

Original Article**The potential therapeutic effect of nitric oxide modulators in experimentally-induced gastric ulcers****Ebtehal El-Demerdash¹, Hala O. El-Mesallamy², Noha M. Abu-Zaid³, Mohamed Z. Gad^{2,*}**¹ Pharmacology & Toxicology Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt;² Biochemistry Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt;³ Quality Assurance Department, The Holding Company for Biological Products and Vaccines (VACSERA), Giza, Egypt.

ABSTRACT: Nitric oxide (NO) appears to play a critical role in modulating gastric mucosal defense. Administration of NO donors has been reported to protect the gastrointestinal mucosa against damage induced by several irritants. However, the possible role of NO in healing existing ulcers must be clarified further. Therefore, the present study was designed to assess the effect of modulation of NO on the healing of an indomethacin-induced peptic ulcer using a NO precursor, L-arginine, and a competitive inhibitor of NO synthase, L-NAME. Results of administering L-arginine were compared to those using nitroglycerin (NTG), an NO donor. Rats were injected with a single oral dose of indomethacin (30 mg/kg) and then treated with L-arginine (200 mg/kg, *i.p.*), NTG (1 mg/kg, *i.p.*) or L-NAME (15 mg/kg, *i.p.*) once daily for 7 d starting 4 h after the indomethacin injection. Gross lesion examination and histological assessment were done. NO, prostaglandin (PGE₂), and mucin content in gastric tissue were detected. In addition, oxidative stress markers including glutathione (GSH) and lipid peroxides were measured. L-arginine and NTG almost completely healed indomethacin-induced ulceration as indicated by macroscopic and histological examination, restoration of normal levels of NO and GSH, and a significant attenuation of the increase in PGE₂ and lipid peroxides induced by indomethacin. In contrast, L-NAME was found to exacerbate mucosal damage. In conclusion, the present study provides further evidence for the role of NO in gastric ulcer healing and it suggests an alternative path to treating the universal problem of non-steroidal anti-inflammatory-drug-induced gastropathy.

Keywords: Indomethacin, L-arginine, nitroglycerin, L-NAME, nitric oxide, gastric ulcer

*Address correspondence to:

Dr. Mohamed Z. Gad, Biochemistry Department, Faculty of Pharmacy, The German University in Cairo, El-Tagmoa El-Khamis, New Cairo, Egypt.
e-mail: mohamed.gad@guc.edu.eg

1. Introduction

Peptic ulcers represent a serious medical problem due to their frequency among different socioeconomic classes. They affect 5-10% of the world's population and are characterized by ulceration of the stomach, duodenum, or both (1). Peptic ulcers are generally believed to result from an imbalance between aggressive factors and counteracting defense mechanisms. Foremost among aggressive factors is the use of non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs have been found to produce gastroduodenal ulcers in 25% of users, often with bleeding and perforation (2).

A diminished mucosal blood flow is generally believed to be a major factor underlying the mechanism of NSAID-induced gastric damage (3). Microcirculatory perfusion comprises the epithelial defense mechanism that provides energy, oxygen, nutrients, gastrointestinal (GI) peptide hormones, as well as other substrates necessary for maintaining epithelial cell integrity. In addition, the circulating blood in the surface mucosa removes waste materials and irritating substances and maintains mucus production and bicarbonate secretion. Thus, it helps to sustain the mucosal barrier (4). Furthermore, surface mucosal blood flow has been found to play an important role in the ulcer healing process (1,5). Therefore, a reduced blood flow leads to the mucosa's increased vulnerability to damaging agents.

The key factor in modulating microcirculation is nitric oxide (NO). When the mucosa is exposed to an irritant, a rapid increase in mucosal flow occurs. This response is initiated by sensory nerves underlying the epithelium, and its stimulation results in activation of endothelial nitric oxide synthase (eNOS) and subsequent production of NO. Vascular smooth muscle relaxes and mucosal blood flow thus increases (6). Moreover, Ma and Wallace (7) determined the relative effects of endothelial and inducible NOS on gastric ulcer healing and found that eNOS plays a significant role in gastric ulcer healing while its inducible isoform do not. Several studies have reported that administration of NO donors may protect the GI mucosa against

damage induced by several irritants, suggesting the potential use of these compounds in situations in which the GI mucosa is exposed to noxious substances or in which mucosal defense is impaired (8-10). Furthermore, a recent study used the NO precursor L-arginine to augment the gastroprotective effect of simvastatin, an antihyperlipidemic drug (11). However, the possible role of NO in treating the existing ulcers must be clarified further.

The present study was thus designed to assess the effect of modulation of NO on healing indomethacin-induced peptic ulcers using the NO precursor L-arginine and a competitive inhibitor of NOS, L-NAME. Results of administering L-arginine were also compared to those of administering nitroglycerin (NTG), an NO donor. Ulcer healing was evaluated macroscopically and histologically, and changes in PGE₂, NO, and mucin were also detected. In addition, the pathogenesis of NSAID-induced GI damage is also known to depend on hyperproduction of reactive oxygen species (ROS) and thus induction of oxidative stress injury (10). Thus, the present study evaluated the effect of modulation of NO on the oxidative stress induced by indomethacin.

2. Materials and Methods

2.1. Drugs and chemicals

Indomethacin (Liometacen ampule) was obtained from the Nile Company for Pharmaceuticals and Chemical Industries, Cairo, Egypt. A single dose of 30 mg/kg was given by oral intubation in order to induce ulcers in accordance with the study of Bhattacharya *et al.* (12). L-Arginine (Merck, Germany) was dissolved in saline and used in a dose of 200 mg/kg (*i.p.*). This dose is suggested to protect against ulcer induction (13). NTG was obtained from G. Pohl-Boskamp GmbH & Co., (Hohenlockstedt, Germany) and used in a dose of 1 mg/kg (*i.p.*) (14). L-NAME (Sigma Chemical Co., St. Louis, Mo, USA) was dissolved in saline and injected in a dose of 15 mg/kg (*i.p.*) (7).

