SE	0.41 ± 0.03	0.51 ± 0.07	0.58 ± 0.07	0.38 ± 0.05	0.18[d] ± 0.08	0.74[e] ± 0.09	1.32[de] ± 0.28	0.72[e] ± 0.19

ASA: aspirin; I3C: indole-3-carbinol; GSH: reduced glutathione; NC: normal controls; OMP: omeprazole.
[a]Values are expressed as mean ± SE.
[b]$p < 0.05$ compared with the control group.
[c]$p < 0.01$ compared with the control group.
[d]$p < 0.001$ compared with the control group.
[e]$p < 0.001$ compared with the ASA group.
[f]$p < 0.01$ compared with the ASA group.

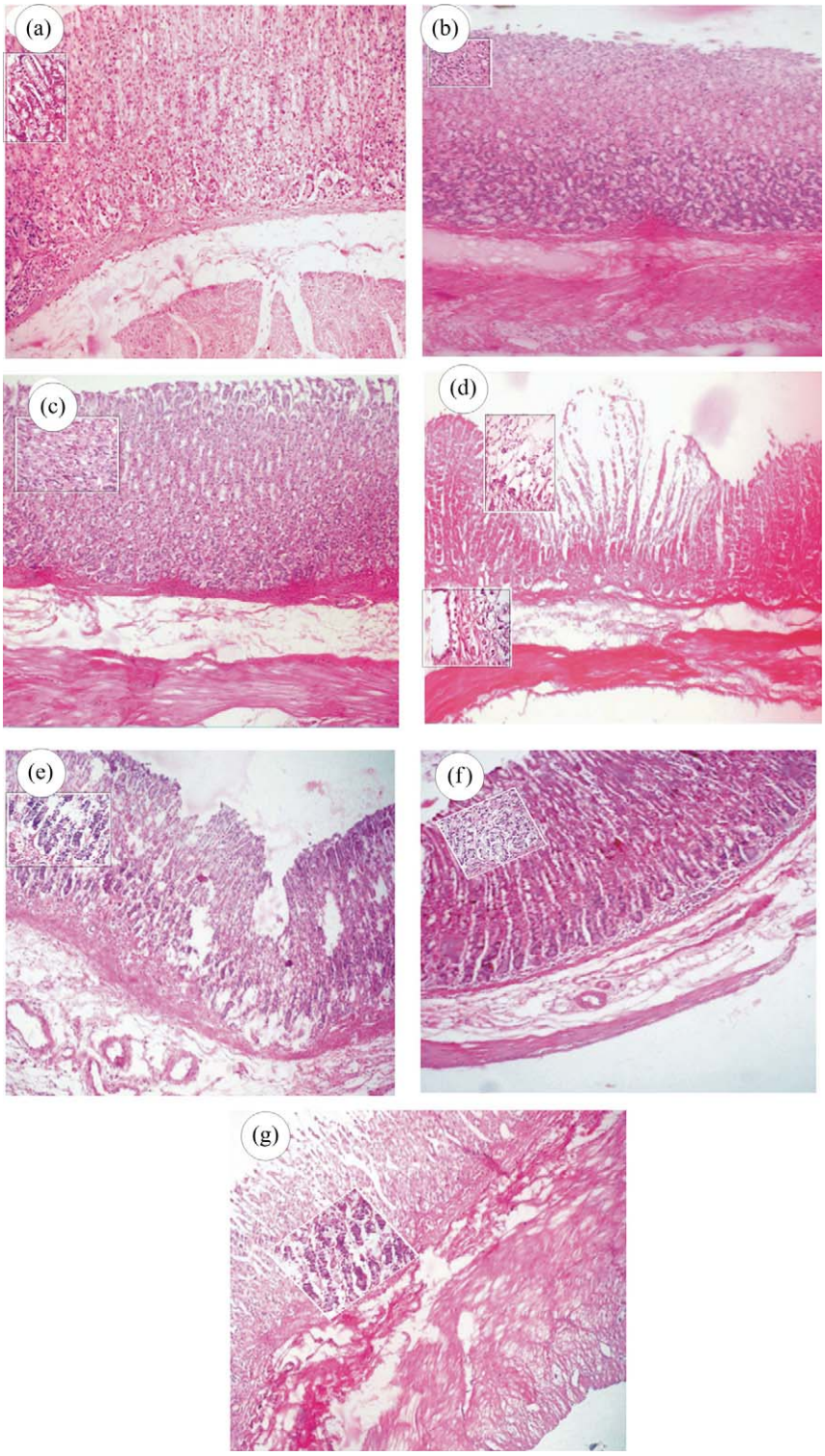

Figure 2. Histological changes of hematoxylin and eosin stain in stomach tissue of normal control (photomicrograph (a)), OMP (photomicrograph (b)), I3C (photomicrograph (c)), ulcerated ASA rats (photomicrograph (d)), OMP-treated tissue (photomicrograph (e)), I3C-treated tissue (photomicrograph (f)) and OMP + I3C-treated tissue (photomicrograph (g)) (100× and 400× magnifications). ASA: aspirin; I3C: indole-3-carbinol; OMP: omeprazole.

