

Sildenafil citrate protects against gastric mucosal damage induced by indomethacin in rats

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Abstract. – BACKGROUND AND OBJECTIVES: The present study was conducted to investigate the possible gastroprotective effect of sildenafil citrate, a selective inhibitor of cyclic guanosine monophosphate-specific phosphodiesterase, against indomethacin-induced gastric damage in rats. Further, the study was extended to investigate some possible mechanisms underlying this effect.

MATERIALS AND METHODS: Forty rats were assigned to vehicle (saline), control (indomethacin, 30 mg/kg, p.o.), ranitidine (50 mg/kg, p.o.), sildenafil (5 mg/kg, p.o.) and sildenafil (10 mg/kg, p.o.); the drugs were administered 30 minutes prior to indomethacin. Four hours after indomethacin administration, all rats were sacrificed and the gastric juices were collected. Then, each stomach was opened and macroscopically examined for gastric lesions and longitudinal sections were used for biochemical and histopathological analysis.

RESULTS: Our results indicated that indomethacin induced marked ulceration in the gastric mucosa, in addition to an increase in gastric acidity as compared to saline group ($p \leq 0.05$). Furthermore, indomethacin group showed lower concentration of mucin and reduced glutathione, whereas, lipid peroxides and tumor necrosis factor- α (TNF- α) were elevated in the stomach homogenate. Pre-treatment with sildenafil (5 mg/kg) significantly reduced gastric acid secretion, ulcer score and lipid peroxides production without effect on mucin, TNF- α , or nitric oxide (NO). The higher dose of sildenafil (10 mg/kg) provided similar results with the exception of increasing tissue NO ($p \leq 0.05$).

CONCLUSIONS: We concluded that sildenafil can protect the gastric mucosa against the aggressive effect of indomethacin via increasing NO and inhibiting lipid peroxidation. Therefore, sildenafil might be helpful in preventing the gastric adverse effects of non-steroidal anti-inflammatory drugs in a clinical setting.

Key Words:

Mucosal damage, Indomethacin, Sildenafil citrate, Ranitidine, Rats.

COX = cyclooxygenase
GSH = reduced glutathione
IL = interleukin
iNOS = inducible nitric oxide synthase
KATP = ATP sensitive potassium channel
L-NAME = N (G)-nitro-L-arginine methyl ester
LP = lipid peroxides
NO = nitric oxide
NSAIDs = nonsteroidal anti-inflammatory drugs
ODQ = 1H-[1,2,4] oxadiazolo[4,3-a]quinoxalin-1-one
PDE = phosphodiesterase
PG = prostaglandins
ROS = reactive oxygen species
sGC = soluble guanylate cyclase
TNF- α = tumor necrosis factor- α

Introduction

Gastric ulcers associated with the use of non-steroidal anti-inflammatory drugs (NSAIDs) remain a major clinical problem and considered to cause a substantial socioeconomic burden and negatively impacts the quality of life¹. NSAIDs are known to be aggressive agents and cause damage in the gastric mucosa. Although the inhibition of cyclooxygenase (COX), which leads to depletion of endogenous prostaglandins (PGs), is a major pathogenic factor, it is unlikely that PG deficiency alone is sufficient to initiate the process that ultimately results in gastric ulceration². Evidence has been produced that leukocyte adherence to the vascular endothelium³, superoxide radicals and protease liberation may be relevant pathogenic mechanisms in NSAIDs gastropathy⁴.

In addition, diminished mucosal circulation has been blamed as one of the etiological factors in gastric ulcer formation. Like PGs, the L-arginine/nitric oxide (NO) pathway is a major protective system in gastric mucosa⁵ via relaxation of the arterial smooth muscles. Accumulating evidence from both animal and human studies indicates that NO plays key roles in normal wound

Abbreviations

cAMP = cyclic adenine monophosphate
cGMP = cyclic guanosine monophosphate
cNOS = constitutive nitric oxide synthase

repair. The beneficial effects of NO on wound repair may be attributed to its functional influences on angiogenesis and inflammation⁶, possibly through a reduction of leukocyte adhesion and maintenance of gastric blood flow⁷.

In fact, there are many antiulcerogenic drugs, among which the most effective are the proton pump inhibitors. However, these drugs do not always provide an effective treatment of ulcer⁸. Therefore, treatment of ulcers is still an important problem, and new drugs are still needed for the treatment of gastric ulcers.

Sildenafil is a commonly prescribed drug for the treatment of male erectile dysfunction and is occasionally used to reduce pulmonary arterial hypertension and to alleviate the symptoms associated with Raynaud's phenomenon⁹. Sildenafil is a selective and potent inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase (PDE₅), which catalyzes the hydrolysis of the intracellular cGMP generated by NO and has a relaxant effect on the smooth muscle cells of the arterioles. Several reports highlighted that sildenafil could be beneficial in preventing acetic acid-induced colonic inflammation¹⁰ and indomethacin induced intestinal¹¹ and gastric injury^{7,12}.