Bovine serum albumin (BSA), *n*-butanol, 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), reduced glutathione (GSH), Folin reagent, Griess reagent, 1,1,3,3-tetramethoxypropane, thiobarbituric acid (TBA), trichloroacetic acid, and vanadium(III) chloride were all purchased from Sigma Chemical Co., Ltd. All other chemicals were of analytical grade.

2.2. Animals

This study involved eighty-five male albino rats weighing 150-200 g obtained from the Nile Co. for Pharmaceutical and Chemical Industries. The animals were housed in cages with wide mesh wire bottoms to prevent coprophagy and kept on a standard diet and water *ad libitum*. The standard food pellets (El-Nasr

Co., Abu Zaabal, Egypt) contained a mixture of no less than 20% protein, 5% fiber, 3.5% fat, 6.5% ash, and vitamins. Rats were deprived of food but had free access to water 24 h before ulcer induction. The study protocol was approved by the local ethics committee.

2.3. Experimental design

Rats were divided into six groups. To induce gastric ulcers, the 1st and 2nd groups were given a single dose of indomethacin 30 mg/kg by oral intubation. The 1st group was sacrificed 4 h after indomethacin injection and designated the group with indomethacin-induced ulceration on day 0. The 2nd group was injected *i.p.* with saline, once daily for 7 d starting 4 h after indomethacin injection and this group was designated the group with indomethacin-induced ulceration on day 7. The 3rd, 4th, and 5th groups were injected *i.p.* with L-arginine (200 mg/kg, *i.p.*), NTG (1 mg/kg, *i.p.*), or L-NAME (15 mg/kg, *i.p.*) once daily for 7 d starting 4 h after the indomethacin injection. The last group was the control group and was given saline only.

After 7 d of treatment, rats were sacrificed under ether anesthesia. Their stomachs were excised, opened along the greater curvature, rinsed extensively with saline to remove attached debris, and then pinned flat on cardboard for evaluation of gross lesions. The length of individual lesions in the mucosa was measured and the sum of lengths of all lesions in each stomach served as the ulcer index (15). The severity of gastric lesions was scored in accordance with the method of Yamamoto *et al.* (16) as follows: **1** (ulcerated area: 1-6 mm²), **2** (ulcerated area: 7-12 mm²), **3** (ulcerated area: 13-18 mm²), **4** (ulcerated area: 19-24 mm²), and **5** (ulcerated area: > 24 mm²).

Immediately after gross lesion examination, the stomach was placed on an ice-cold surface. The stomach tissue was cut into pieces, weighed, and homogenized in 0.1 M Tris-HCl buffer (pH 7.4) to obtain 20% (w/v) homogenate. Tissue homogenate was used for determination of GSH, lipid peroxides, and PGE₂. Part of the homogenate was centrifuged at 1,000 × g for 10 min to remove cell debris and nuclei and the resultant supernatant was used for estimation of NO. Specimens from stomachs of each group were fixed in alcoholic fixative material for histological examination and mucin assessment.

2.4. Biochemical analysis

Oxidative stress markers, GSH, and lipid peroxides were estimated by the methods of Ellman (17) and Uchiyama and Mihara (18), respectively. As an index of NO production, total nitrate/nitrite was estimated in the cytosolic fraction after protein precipitation using an equal volume of ethanol in accordance with the method of Miranda (19). In accordance with the

method described elsewhere (20), PGE₂ was determined by competitive enzyme-linked immunosorbent assay (ELISA) using a PGE₂ kit (Parameter™) provided by R & D Systems Inc., USA. Tissue protein was assessed in accordance with the method of Lowry *et al.* (21).

2.5. Histological assessment

Sections of 5 µm were obtained and stained with haematoxylin and eosin (H&E) stain for standard histological examination in accordance with the method of Drury and Wallington (22). Qualitative assessment of mucin was carried out in accordance with the method of McManus (23).

2.6. Statistical analysis

Data are presented as mean ± S.E.M. For statistical analysis of the data, multiple comparisons were carried out using one-way analysis of variance (ANOVA) followed by a Tukey-Kramer test for post-hoc analysis. Statistical significance was acceptable at a level of $p < 0.05$. Data analysis was achieved using the software program GraphPad InStat.

3. Results

3.1. Macroscopic and histological examination of gastric ulcers

Oral injection of a single dose of indomethacin in rats fasted for 24 h caused gastric ulceration in the glandular portion of the stomach. The group with indomethacin-induced ulceration on day 7 had a significantly higher number of ulcers than did the group with indomethacin-induced ulceration on day 0. However, there was no significant difference in the ulcer index and ulcer score for the two groups with ulceration (Table 1). Treatment of rats with either L-arginine or NTG after ulcer induction tended to return the stomach mucosa to normal (Table 1). In contrast, treatment with L-NAME for 7 d after ulcer induction further aggravated ulcer formation, as reflected by a 29% increase in the

number of ulcers, a 22% increase in ulcer index, and a 26% increase in the ulcer score for rats receiving this treatment in comparison to those receiving indomethacin (Table 1). In addition, indomethacin caused the death of three animals. The highest number of animal deaths, five animals, occurred in the group treated with indomethacin + L-NAME; these deaths were attributed to severe hemorrhaging as was revealed by gross examination of the gastric ulcers. The lowest number of deaths, one animal, occurred in the group treated with indomethacin + L-arginine. No deaths occurred in the control group.