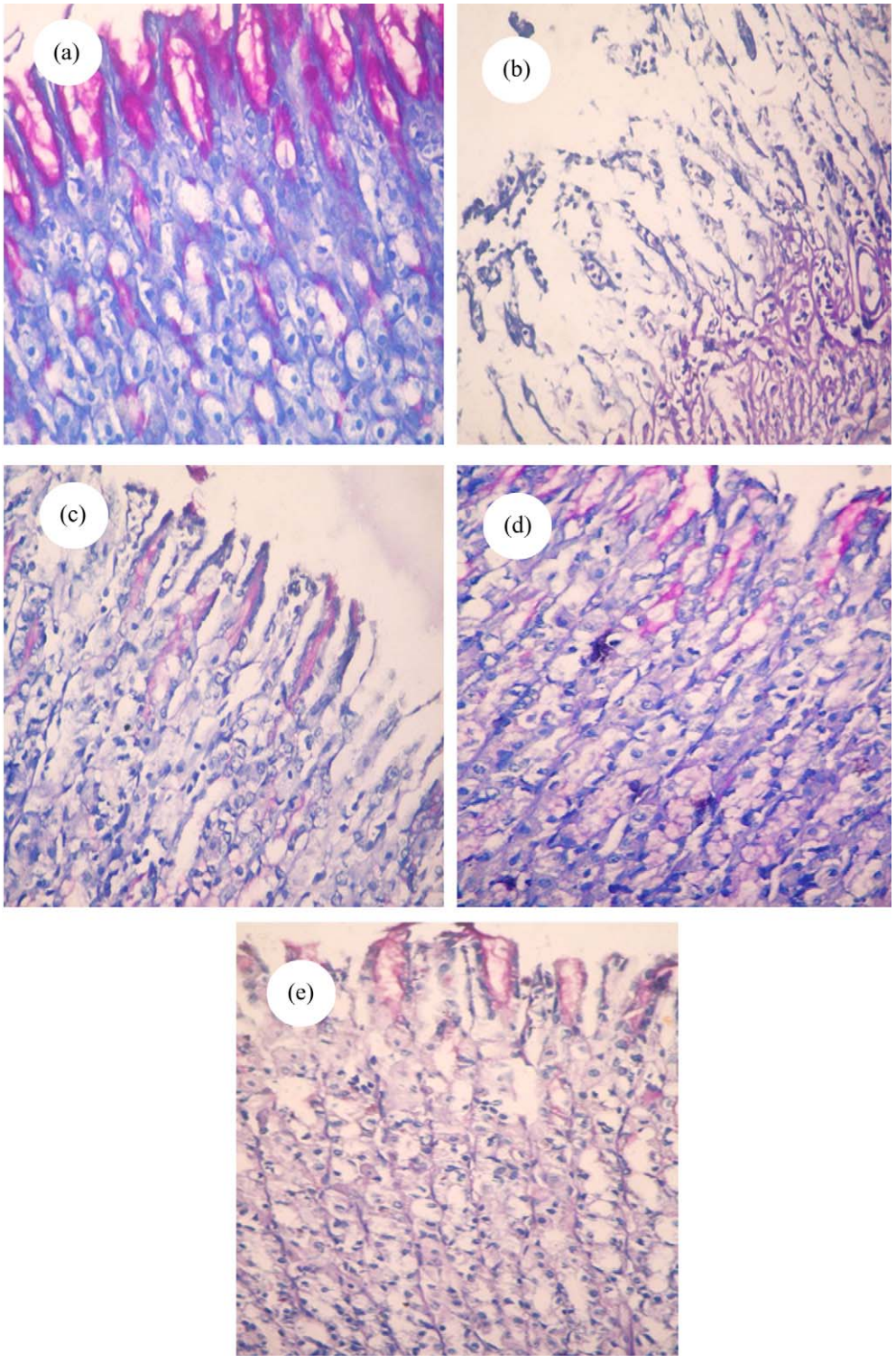

Figure 3. Histochemical changes of combined alcian blue periodic Schiff technique in normal control stomach tissue (photomicrograph (a)), ulcerated ASA rats (photomicrograph (b)), OMP-treated tissue (photomicrograph (c)), I3C-treated tissue (photomicrograph (d)) and OMP + I3C-treated tissue (photomicrograph (e)) (400× magnification). ASA: aspirin; I3C: indole-3-carbinol; OMP: omeprazole.