In the present study, we aimed to examine the gastroprotective effect of sildenafil citrate against gastric damage induced by indomethacin in rats. Further, the present study was extended to investigate the role of NO, mucin production and the possible anti-inflammatory or antioxidant effects in promoting the action of sildenafil.

Materials and Methods

Animals

Forty male Wistar rats weighing 150-200 g were used in the present study. Rats were housed in stainless steel cages with free access to food and water under controlled laboratory conditions (temperature 25±3 and normal light-dark cycle). Rats were habituated to the experiment condition 10 days before conduction of the experiment. All experiments were performed in accordance with the national guidelines for the use and care of laboratory animals. Experimental protocols were done under approval from Institutional Animal Use and Care Committee at the faculty of Pharmacy, Suez Canal University.

Drugs

Indomethacin powder was a gift from Nile Pharmaceutical Co. (Cairo, Egypt) and was dissolved in 1% aqueous solution of tween-80. Ranitidine HCl and sildenafil citrate were kindly provided by Medical Union Pharmaceuticals (MUP, Ismailia, Egypt). Ranitidine HCl was prepared in distilled water and sildenafil citrate was dissolved in 1% aqueous solution of tween-80.

Experimental Groups

Rats were randomly divided into 5 groups, 8 per each. Rats were assigned to vehicle (saline), control (indomethacin, 30 mg/kg, p.o.), ranitidine (50 mg/kg, p.o.), sildenafil (5 mg/kg, p.o.), sildenafil (10 mg/kg, p.o.). The drugs were administered 30 minute prior to indomethacin using an oral tube. However, rats from the control group received 0.5 ml of distilled water.

Induction of Experimental Gastric Lesion

Rats were fasted overnight before starting the experiments. Rats received an acute dose of indomethacin (30 mg/kg) by oral gavage.

Anesthesia

Four hours after administration of indomethacin, rats were anesthetized in a jar with a tight-fitting lid containing an appropriate amount of ether and scarified by cervical dislocation. After that, a laparotomy was performed and the stomach of each rat was ligated at the pylorus part and then dissected.

Acid Determination

Gastric acidity was assessed by the method of Aguwa et al¹³. Briefly, after ligation of the pylorus, the gastric contents were collected by washing with 1 ml of saline and centrifuged at 3000 rpm for 10 min. The total acid concentration was determined in the supernatant by titration to pH=7 with 0.01 N NaOH using phenolphthalein as indicator.

Quantification of Ulceration

The glandular portion comprising of the fundic and corpus region of each stomach was opened longitudinally along the greater curvature and examined macroscopically. The number and

severity of lesions in the glandular mucosa were scored from 0 to 5 according to the method of Clement et al¹⁴.

Score	Macroscopic Feature
0	No lesion
0.5	Diffuse hyperemia
1	1 to 2 small ulcers
1.5	3 to 6 small ulcers
2	7 to 10 small ulcers
2.5	More than 10 small ulcers
3	1 marked ulcer plus 0 to 4 small ulcers
3.5	1 marked ulcer plus 5 or more small ulcers
4	2 marked ulcers plus 0 to 4 small ulcers
4.5	2 marked ulcers plus 5 or more small ulcers
5	3 or more marked ulcers

Mucin Assay

Following a reported method, free mucin in the gastric tissue was estimated. Briefly, 0.5 g of the glandular segment of stomach was added to 10 ml of 0.1% alcian blue solution (prepared in 0.16 M sucrose buffered with 0.05 M sodium acetate; final pH was adjusted to 5.5). The stomach tissue was left to be stained for 2 hours in the alcian blue solution. After that, the uncomplexed dye was removed by two washes with 0.25 M sucrose. The complexed dye was then eluted by immersion in 5 ml of 0.5 M magnesium chloride for 2 hours. Dye extract was shaken with equal volume of diethyl ether and then centrifuged at 3600 rpm for 10 min. The optical density of the aqueous layer was read at 598 nm. Results were obtained as mg/g tissue¹⁵.

Preparation of Tissue Homogenate

Longitudinal sections weighting 0.5 g from each stomach were homogenized in phosphate-buffered saline (pH=7.4) using a glass-teflon homogenizing tube (Glas Col homogenizer system, Vernon Hills, TX, USA). The homogenate was centrifuged at 2500 rpm for 10 min and the supernatant was carefully removed from the pellet and used for biochemical analyses.

Biochemical Analysis

Determination of TNF- α

Tumor necrosis factor- α was determined according to Mysliwska et al¹⁶, using a commercial-

ly available ELISA Kit (Biosource®, Camarillo, CA, USA) following the instructions of the manufacturer. TNF- α was expressed as pg/g tissue.