As shown in Figure 1A, specimens from control rats revealed the normal histological structure of the glandular stomach for both the fundus and pylorus. Indomethacin treatment induced severe macroscopic gastric mucosal damage that was not accompanied by self-healing during the 7 d of the experiment. Gastric mucosal damage was characterized by focal necrosis and ulceration (Figure 1B). The inner area of the mucosa and lamina propria showed inflammatory cell infiltration as well as focal aggregation (Figure 1C). Edema with severe congested blood vessels was also detected in the lamina propria and submucosa. After treatment with L-arginine for 7 d, the gastric mucosa had an almost normal mucosal layer with little inflammatory cell infiltration. However, edema and congested blood vessels were detected in the lamina propria along with edema present only in the muscularis (Figure 1D). The stomachs of rats receiving NTG showed only focal desquamation in the lining mucosal epithelium along with little inflammatory cell infiltration in the lamina propria, although the muscularis was still edematous (Figure 1E). In contrast, rats treated with L-NAME had multiple gastric ulcers and necrobiotic changes were noted in the mucosal epithelium and glandular structure along with inflammatory cell infiltration and congested blood vessels in the lamina propria (Figure 1F).

Qualitative assessment of mucin revealed that specimens from the control group showed positive reaction to periodic acid Schiff's staining (PAS) in the periphery and in the mid zone of the glandular mucosal layer of the stomach, although the groups

Table 1. Gross examination of the effect of daily administration of L-arginine (200 mg/kg, *i.p.*), NTG (1 mg/kg, *i.p.*), or L-NAME (15 mg/kg, *i.p.*) for 7 d on gastric ulcers induced with indomethacin in rats

Group ^a	No. of dead rats	No. of ulcers	Ulcer index (mm)	Ulcer score
Control	0/15	0	---	---
Indomethacin (group with indomethacin-induced ulceration on day 0)	0/10	10.80 ± 0.49	17.0 ± 0.79	3.40 ± 0.16
Indomethacin (group with indomethacin-induced ulceration on day 7)	3/15	13.25 ^a ± 0.75	19.0 ± 1.45	3.63 ± 0.26
Indomethacin + L-arginine	1/15	0	---	---
Indomethacin + NTG	2/15	0	---	---
Indomethacin + L-NAME	5/15	17.11 ± 0.66 ^{***}	23.2 ± 1.15 ^{***}	4.55 ± 0.18 ^{***}

^a Values given are means of 10-15 observations ± S.E.M. Gastric lesions were scored according to their severity on a scale of 1 to 5: **1** (ulcerated area: 1-6 mm²), **2** (ulcerated area: 7-12 mm²), **3** (ulcerated area: 13-18 mm²), **4** (ulcerated area: 19-24 mm²), and **5** (ulcerated area: > 24 mm²). ^{***} Significant difference with respect to groups with indomethacin-induced ulceration on day 0 and 7, respectively, at $p < 0.05$ using ANOVA followed by Tukey-Kramer for multiple comparisons.