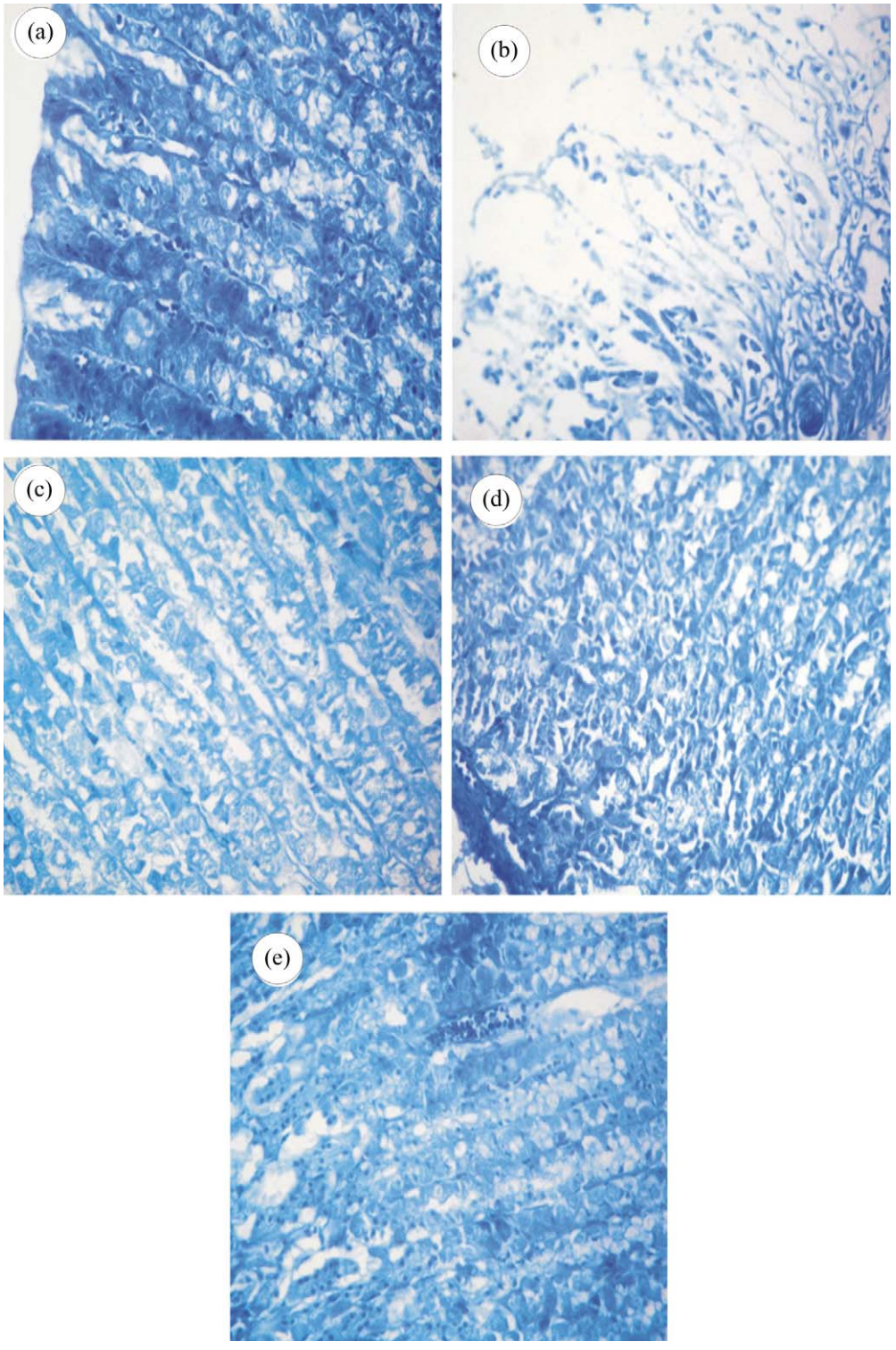

Figure 4. Histochemical changes of bromophenol blue stain in normal control stomach tissue (photomicrograph (a)), ulcerated ASA rats (photomicrograph (b)), OMP-treated tissue (photomicrograph (c)), I3C-treated tissue (photomicrograph (d)) and OMP + I3C-treated tissue (photomicrograph (e)) (400× magnification). ASA: aspirin; I3C: indole-3-carbinol; OMP: omeprazole.