Determination of Tissue Nitric Oxide

Nitrite was determined as an oxidation product and indicator of NO synthesis as described previously¹⁷. The method is based on the addition of Griess reagent which converts nitrite into deep purple azo chromophore. The color intensity was measured using a UV-visible spectrophotometer (UV1601-PC, Shimadzu, Tokyo, Japan). Nitric oxide level was expressed as mol/g tissue.

Determination of Lipid Peroxides

Lipid peroxides (LPs) were assessed according to the spectrophotometric method of Ohkawa et al¹⁸. LPs react with thiobarbituric acid leading to the production of a red pigment at pH = 3.5. The reaction product is quantitatively extracted from the solution with *n*-butanol-pyridine mixture and estimated by measuring the absorption at 532 nm. 1,1,3,3, Tetramethoxypropane was used as a standard. Tissue LPs was expressed as μ mol/g tissue.

Estimation of GSH Concentration in Gastric Tissues

Reduced glutathione in the tissue homogenate was measured according to the colorimetric method of Sedlak and Lindsay¹⁹. The reduced form of glutathione reacts with Ellman's reagent to form a colored product, which could be quantitatively determined at 412 nm in the UV region. Reduced glutathione level in the stomach homogenates was expressed as μ g/g tissue.

Histopathological Examination

For histological assessment, the glandular area of each stomach was fixed in 10% phosphate-buffered paraformaldehyde solution and prepared for staining with, haematoxylin and eosin and then examined under a light microscope. The specimens were assessed according to the criteria of Laine and Weinstein²⁰. In a brief, a 1 cm length of each histological section was assessed for epithelial cell loss (a score of 0-3), edema in the upper mucosa (a score of 0-4), hemorrhagic damage (a score of 0-4) and presence of inflammatory cells (a score of 0-3). The sections the sections were assessed by an experienced pathologist without the knowledge of treatments.

Statistical Analysis

Data was expressed as mean $\pm$ SEM and statistically analyzed using SPSS program version 16 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance, ANOVA, followed by Bonferroni's multiple comparisons test were used for the statistical analysis. Non parametric ANOVA was used when appropriate. For all comparisons, differences were considered significant at $p \leq 0.05$.

Results

In the present study, oral administration of indomethacin (30 mg/kg) induced marked increase in the ulcer score as compared to saline group (5 ± 0 vs. 0, $p \leq 0.05$, Figures 1). Pretreatment with ranitidine attenuated the ulcer score as compared to indomethacin group. Moreover, the mean ulcer score was attenuated by pretreatment with sildenafil (5 and 10 mg/kg) as compared to indomethacin group.

Indomethacin treatment induced a significant increase in the total acid concentration (3.4 ± 0.16 g/L) as compared with saline group (1.3 ± 0.1 g/L, $p \leq 0.05$, Figure 2). Furthermore, ranitidine suppressed the acid production as compared to indomethacin group ($p \leq 0.05$). Similar to ranitidine, pretreatment with sildenafil (5 and 10 mg/kg) markedly suppressed acid production as compared to indomethacin group ($p \leq 0.05$, Figure 2).

Furthermore, the stomach of indomethacin-treated rats showed a significant decrease in the mucin concentration as compared to saline group. Pretreatment with ranitidine significantly restored tissue mucin concentration in the gastric mucosa as compared to indomethacin group. However, pretreatment with sildenafil (5 and 10 mg/kg) did not show similar results ($p \leq 0.05$, Figure 3).

The biochemical parameters measured in the stomach tissue differed significantly in indomethacin-treated rats as compared with saline-treated rats; this with the exception of NO. GSH was significantly decreased. However, LPs and TNF- α were significantly elevated in the gastric tissues of indomethacin group ($p \leq 0.05$). Nevertheless, ranitidine produced a non significant increase in TNF- α level as compared to indomethacin group. However, sildenafil (5 and 10 mg/kg) could not decrease the elevated TNF- α level significantly ($p \leq 0.05$, Figure 4A). Differently, both ranitidine and sildenafil (10 mg/kg) could induce a significant