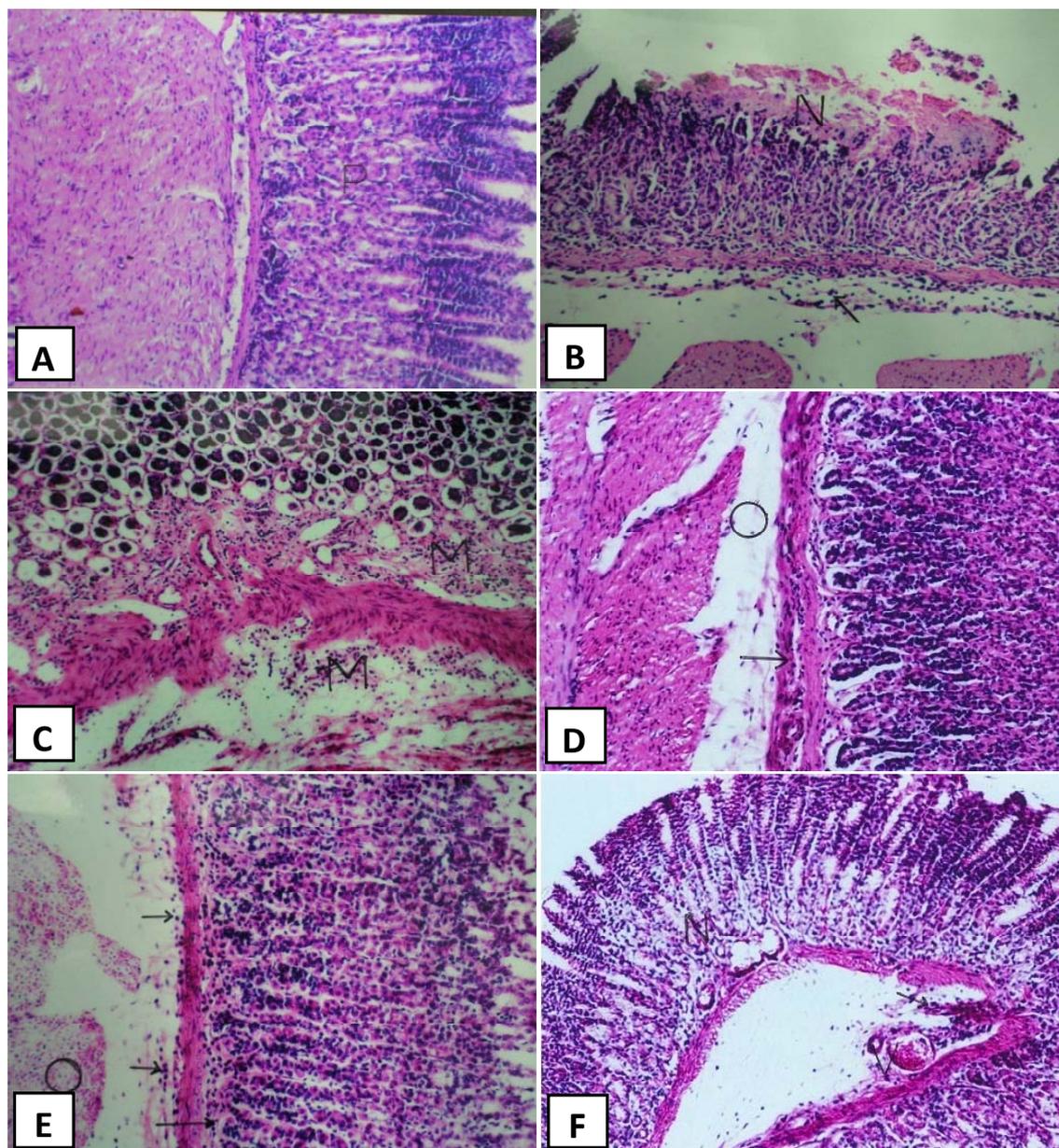


Figure 1. Photomicrographs of sections in the glandular stomach stained with hematoxylin-eosin stain. Panel A ($\times 40$) represents a section taken from the stomach of a rat treated with saline and shows the normal histological structure of mucosal layer (P). Panels B and C represent sections taken from the stomach of a rat treated with indomethacin and panel B ($\times 40$) shows focal necrosis and ulceration in the mucosal layer (N) and panel C ($\times 24$) shows inflammatory cell infiltration in the base of mucosal layer and lamina propria (M). Panel D ($\times 40$) represents a section taken from the stomach of a rat treated with indomethacin and L-arginine (200 mg/kg, *i.p.*) for 7 d and shows a normal mucosal layer with little inflammatory cell infiltration in the lamina propria (arrow) and edema (O). Panel E ($\times 40$) represents a section taken from the stomach of a rat treated with indomethacin and NTG (1 mg/kg, *i.p.*) for 7 d and shows desquamation of the mucosal epithelium and little inflammatory cell infiltration in the lamina propria (arrow) and muscular edema (O). Panel F ($\times 40$) represents a section taken from the stomach of a rat treated with indomethacin and L-NAME (15 mg/kg, *i.p.*) for 7 d and shows necrotic changes in the mucosal layer and glands (N) with hyperemia in blood vessels and inflammatory cell infiltration in the lamina propria (arrow).

with indomethacin-induced ulceration on day 0 and 7 showed a positive PAS reaction in the ulcerated area of the mucosa (Figures 2A-2C). Rats treated with L-NAME showed a positive PAS reaction in the ulcerated area of mucosal epithelium (Figure 2F). After treatment with either L-arginine or NTG, positive PAS was noted in the edges of the mucosa (Figures 2D and 2E).

3.2. Stomach content of NO and PGE₂

In comparison to the control group, a single dose of indomethacin induced a significant reduction in NO content of 28% 4 h after injection and a similar reduction of 40% 7 d after injection. Treatment of animals with L-arginine after ulcer induction significantly increased NO content by 80% in comparison to the group with indomethacin-induced ulceration on day 7 and even caused significant elevation of 8% above normal (Table 2). Animals treated with NTG also had a significant