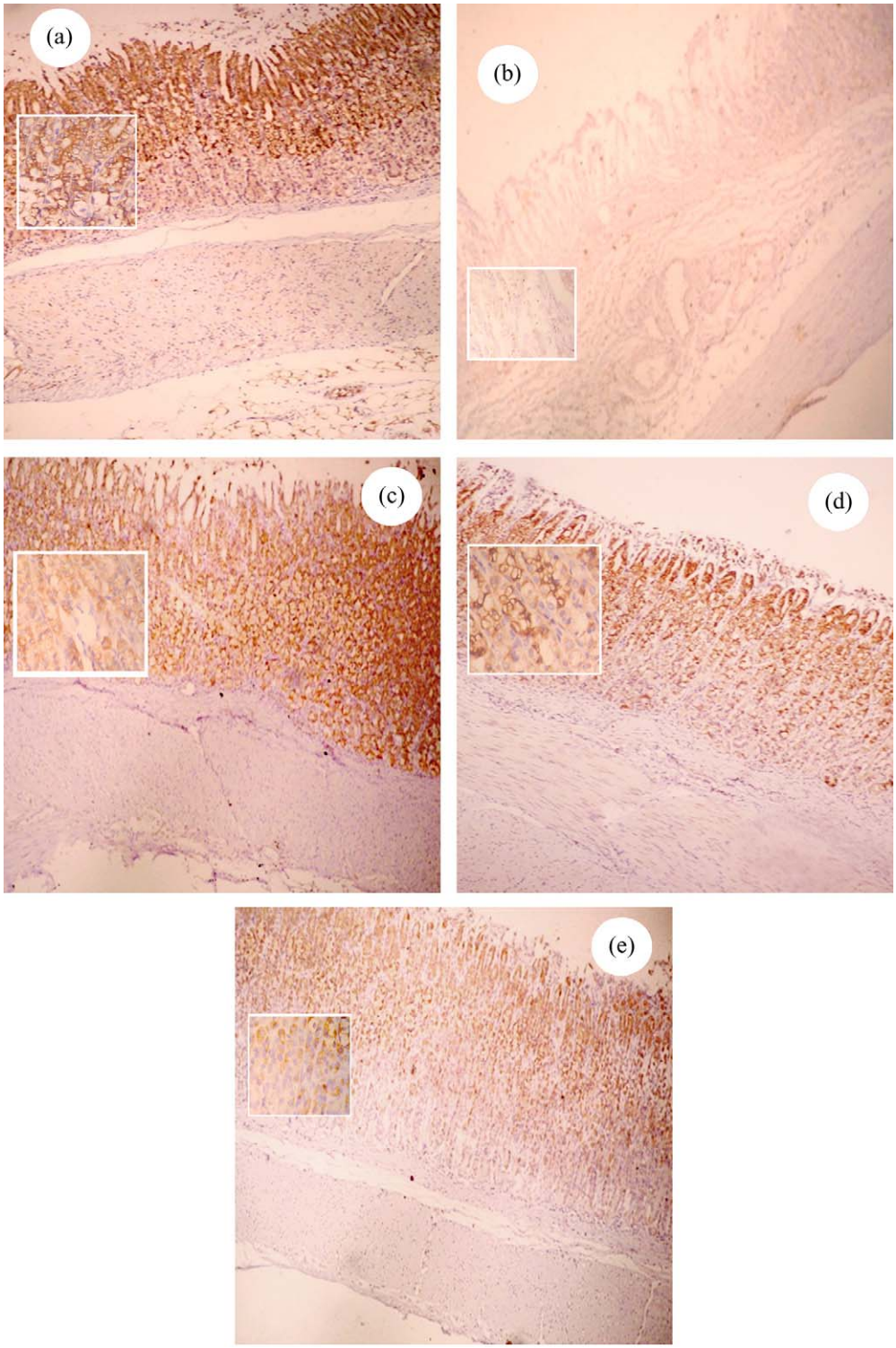

Figure 5. Immunoreactivity of COX2 in normal control stomach tissue (photomicrograph (a)), ulcerated ASA rats (photomicrograph (b)), OMP-treated tissue (photomicrograph (c)), I3C-treated tissue (photomicrograph (d)), and OMP + I3C-treated tissue (photomicrograph (e)) (100× and 400 × magnifications). ASA: aspirin; COX-2: cyclo-oxygenase-2; I3C: indole-3-carbinol; OMP: omeprazole.

Table 7. COX-2 expression in normal control and ulcerated groups at 7 days

	NC	ASA	ASA + OMP	ASA + I3C	ASA + OMP + I3C
Peptic cells	+	−	++	++++	+++
Oxyntic gland cells	+	−	++	++++	+++

ASA: aspirin; COX-2: cyclo-oxygenase-2; I3C: indole-3-carbinol; NC: normal controls; OMP: omeprazole.
+: values express stained cells between 1% and 10%; ++: values express stained cells between 11% and 30%.; +++: values express stained cells more than 31%; ++++: expresses stained cells more than 70%; −: values express no stain.

Discussion

Previous studies showed that ASA as one of the commonly used NSAIDs, which induced gastric ulcer and delayed its healing (Hawkey, 2000). As most of the commonly used pharmaceutical treatments of gastric ulcer have some rational side effects or the incidence of returning the ulcer after treatment discontinuation (Szabo and Vincze, 2000), there must be a need for some bioactive natural alternatives combined with the perused treatments to subside the adverse effects of NSAIDs. The present study aims to compare the anti-ulcer effect of I3C in comparison or in combination with OMP in ASA-induced ulcerated rat model.

The present investigation elucidates a significant increase in the acidic activity, ulcer index and percentage of ulceration with a decrease in pH value in ASA-ulcerated rats. Severe red coloration, with hemorrhagic streaks in the dissected stomach of ASA-treated rats was also observed. In general, the formation of gastric mucosal lesions may be due to several mechanisms, as the reduction in gastric blood flow, which results in a rapid decrease in the pH within the mucoid cap, causing the formation of hemorrhagic erosions (Wallace, 2005). This contributes to the development of necrosis, hemorrhage and to the solubilization of mucus constituents in stomach. In addition to the increased pepsin, acid secretion and flux of Na^+ and K^+ was noted (Szabo, 1987). This is accompanied with decreased mucin activity by means of a decrease in histamine and back diffusion of H^+ ions that produced autodigestion of the gastric mucosa and breakdown of the gastric mucosal barrier (Jaikumar et al., 2010).