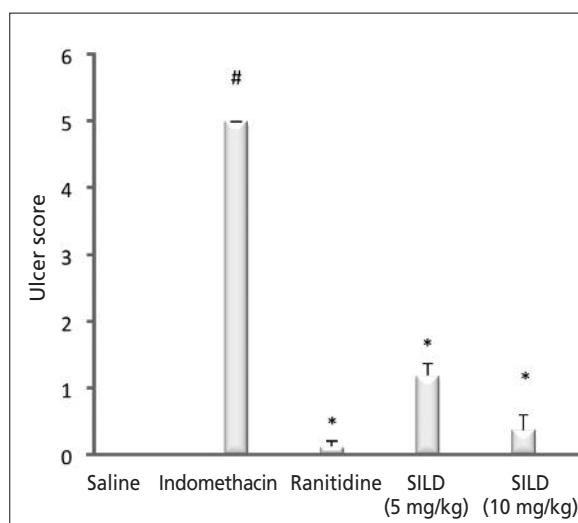


Figure 1. Ulcer scores in the gastric juice of the experimental groups. Administration of indomethacin (30 mg/kg, p.o.) induced a significant increase in the ulcer score as compared to saline group. Pretreatment with ranitidine (50 mg/kg, p.o.) or sildenafil (SILD, 10 mg/kg, p.o.) suppressed ulcer score as compared to indomethacin group. Results were expressed as mean $\pm$ SD and analyzed using Kruskal-Wallis test followed by Mann-Whitney test for multiple comparisons ($n=8$). #Significantly different from saline group at $p \leq 0.05$. *Significantly different from indomethacin group at $p \leq 0.05$.

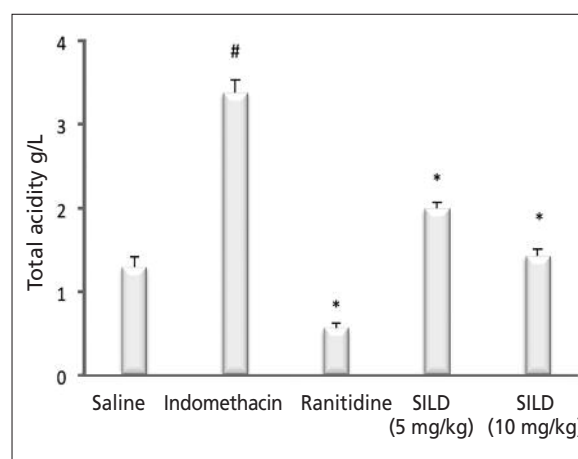


Figure 2. Total acidity in the gastric juice of the experimental groups. Administration of indomethacin (30 mg/kg, p.o.) induced a significant increase in the total acidity as compared to saline group. Pretreatment with ranitidine (50 mg/kg, p.o.) or sildenafil (10 mg/kg, p.o.) suppressed acid production in the gastric juice as compared to indomethacin group. Results were expressed as mean $\pm$ SEM and analyzed using one-way ANOVA followed by Bonferroni's multiple comparisons test ($n=8$). #Significantly different from saline group at $p \leq 0.05$. *Significantly different from indomethacin group at $p \leq 0.05$.

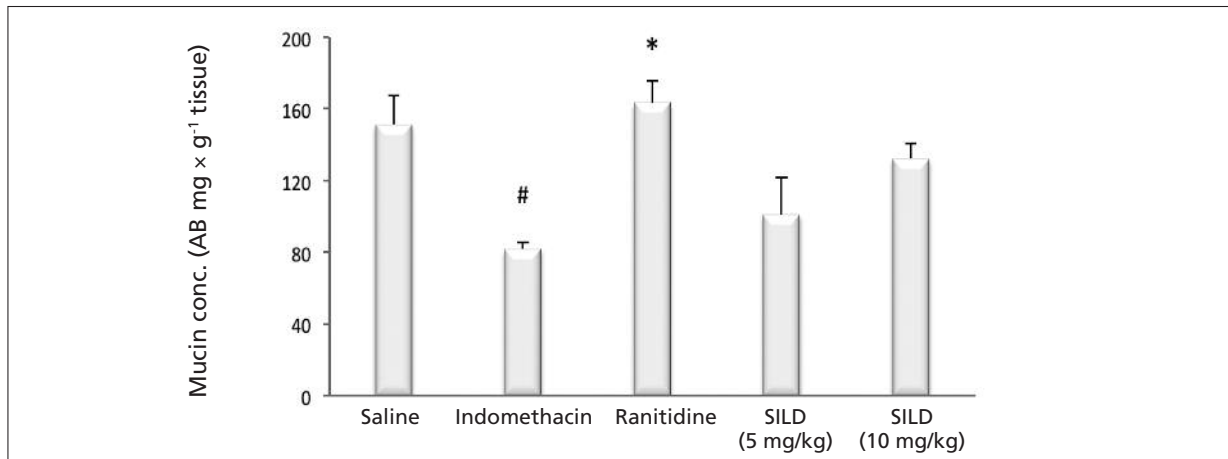


Figure 3. Mucin concentration in the gastric tissues in the experimental groups. Administration of indomethacin (30 mg/kg, p.o.) induced a significant decrease in mucin concentration as compared to saline group. Pretreatment with ranitidine (50 mg/kg, p.o.) normalized the gastric mucin level. Sildenafil (10 mg/kg) did not produce a significant change. Results were expressed as mean $\pm$ SEM and analyzed using one-way ANOVA followed by Bonferroni's multiple comparisons test ($n=8$). [#]Significantly different from saline group at $p \leq 0.05$. ^{*}Significantly different from indomethacin group at $p \leq 0.05$.

increase in tissue NO level as compared to indomethacin-group ($p \leq 0.05$, Figure 4B).