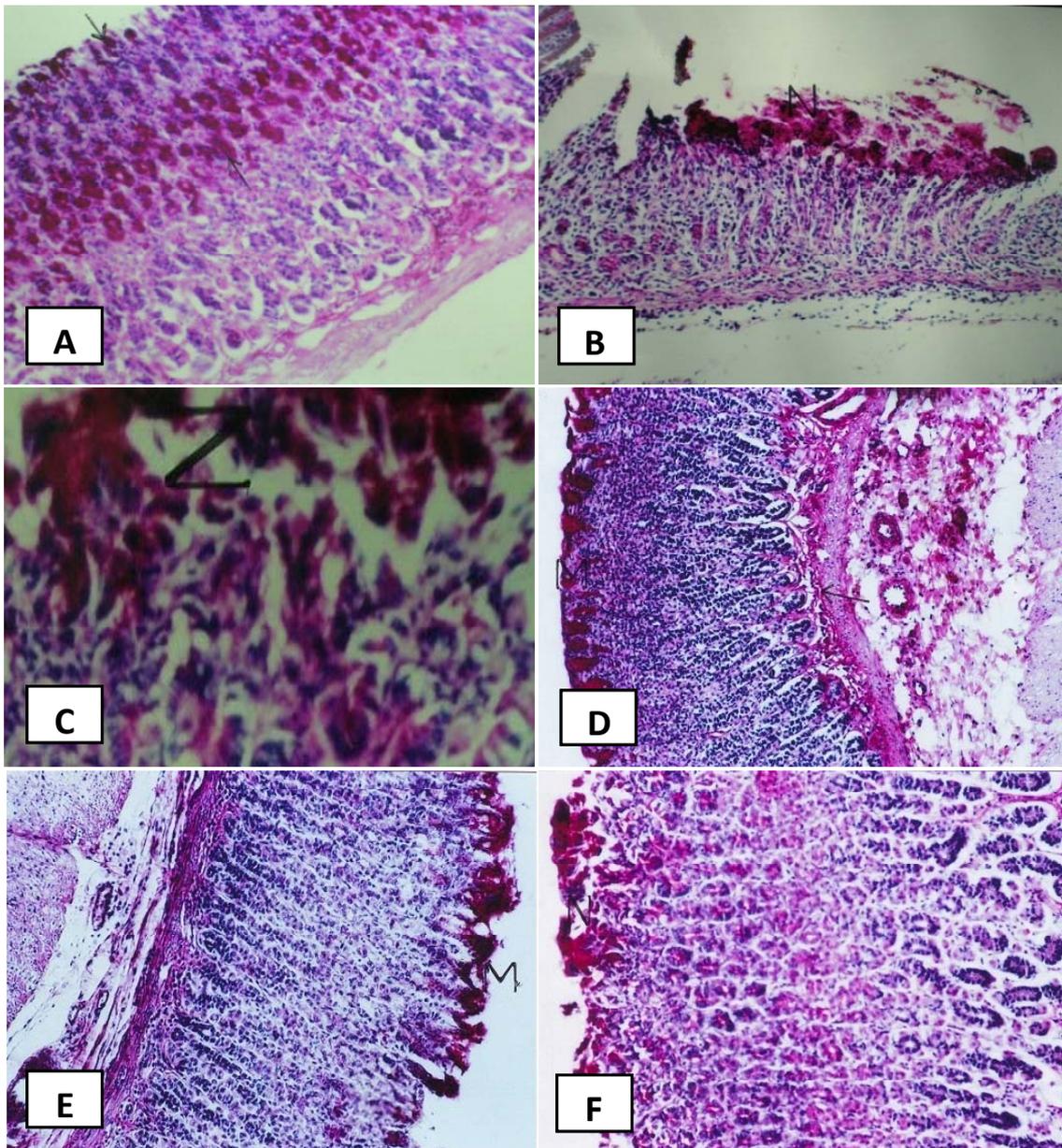


Figure 2. Photomicrographs of sections in the glandular stomach after periodic acid Schiff's staining (PAS). Panel A ($\times 40$) represents a section taken from the stomach of a rat treated with saline and shows a positive PAS reaction in the peripheral and mid zonal portions of the mucosa. Panels B and C ($\times 40$) represent sections taken from the stomach of rats treated with indomethacin after 4 h and 7 d, respectively, and the panels show a positive PAS reaction around the necrotic and ulcerated area of the mucosal layer. Panels D and E ($\times 40$) represent a section taken from the stomach of rats treated with indomethacin and either L-arginine or NTG, respectively, and show a positive PAS reaction in the edges of the mucosa. Panel F ($\times 40$) represents a section taken from the stomach of a rat treated with indomethacin and L-NAME and shows a positive PAS reaction in the ulcerated area of the mucosa.

Table 2. Effect of daily administration of L-arginine (200 mg/kg, *i.p.*), NTG (1 mg/kg, *i.p.*), or L-NAME (15 mg/kg, *i.p.*) for 7 d on NO content and PGE₂ level in gastric tissue of rats subjected to indomethacin-induced gastric ulcers

Group ^a	NO ($\mu\text{mol/g}$ tissue)	PGE ₂ (pg/mg protein)
Control	381.5 \pm 12.94	254.2 \pm 7.35
Indomethacin (group with indomethacin-induced ulceration on day 0)	274.1 \pm 3.78*	78.3 \pm 2.00*
Indomethacin (group with indomethacin-induced ulceration on day 7)	229.7 \pm 2.57***	330.4 \pm 6.06***
Indomethacin + L-arginine	413.2 \pm 5.44*****	266.5 \pm 4.86*****
Indomethacin + NTG	327.5 \pm 5.58*****	288.1 \pm 1.43*****
Indomethacin + L-NAME	184.2 \pm 9.21*****	394.8 \pm 4.34*****

^a Values given are means of 8-10 observations \pm S.E.M. ***** Significant difference with respect to the control group and groups treated with indomethacin on d 0 and d 7, respectively, at $p < 0.05$ using ANOVA followed by Tukey-Kramer for multiple comparisons.

increase in NO content of 42.5% in comparison to the group with indomethacin-induced ulceration on day 7. However, the level was still significantly lower than normal. It was about 86% of that for the control group (Table 2). On the other hand, treatment of rats with L-NAME caused a further lowering of NO content (20%) in comparison to the group with indomethacin-induced ulceration on day 7 (Table 2).

A single dose of indomethacin induced a significant decrease in PGE₂ that reached 78.31 pg/mg protein after 4 h. However, the group with ulceration on day 7 had a significant increase in PGE₂ of 30% in comparison to the control group. Treatment with either L-arginine or NTG for 7 d after ulcer induction induced a significant decrease in the PGE₂ level of 19 and 13%, respectively, in comparison to the group with indomethacin-induced ulceration on day 7. Worthy of mention is the fact that the PGE₂ level in the group treated with NTG was still significantly higher than the control group while there was no significant difference in this level for the group treated with L-arginine and the control group (Table 2). In the group treated with L-NAME, a further significant increase in the PGE₂ level of 19% was observed in comparison to the group with indomethacin-induced ulceration on day 7 (Table 2).

3.3. Oxidative stress markers (GSH and lipid peroxides)

The control level of GSH was found to be 3.65 ± 0.104 $\mu\text{mol/g}$ of tissue. Table 3 shows that in comparison to the control group indomethacin induced a significant decrease in the GSH level of about 40% 4 h and 7 d after injection. Both L-arginine and NTG treatment for 7 d succeeded in increasing GSH content by 1.6- and 1.4-fold, respectively, in comparison to GSH content in the group with indomethacin-induced ulceration on day 7. However, the level of GSH in the group treated with NTG was still significantly lower than normal. In contrast, treatment with L-NAME resulted in a further decrease in GSH content of 26% in comparison to the group with indomethacin-induced ulceration on day 7.

Assessment of stomach lipid peroxides revealed that the control level was 13.73 ± 0.63 nmol/g tissue. Table 3 illustrates the effects of different treatments on the level of stomach lipid peroxides. In comparison to

the control value, the level of lipid peroxides increased up to 149 and 157% in the groups with indomethacin-induced ulceration on day 0 and 7, respectively. The effect of indomethacin was significantly attenuated by treatment with either L-arginine or NTG (reaching 48 and 35%, respectively) in comparison to the group with indomethacin-induced ulceration on day 7. However, the level of stomach lipid peroxides was still significantly higher than normal. In contrast, animals treated with L-NAME had a further significant increase in lipid peroxides of 57% in comparison to the group with indomethacin-induced ulceration on day 7.