Prostaglandin E2 (PGE2) is one of the major protective factors in gastric tissue, which inhibits gastric acid secretion. PGE2 is significantly declined with ASA treatment. Thus, PGE2 might indirectly take part in ulcer relapse via acid secretion (Wang et al., 2007). Moreover, COX, which exists in three isoforms COX-1, COX2 and COX-3, is the key enzyme for the synthesis of prostaglandins. Administration of ASA was accompanied by the suppression of COX-1 and COX-2 activity in the gastric mucosa (Brzozowski et al., 2001a). These results were in accordance with the results of the immunohistochemical stain of COX-2 in the present study in ASA-ulcerated rats, which supports the suppression of COX-2 activity in ASA-ulcerated gastric mucosa. This may be related to the inhibition of prostaglandin synthesis that probably weakened the function of the gastric mucosal defense (Wallace, 2005). The histological examination of gastric mucosal tissue of ASA group in the present study revealed sharply damaged mucosal epithelium reaching the submucosal layer with hemorrhage and discontinuity in lining epithelium. These consequences may be related to the back-diffusion of acid into the mucosa, which directly leads to vascular leakage and aggressive damaging effect in the basement membrane of both epithelial and mucosal cells in the gastric wall (Jainu et al., 2006). The significant increase in serum total protein and albumin in ASA-ulcerated rats at 7 days in this study was supported by Prakash and Gunasekaran (2010), who stated that ASA causes leakage of plasma protein into gastric juice. This reflects the increased protein concentration in ASA-treated rats. Weberg et al. (1990) stated that the level of protein was significantly decreased in the gastric mucosal tissue. This indicated that acute stress caused the corrosion of gastric mucosa, resulting in the disruption of gastric mucosal cells. The net effect would be the loss of protein from gastric tissue leading to their leakage into the serum. Furthermore, plasma proteins largely consist of albumin and globulins such as immunoglobulins, carrier proteins and acute phase reactants. So, elevated proteins may be due to an increase in multiple immunoglobulins due to chronic infection or stress caused by ulcer.

The results of this study demonstrated a nonsignificant decrease in RBCs, HCT and Hb levels in ASA-ulcerated rats. These results were in accordance with Sunday et al. (2009) who showed that ASA had

no effect on packed cell volume, Hb % and RBC. In contrary to this, Langman et al. (1994) reported that ASA alters iron uptake from the gastrointestinal tract. This effect was coupled with acute or chronic blood loss due to gastrointestinal tract erosion induced by ASA, which is believed to cause iron deficiency anemia. On the other hand, ASA is used in the prevention of the formation of thrombosis in the myocardium coronary blood vessel by inhibiting platelet aggregation. This effect may also be one of the side effects of ASA as preoperative ASA administration increases blood loss during bleeding in sensitive operations (Douketis et al., 2008). This was clearly demonstrated in the present study by the significant decline in the mean values of PLTs and monocyte levels in ASA-treated rats. In addition, the decrease in gastric tissue GSH activity in the present study was supported by Tanaka et al. (2001), who explained that gastric mucosal oxidative stress is important in the pathogenesis of ASA-induced gastrotoxicity. From the inspection of the data represented in this study, it is clear that OMP, I3C or OMP + I3C treatments attenuated the damage caused by ASA represented by the significant decrease in stomach weight, acidity, followed by the disappearance of ulcers and hemorhagic streaks in the dissected treated stomachs. This was accompanied with increase in pH value, GSH levels, PLTs count