In comparison to indomethacin group, ranitidine and the two doses of sildenafil could suppress the production of LPs in the gastric tissue ($p \leq 0.05$, Figure 5A). However, these pharmacological treatments did not increase GSH level significantly (Figure 5B).

Histopathological examination of the gastric mucosa revealed that oral administration of in-

domethacin (30 mg/kg) induced marked erosion to the mucosal epithelia coupled with edema, congestion and bleeding. In addition, inflammation was manifested by higher number of leukocytes in the field (mainly, neutrophils and macrophages). Pretreatment with ranitidine improved the histopathological picture; the edema was mild and erosion was limited to the superficial epithelia. Pretreatment with sildenafil (5 and 10 mg/kg) decreased the degree of hemorrhage

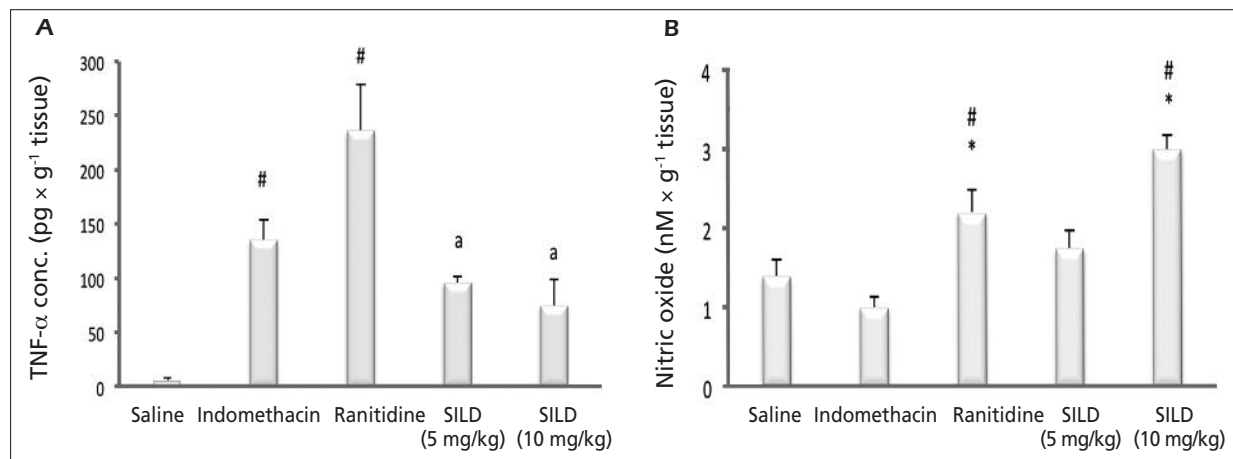


Figure 4. Tumor necrosis factor- α (TNF- α) (**A**) and nitric oxide (**B**) in the gastric tissues of the experimental groups. Administration of indomethacin (30 mg/kg, p.o.) induced a significant increase in TNF- α as compared to saline group. Sildenafil could not change TNF- α significantly. Indomethacin group did not show a significant change in nitric oxide level as compared to saline group. Pretreatment with ranitidine (50 mg/kg, p.o.) and sildenafil (10 mg/kg, p.o.) produced a significant increase in nitric oxide level. Results were expressed as mean $\pm$ SEM and analyzed using one-way ANOVA followed by Bonferroni's multiple comparisons test ($n=8$). [#]Significantly different from saline group at $p \leq 0.05$. ^{*}Significantly different from indomethacin group at $p \leq 0.05$. ^aSignificantly different from ranitidine group at $p \leq 0.05$.

and lessened leukocytic attraction as compared to indomethacin group (Figure 6 and Table I).

Discussion

In the current study, administration of acute dose of indomethacin (30 mg/kg, p.o) induced marked ulceration in gastric mucosa, in addition to an increase in ulcer score and gastric acidity and a significant decrease in gastric mucin content. These results came on line with those obtained recently²¹. In addition, treatment with indomethacin significantly increased LPs concentration and decreased GSH level in stomach tissues and that extend findings of others^{22,23}. These results indicate a role of toxic oxygen radicals in the etiopathogenesis of indomethacin-induced gastric damage.