4. Discussion

NO appears to play an important role in gastric mucosal defense (5). However, the possible role of NO in treating existing ulcers must be clarified further. The present study assessed the effect of modulation of NO on peptic ulcer healing using a NO precursor, L-arginine, an NO donor, NTG, and a competitive inhibitor of NOS, L-NAME. Indomethacin was chosen to induce gastric ulcers because it is widely used as an experimental model to study the pathophysiological mechanisms underlying the formation of mucosal lesions and to evaluate the potential therapeutic effects of drugs (24). A single dose of indomethacin produced marked damage to the gastric mucosa, as macroscopic and histological examinations revealed. Similar results have been previously reported (12,25,26). To better understand the role played by NO in healing indomethacin-induced gastric ulcers, the concentration of NO was measured in the gastric tissue and results revealed that indomethacin significantly reduced the gastric NO content in comparison to the NO content in the control group. The reduction in NO content was significantly higher 7 d after injection than 4 h after injection. This reduction in NO content might be explained by the fact that indomethacin induces a reduction in eNOS activity (27).

The reduction in endogenous NO tissue content by indomethacin was supported by studying the effect of L-arginine, NTG, and L-NAME on gastric ulcer healing. Macroscopic and histological examination revealed that L-arginine and NTG accelerated the healing

Table 3. Effect of daily administration of L-arginine (200 mg/kg, i.p.), NTG (1 mg/kg, i.p.), or L-NAME (15 mg/kg, i.p.) for 7 d on oxidative stress markers in rats subjected to indomethacin-induced gastric ulcers

Group ^a	GSH ($\mu\text{mol/g}$ tissue)	Lipid peroxides (nmol/g tissue)
Control	3.65 ± 0.10	13.73 ± 0.63
Indomethacin (group with indomethacin-induced ulceration on day 0)	$2.22 \pm 0.07^*$	$34.17 \pm 0.57^*$
Indomethacin (group with indomethacin-induced ulceration on day 7)	$2.15 \pm 0.04^*$	$35.28 \pm 0.61^*$
Indomethacin + L-arginine	$3.39 \pm 0.11^{***}$	$18.34 \pm 0.74^{*****}$
Indomethacin + NTG	$3.04 \pm 0.05^{*****}$	$22.84 \pm 0.66^{*****}$
Indomethacin + L-NAME	$1.58 \pm 0.02^{*****}$	$55.49 \pm 1.48^{*****}$

^a Values given are means of 8-10 observations \pm S.E.M. ***** Significant difference with respect to the control group and groups treated with indomethacin on day 0 and 7, respectively, at $p < 0.05$ using ANOVA followed by Tukey-Kramer for multiple comparisons.

of indomethacin-induced ulcers but that L-NAME exacerbated mucosal damage. In addition, both L-arginine and NTG significantly restored the gastric NO level while L-NAME induced a further decrease in NO tissue content. As a consequence of the increased NO level, the complete recovery of gastric mucosa as a result of L-arginine or NTG probably depends on either drug's direct vasodilating action. This action increases mucosal blood flow and promotes angiogenesis, a key factor in ulcer healing (13).

Furthermore, NO not only improves blood flow and angiogenesis but also plays a role in stimulating mucus secretion, which is believed to constitute one of the primary levels of mucosal defense (28). Therefore, mucus secretion was assessed in the present study. However, treatment with indomethacin alone and with L-NAME was unexpectedly found to increase mucin in the ulcerated area. This interesting result agrees with the finding of Luo *et al.* (29), who found that mucus thickness at the ulcer margin increased profoundly after ulcer induction. This could be a positive biological feedback mechanism to protect the ulcer from further ulceration (20,29). Treatment with either L-arginine or NTG once daily for 7 d after ulcer induction clearly increased the mucus layer at the edges of the gastric mucosa. The increase in mucus production usually assists the healing process by protecting the ulcer crater against irritant stomach secretions, thereby enhancing the rate of the local healing process (12).

Since the mechanism of NSAID-induced gastric damage is generally believed to be related to inhibition of gastric PG generation (3), studying the effect of NO donors and inhibitors on the level of gastric PGs generated was crucial. A single dose of indomethacin induced a significant decrease in PGE₂ that reached 78.31 pg/mg protein after 4 h, a finding that agrees with several previous studies (3,25). However, 7 d after indomethacin injection the PGE₂ level was found to have significantly increased (30%) in comparison to the PGE₂ level in the control group. This finding agrees with a study by Luo *et al.* (29) that reported an increase in PGE₂ formation at the ulcer margin 7 d after ulcer induction in comparison to the control group.

Indeed, peptic ulcer healing is an active and complex process that includes the reconstruction of the mucosa by formation of granulation tissue at the ulcer base, formation of new blood vessels, and re-establishment of the glandular architecture (30). PGs generated particularly at the ulcer margin by COX-2 appear to play a crucial role in ulcer healing by triggering cell proliferation, promoting angiogenesis, and repairing mucosal injury (31). In contrast, PGs generated by COX-1 are constitutively expressed in intact gastric mucosa to regulate mucosal blood flow and epithelial secretions. Of interest is the fact that PGs generated from COX-1 suppress COX-2 activity in the GI tract. When the mucosa is exposed to potentially

damaging agents or when ulceration occurs, COX-2 is rapidly up-regulated. Therefore, higher amounts of PGE₂ are detected at the site of ulceration than in non-ulcerated mucosa (32,33). Thus, an important finding of the present study is that histopathological examination of the rats with ulcers revealed the presence of multiple sites of inflammatory cell infiltration in the base of the mucosal layer and lamina propria, providing a suitable inflammatory environment for COX-2 expression and consequently resulting in a significant increase in PGE₂ generation as was detected.