and monocyte percentage. These results were in accordance with Scheiman et al. (2006) who proved that proton pump inhibitors (PPIs), including OMP, are effective in preventing NSAID-induced gastric injury as well as in promoting the healing of NSAID-induced ulcers by inhibition of acid secretion. Besides, PPIs can protect the gastric mucosa through mechanisms related to the reduction in tissue oxidative damage. Therefore, OMP counteracted tissue oxidation and produced reduction in mucosal cell proliferation associated with NSAID. But on the other hand, OMP does not influence mucosal PGE2 production (Fornai et al., 2011). It is very important to bear that OMP efficiently scavenges hydroxyl radicals but not superoxide radical. Since, the main danger for hydrogen peroxide arises from its ability to cross cell membranes rapidly. Once inside the cells, it can probably react with Fe^{++} and Cu^{++} ions to form hydroxyl radicals, which may be the origin of many toxic insults (Simon et al., 2006). Additionally, Szabo and Vincze (2000) elucidated that the healing rates of ulcer with the PPI decreased after treatment discontinuation and that the percentage of the recurrence of ulcer is between 40% and 80% in most of the studies. Therefore, OMP is not the only drug of choice for ulcer treatment. Research of naturally occurring antioxidant compounds in edible plants reduces the risk of gastrointestinal cancer seems to have some potential source for ulcer-related problems and it could make a substantial contribution to drug development by providing novel chemicals to these drugs (Brzozowski et al., 2001b). I3C is a naturally occurring hydrolysis product of glucobrassicin found in vegetables of the *Cruciferae* family such as broccoli, brussels sprouts and cauliflower. Epidemiological studies suggest that high dietary intake of cruciferous vegetables is associated with lower cancer risk (Verhoeven et al., 1997). In the present investigation, ASA-ulcerated rats showed a decrease in the mucin content represented by combined alcian blue periodic Schiff technique and sites of protein content demonstrated by bromophenol blue stain, which began to be attenuated by the I3C, or the combined treatments of I3C and OMP. An improvement in mucus production guides the healing process by protecting the ulcer crater against the endogenous aggressors, such as stomach secretions and oxidants, as well as against exogenous damaging agents, such as NSAIDs. The ulcer prevention or healing by I3C was associated with an increase in the mucus layer in the gastric mucosa (Chang et al., 2005). The PAS (Periodic acid Schiff staining Technique) staining method confirmed the role of I3C enhancing the mucus level and protecting the inflammatory cytokine-mediated oxidative damage to gastric mucosa. Thus, the antiulcerogenic activity of I3C may involve its beneficial effect on both offensive and defensive gastric mucosal factors (Choi et al., 2010). In addition, I3C is able to inhibit chemically induced neoplasia in forestomach by selective beneficial alteration of phase I cytochrome P-450 and induction of phase II detoxification enzymes (Manson et al., 1997; Zhang, 2004). GSH is an important tripeptide thiol, which in addition to being the substrate for glutathione *S*-transferases (GSTs), maintains cellular oxidation–reduction balance and protects cells against free radical species. Thus, determination of tissue GSH levels in combination with GST activities was used to evaluate the detoxifying potential of anticarcinogens (Van Lieshout et al., 1997). I3C in cruciferous vegetables induced gastric GST production (Verhoeven et al., 1997). These results were in accordance with our results that signified the increase in stomach GSH level after I3C administration to ulcerated rats more than those under OMP treatment. In addition, I3C has been shown to have protective