The increase in the production of reactive oxygen species (ROS) does not fully account for the etiopathogenesis of indomethacin-induced gastric damage. It was also demonstrated that indomethacin produced a significant increase in TNF- α in gastric tissues which is one of the aggressive factors in ulcerogenesis²⁴ and this came on line with our findings. There is an evidence that TNF- α stimulates neutrophil infiltration into gastric mucosa and its overproduction increases the risk of gastric ulcer and cancer. Furthermore, it was proved that there is a relation between TNF- α and PGE₂. It was found that exogenous adminis-

tration of PGE₂ produced an anti-ulcer effect by preventing the indomethacin-induced TNF- α increase²⁵. It was also suggested that TNF- α is a potent stimulator of inducible NO expression²⁶.

In the current study, indomethacin group did not show a significant difference in NO content as compared to the normal group. Some of the previous researches reported that indomethacin was found to produce ulcers via increasing gastric acid secretion and decreasing NO synthesis²⁷. In contrast, others suggested that NO acts in gastric defense mechanisms by regulating the gastric mucosal blood flow, gastric mucus secretion and increase in PG biosynthesis²⁸. To solve these contradictory results, NO seems to play a biphasic role in the ulcerogenic response of the gastrointestinal mucosa depending on the nitric oxide synthase (NOS) isozyme, a protective effect of constitutive NOS (cNOS)/NO and a proulcerogenic effect of inducible NOS (iNOS)/NO²⁹. Collectively, indomethacin has been reported to induce gastric mucosal injury via a COX-dependent decrease in PG production³⁰, a direct COX-independent cytotoxic action³¹ and ROS-dependent signal transduction stimulation³².

The present study showed that sildenafil (5 mg/kg) significantly decreased gastric acid concentration, lipid peroxidation and gastric injury without effect on mucin, GSH, or NO. However, sildenafil (10 mg/kg) showed similar results except that it increased NO level. It was suggested that sildenafil

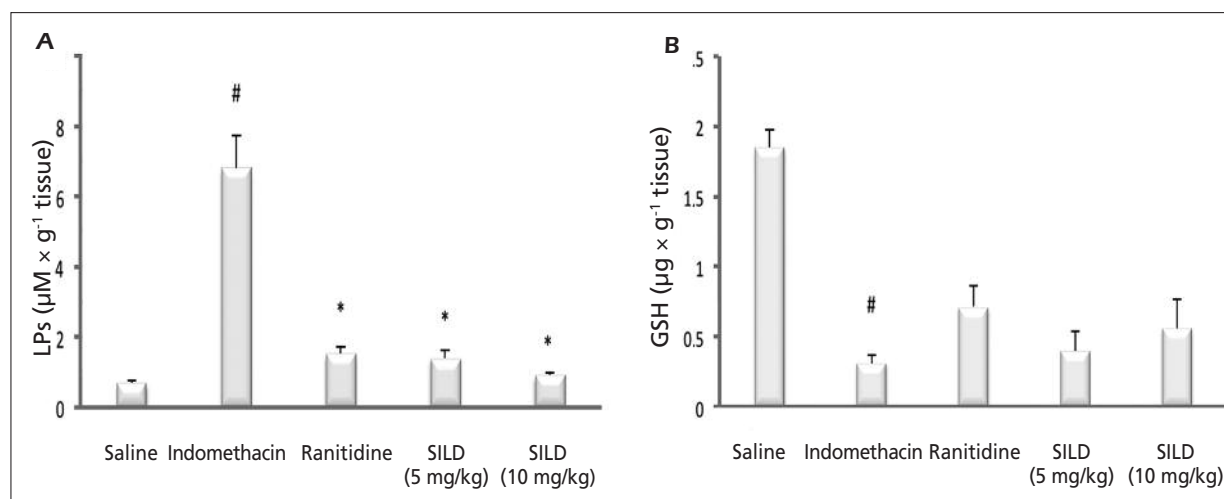


Figure 5. Lipid peroxides (A) and glutathione (B) in the gastric tissues of the experimental groups. Administration of indomethacin (30 mg/kg, p.o.) induced a significant increase in lipid peroxides and decreased glutathione level as compared to saline group. Pretreatment with ranitidine (50 mg/kg, p.o.) and sildenafil (10 mg/kg, p.o.) suppressed lipid peroxides level as compared to indomethacin group without affecting GSH level. Results were expressed as mean $\pm$ SEM and analyzed using one-way ANOVA followed by Bonferroni's multiple comparisons test ($n=8$). [#]Significantly different from saline group at $p \leq 0.05$. ^{*}Significantly different from indomethacin group at $p \leq 0.05$.