With regard to the effect of NO modulation on the elevated level of PGE₂, the current study revealed that treatment with either L-arginine or NTG for 7 d after ulcer induction inhibited the inflammatory signs associated with indomethacin to different degrees. Rats treated with L-arginine had a normal PGE₂ level, while those treated with NTG had a significant decrease in the elevated PGE₂ level. However, this level was still significantly higher than normal. In contrast, treatment with L-NAME further increased the PGE₂ level. These results can be explained by the inhibitory role of NO in the inflammation process (34). NO is an important modulator of leukocyte adherence to the vascular endothelium, a rate-controlling step in the inflammatory process, and using NO donors results in a reduction in myeloperoxidase activity (an indicator of the neutrophil count) and thus tissue injury (35). NO also appears to down-regulate the release of a number of inflammatory mediators from mast cells (36). Similarly, Calatayud *et al.* (37) found that transdermal NTG protected against indomethacin-induced ulceration by maintenance of mucosal blood flow and reduction of leukocyte endothelial cell rolling and adherence. In contrast, Barrachina *et al.* (34) found that NOS inhibitors enhanced inflammation by increasing leukocyte endothelium interactions.

Several studies have recognized the role of oxidative stress in the development of mucosal ulceration caused by NSAIDs. Therefore, the present study involved two potential oxidative stress markers, namely GSH and malondialdehyde (MDA). Indomethacin was found to induce a significant decrease in the gastric content of GSH along with a significant increase in gastric MDA content in both groups with indomethacin-induced ulceration on day 0 and 7 in comparison to gastric content of GSH and MDA in the control group. These findings might be attributed to the pro-oxidant activity of indomethacin, which initiates generation of ROS and thus interferes with the endogenous antioxidant systems of mucosal cells (38). The decreased concentration of GSH may also contribute to increased lipid peroxidation as the loss of this important cellular antioxidant will lead to the accumulation of ROS (39). Treatment with either L-arginine or NTG for 7 d after ulcer induction significantly increased gastric GSH content and decreased the MDA level, but these values did not

return to normal. In contrast, L-NAME caused a further reduction in GSH content and significant elevation in the MDA level in comparison to the GSH content and MDA level in the group with indomethacin-induced ulceration on day 7. These findings are in accordance with previous studies that investigated the protective role of NO during the induction of gastric ulcers. Kwiecien *et al.* (40) found that NO donors reduced gastric lesions and they found that this effect was accompanied by a fall in oxidative stress parameters. That said, NOS inhibitors have also been found to induce an increase in gastric mucosal lipid peroxidation (6).

In conclusion, L-arginine and NTG almost completely healed indomethacin-induced ulceration. Less healing by NTG than by L-arginine may be a result of the different doses. However, the advantages of L-arginine as a natural dietary supplement and immediate precursor of NO should not be ignored. Thus, the present study provides further evidence for the role of NO in gastric ulcer healing and it suggests an alternative path to treating the universal problem of NSAID-induced gastropathy.

Acknowledgements

The authors wish to thank Professor Adel B. Kholoussy (Department of Pathology, Faculty of Veterinary Medicine, Cairo University) for his help with the pathology portion of this work.

References

- Spechler SJ. Peptic ulcer disease and its complications. In: *Gastrointestinal and Liver Disease Pathophysiology/Diagnosis/Management* (Feldman M, Friedman LS, Sleisenger M, eds.). 7th ed., Saunders Company, Philadelphia, USA, 2002; pp. 747-781.
- Sivri B. Trends in peptic ulcer pharmacotherapy. *Fundam Clin Pharmacol.* 2004; 18:23-31.
- Santos CL, Souza MH, Gomes AS, Lemos HP, Santos AA, Cunha FQ, Wallace JL. Sildenafil prevents indomethacin-induced gastropathy in rats: Role of leukocyte adherence and gastric blood flow. *Br J Pharmacol.* 2005; 146:481-486.
- Petersson J, Phillipson M, Jansson EA, Patzak A, Lundberg JO, Holm L. Dietary nitrate increases gastric mucosal blood flow and mucosal defense. *Am J Physiol Gastrointest Liver Physiol.* 2007; 292:G718-G724.
- Gyires K. Gastric mucosal protection: From prostaglandins to gene-therapy. *Curr Med Chem.* 2005; 12:203-215.
- Hsu DZ, Liu MY. Involvement of nitric oxide in gastrin protection of epinephrine in endotoxin intoxication in rats. *Toxicology.* 2004; 204:203-208.
- Ma L, Wallace JL. Endothelial nitric oxide synthase modulates gastric ulcer healing in rats. *Am J Physiol Gastrointest Liver Physiol.* 2000; 279:G341-G346.
- Gürbüz V, Alican I, Berrak, Yegen C, Bozkurt A, Oktar B, Haklar G, Yüksel M, Kurtel H. Role of nitric oxide in indomethacin-induced gastric mucosal dysfunction in the rat. *Exp Physiol.* 1999; 84:319-332.
- Khattab MM, Gad MZ, Abdallah D. Protective role of nitric oxide in indomethacin-induced gastric ulceration by a mechanism independent on gastric acid secretion. *Pharmacol Res.* 2001; 43:463-467.
- Dengiz GO, Odabasoglu F, Halici Z, Suleyman H, Cadirci E, Bayir Y. Gastroprotective and antioxidant effects of amiodarone on indomethacin-induced gastric ulcers in rats. *Arch Pharm Res.* 2007; 30:1426-1434.
- Heeba GH, Hassan MK, Amin RS. Gastroprotective effect of simvastatin against indomethacin-induced gastric ulcer in rats: Role of nitric oxide and prostaglandins. *Eur J Pharmacol.* 2009; 607:188-193.
- Bhattacharya S, Banerjee D, Bauri AK, Chattopadhyay S, Bandyopadhyay SK. Healing property of piper betel phenol, allylpyrocatechol against indomethacin-induced stomach ulceration and mechanism of action. *World J Gastroenterol.* 2007; 13:3705-3713.
- Konturek SJ, Brzozowski T, Majka J, Pytko-Polonczyk J, Stachura J. Inhibition of nitric oxide synthase delays healing of chronic gastric ulcers. *Eur J Pharmacol.* 1993; 239:215-217.
- Sen S, Doger FK, Sen S, Aydin ON, Karul A, Dost T. The protection effect of nitroglycerin on gastrointestinal and renal side effects of lornoxicam in rats. *Eur J Pharmacol.* 2006; 41:191-197.
- Valcavi U, Caponi R, Brambilla A, Palmira M, Minoja F, Bernini F, Musanti R, Fumagalli R. Gastric antisecretory, antiulcer and cytoprotective properties of 9-hydroxy-19,20-bis-nor-prostanoic acid in experimental animals. *Arzneimittelforschung.* 1982; 32:657-663.
- Yamamoto O, Okada Y, Okabe S. Effects of a proton pump inhibitor, omeprazole, on gastric secretion and gastric and duodenal ulcers or erosions in rats. *Dig Dis Sci.* 1984; 29:394-401.
- Ellman M. A spectrophotometric method for determination of reduced glutathione in tissues. *Anal Biochem.* 1959; 74:214-226.
- Uchiyama M, Mihara M. Determination of malondialdehyde precursor in tissues by thiobarbituric acid test. *Anal Biochem.* 1978; 86:271-278.
- Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric oxide.* 2001; 5:62-71.
- Ma L, Wang WP, Chow JY, Lam SK, Cho CH. The role of polyamines in gastric mucus synthesis inhibited by cigarette smoke or its extract. *Gut.* 2000; 47:170-177.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem.* 1951; 64:95-102.
- Drury RA, Wallington EA. *Carlton's Histological Technique.* 5th ed., Oxford University Press, New York, USA, 1980; pp. 139-200.
- McManus JFA. Histological demonstration of mucin after periodic acid. *Nature.* 1946; 158:202.
- Ilahi M, Khan J, Inayat Q, Abidi TS. Histological changes in parts of foregut of rat after indomethacin administration. *J Ayub Med Coll Abbottabad.* 2006; 18:29-34.
- Banerjee D, Bauri AK, Guha RK, Bandyopadhyay SK, Chattopadhyay S. Healing properties of malabaricone B and malabaricone C against indomethacin-induced gastric ulceration and mechanism of action. *Eur J Pharmacol.* 2008; 578:300-312.
- Bhattacharya S, Chaudhuri SR, Chattopadhyay S,