effects due to its oligomerization under acidic conditions and thus, it has been suggested that the observed biological activity may be attributable mainly to these acid condensation products (Grose and Bjeldanes, 1992). Precisely, after ingestion, in the stomach acidic medium, I3C is converted into 3,3-diindolylmethane as a major condensation product. 3,3-diindolylmethane was found to inhibit the growth of human colon adenocarcinoma (Gamet-Payrastre et al., 1998). Besides, inflammation intersects at COX-2 and inducible nitric oxide (iNOS) level (Lee et al., 2003) and is accompanied by activating neutrophils, which results in the overproduction of proinflammatory mediators, including tumor necrosis factor-α, interleukin (IL)-4 and IL-6 (Raghavendran and Srinivasan, 2011). The overproduced proinflammatory mediators upregulate nitric oxide production, which leads to an increase in reactive nitrogen species, lipid peroxidation and cell damage (Hayes and Mc-Lellan, 1999). I3C inhibits nitric oxide production through decreasing iNOS expression in activated macrophages (Chen et al., 2003), possess *in vitro* nitric oxide clearance activity (Wang et al., 2012) and suppresses nucleic acid kappa-light-chain-enhancer of activated B cells activity, which leads to the decreased production of reactive oxygen species (Kim and Milner, 2005). These results were clearly demonstrated by the immunohistochemical stain of COX-2 in this study showing that I3C-treated ulcerated rats produced an increase in the COX-2 production in the stomach tissue verifying the healing process of I3C. These results were in agreement with Gilroy et al. (1999) who explained that COX-2 produces prostaglandins that exert anti-inflammatory actions and play an important role in the healing of gastric ulcers (Shigeta et al., 1998). On the basis of the results made by Hatazawa et al. (2007), they brought about that endogenous prostaglandin subtype 2 derived from COX-2, which plays an important part in the spontaneous healing of gastric ulcers and the upregulation of COX-2 appears to be a defensive and anti-inflammatory response aimed at enhancing mucosal defense.

Conclusion

In this study, we demonstrated that cotreatment of OMP and I3C decreased the risk of ASA-induced gastric mucosal injury in rats. I3C + OMP increased the inhibition of ASA-induced gastric hemorrhage, ulceration, gastric mucosal oxidative stress and inflammation in rats. Moreover, we concluded that combining I3C with OMP in the treatment of gastric ulcer is potentially a new approach for decreasing gastrointestinal injury caused by ASA and other NSAIDs.

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