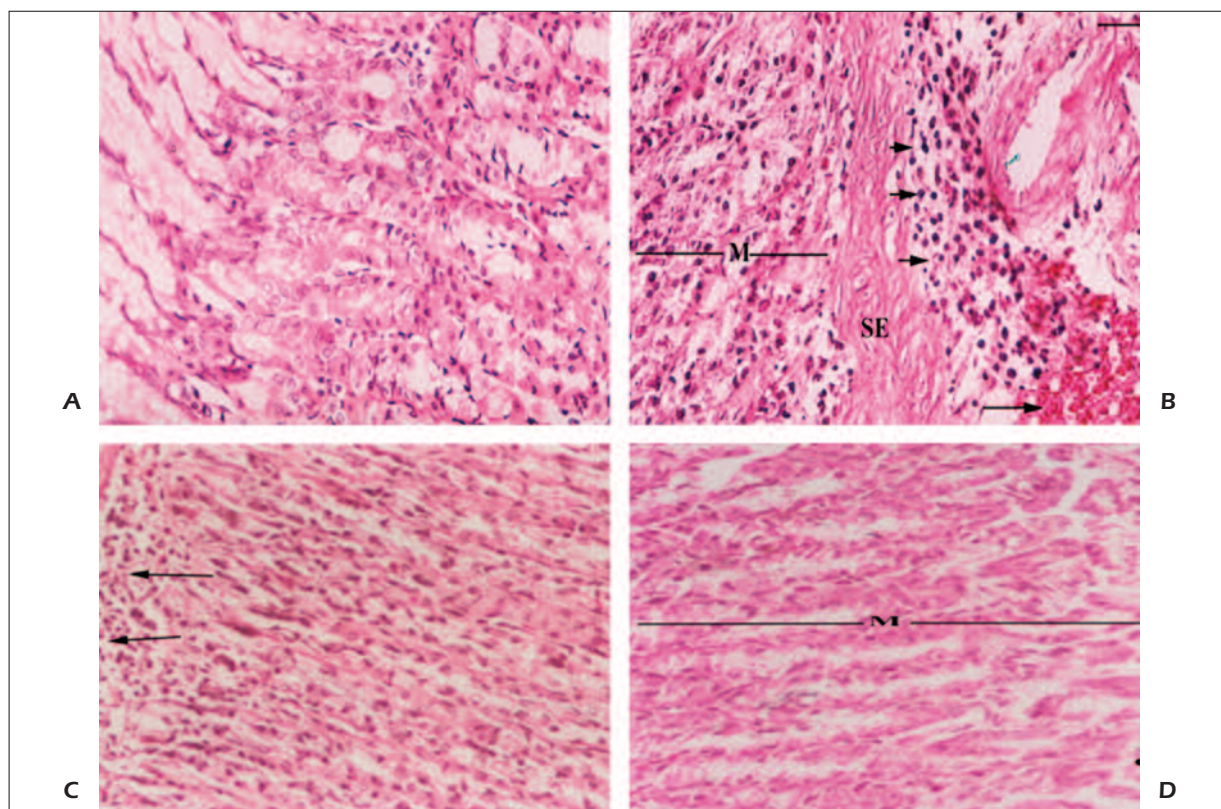


Figure 6. Histological picture of the gastric tissues. **A**, A normal stomach showing normal histological appearance mucosa. **B**, A stomach from indomethacin group showing erosion of mucosa, degeneration and necrosis of gastric gland and hemorrhage. **C**, Ranitidine group shows fairly normal gastric mucosa with slight leukocytic infiltrations (arrows). **D**, A stomach of sildenafil group showing erosion of mucosa with degeneration and necrosis covering of epithelium with congestion of blood vessels of submucosa (H&E $\times 40$).

prevents indomethacin-induced small-intestinal ulceration in rats, via increased production of tissue NO^{8,33} or increased tissue cGMP level without modifying NO content³⁶ and this effect is function-

ally associated with an increase in the secretion of mucus/fluid⁵ and a decrease of hypermotility, resulting in the suppression of bacterial invasion and iNOS expression following indomethacin

Table I. Scoring for the microscopic changes in the gastric tissues in the experimental groups.

Experimental groups	Hemorrhagic damage (score 0-4)	Edema (score 0-4)	Epithelial cell loss (score 0-3)	Inflammatory cells (score 0-3)	Total (scores 14)
Saline	0	0	0	0	0
Indomethacin	4 $\pm$ 0	4 $\pm$ 0	3 $\pm$ 0	3 $\pm$ 0	14 $\pm$ 0 [#]
Ranitidine (50 mg/kg)	0.9 $\pm$ 0.3	1 $\pm$ 0	1.1 $\pm$ 0.1	1.1 $\pm$ 0.1	4.25 $\pm$ 0.16 ^{#*}
Sildenafil (5 mg/kg)	1.1 $\pm$ 0.1	1.5 $\pm$ 0.8	1.6 $\pm$ 0.2	1.6 $\pm$ 0.2	5.5 $\pm$ 0.5 ^{#*}
Sildenafil (10 mg/kg)	2.2 $\pm$ 0.4	2 $\pm$ 0.4	2.2 $\pm$ 0.3	2.4 $\pm$ 0.5	8.87 $\pm$ 0.5 ^{#*}