- Bandyopadhyay SK. Healing properties of some indian medicinal plants against indomethacin induced gastric ulceration of rats. *J Clin Biochem Nutr.* 2007; 41:106-114.
27. Slomiany BL, Piotrowski J, Slomiany A. Endothelin-1, interleukin-4 and nitric oxide synthase modulators of gastric mucosal injury by indomethacin: Effect of antiulcer agents. *J Physiol Pharmacol.* 1999; 50:197-210.
 28. Wallace JL, Ma L. Inflammatory mediators in gastrointestinal defense and injury. *Exp Biol Med (Maywood).* 2001; 226:1003-1015.
 29. Luo JC, Shin VY, Liu ES, So WH, Ye YN, Chang FY, Cho CH. Non-ulcerogenic doses of dexamethasone delays gastric ulcer healing in rats. *J Pharmacol Exp Ther.* 2003; 307:692-698.
 30. Perini RF, Ma L, Wallace JL. Mucosal repair and COX-2 inhibition. *Curr Pharm Des.* 2003; 9:2207-2211.
 31. Tatsuguchi A, Sakamoto C, Wada K, Akamatsu T, Tsukui T, Miyake K, Futagami S, Kishida T, Fukuda Y, Yamanaka N, Kobayashi M. Localisation of cyclooxygenase 1 and cyclooxygenase 2 in *Helicobacter pylori* related gastritis and gastric ulcer tissues in humans. *Gut.* 2000; 46:782-789.
 32. Lesch CA, Gilbertsen RB, Song Y, Dyer RD, Sehriar D, Kraus ER, Sanchez B, Guglietta A. Effect of novel anti-inflammatory compounds on healing of acetic acid-induced gastric ulcer in rats. *J Pharmacol Exp Ther.* 1998; 287:301-306.
 33. Poonam D, Vinay CS, Gautam P. Cyclo-oxygenase-2 expression and prostaglandin E2 production in experimental chronic gastric ulcer healing. *Eur J Pharmacol.* 2005; 519:277-284.
 34. Barrachina MD, Panes J, Esplugues JV. Role of nitric oxide in gastrointestinal inflammatory and ulcerative diseases: perspective for drugs and development. *Curr Pharm Des.* 2001; 7:31-48.
 35. Kubes P, Wallace JL. Nitric oxide as a mediator of gastrointestinal mucosal injury? – Say it ain't so. *Mediators Inflamm.* 1995; 4:397-405.
 36. Salvemini D, Masini E, Anggard E, Mannaioni PF, Vane J. Synthesis of nitric oxide-like factor from L-arginine by rat serosal mast cells: Stimulation of guanylate cyclase and inhibition of platelet aggregation. *Biochem Biophys Res Commun.* 1990; 169:596-601.
 37. Calatayud S, Sanz MJ, Canet A, Bello R, de Rojas FD, Esplugues JV. Mechanisms of gastroprotection by transdermal nitroglycerin in the rat. *Br J Pharmacol.* 1999; 127:1111-1118.
 38. Naito Y, Yoshikawa T, Yoshida N, Kondo M. Role of oxygen radical and lipid peroxidation in indomethacin-induced gastric mucosal injury. *Dig Dis Sci.* 1998; 43:30S-34S.
 39. Othman AI, El-Missiry MA, Amer MA. The protective action of melatonin on indomethacin-induced gastric and testicular oxidative stress in rats. *Redox Rep.* 2001; 6:173-177.
 40. Kwiecień S, Brzozowski T, Konturek PCh, Konturek SJ. The role of reactive oxygen species in action of nitric oxide-donors on stress-induced gastric mucosal lesions. *J Physiol Pharmacol.* 2002; 53:761-773.

(Received March 17, 2010; Revised April 6, 2010; Accepted April 15, 2010)