Gastric ulceration was induced by administration of indomethacin (30 mg/kg, p.o.). Rats were pretreated orally with ranitidine or sildenafil (10 mg/kg). Four hours later, rats were sacrificed and the stomachs were dissected and longitudinal sections were taken and fixed in 10% paraformaldehyde solution and sections were cut along the stomach wall and stained with hematoxylin and eosin and examined for microscopic changes. Indomethacin group showed the highest score for gastric damage. All the implemented pharmacological agents could suppress the total scores as compared to indomethacin group. Data was expressed as mean $\pm$ SD and analyzed using Kruskal-Wallis test ($n = 8$). [#]Significantly different from saline group at $p \leq 0.05$. ^{*}Significantly different from indomethacin group at $p \leq 0.05$.

treatment^{11,35}. The Authors highlighted these effects may account partly for the prophylactic effect of sildenafil against small-intestinal lesions.

Our study demonstrated that sildenafil dose dependently can protect the stomach against indomethacin-induced damage. There are many mechanisms through which sildenafil could exert this effect; it was hypothesized that inhibition of indomethacin-induced leukocyte adhesion to vascular endothelium and maintenance of gastric blood flow are of primary importance⁷. There is evidence that NO inhibits the expression of adhesion molecules on endothelial cells, which is an important step in neutrophil migration³⁶. Thus, it is possible that the gastroprotective effect of sildenafil is mediated by inhibition of leukocyte adherence, and this is a NO-dependent process. In accordance, sildenafil decreased leukocyte efflux in bronchoalveolar lavage fluid in guinea pig models of inflammatory airway disease³⁷.

The evidence that the protective effects of sildenafil were NO dependent includes the following: treatment with L-NAME (N (G)-nitro-L-arginine methyl ester) (a competitive, nonselective NOS inhibitor) abrogated the protective effect of sildenafil, while the protective effect could be restored by co-administration of L-arginine (a substrate of NOS). These observations are consistent with those observed that NO donors accelerated gastric ulcer healing in rats³⁸ while NO-releasing agents protected against experimental NSAIDs-induced damage³⁹. Further evidence was provided by the study of Bianco et al, who reported a curative effect for sildenafil in diabetic gastropathy⁴⁰.

It has been reported that sildenafil ameliorates ethanol-induced gastric hemorrhagic damage, edema and epithelial cell loss. Furthermore, the soluble guanylate cyclase (sGC) inhibitor ODQ (1H-[1,2,4] oxadiazolo[4,3-a]quinoxalin-1-one) reversed the protective effects of sildenafil, demonstrating that the protective mechanism is cGMP dependent⁴¹. Interestingly, the ATP sensitive potassium channel (K_{ATP}) blocker glibenclamide was also capable of reversing sildenafil's gastroprotective effect; these K_{ATP} channels regulate gastric protection⁴². Thus, it appears that inhibition of PDE V by sildenafil increases the survival of cGMP generated in response to endogenous NO and affords protection against alcohol-induced gastric damage, possibly via activation of K_{ATP} channels. Finally, our results agree with studies which concluded that sildenafil may act functionally with amplifying the action of NO with or without increase in its production.

In agreement with our findings, an *in vitro* study demonstrated that sildenafil was unable to decrease hypoxia-induced upregulation of TNF- α and IL-1b mRNA in pulmonary artery⁴³. Similarly, sildenafil did not significantly inhibit any markers of inflammation including TNF- α , IL-4 and IL-5 levels in a murine model of allergic asthma⁴⁴. Differently, another study demonstrated that pretreatment with PDE5 inhibitor zaprinast at a dose of 10 mg/kg blocked lipopolysaccharide-induced increase in serum TNF- α level in mouse skin⁴⁵.

Consistent with our results, sildenafil was found to have anti-lipid peroxidation properties in rat neural cells⁴⁶, renal cells⁴⁷ and in salivary glands by increasing by the synthesis of cGMP and cAMP⁴⁷. Consistently, some Authors observed a significant decrease in colonic lipid peroxidation level and oxidant production in the sildenafil-treated colitis group¹⁰. On the other hand, endogenous antioxidant glutathione was found to be restored in the same group. A possible explanation for this finding was that glutathione was conserved due to a lower level of lipid oxidation. Thus, their results showed the inhibition of tissue lipid peroxidation along with the replenishment of GSH content by sildenafil imply that the compound is beneficial in maintaining oxidant-antioxidant balance. However, the increase of gastric GSH in the current study was not statistically significant.

Conclusions

Our findings provide evidence that sildenafil possesses gastroprotective activity against indomethacin-induced gastric damage via increasing NO and inhibition lipid peroxidation. Therefore, sildenafil might be helpful in preventing the gastric adverse effects of NSAIDs in a clinical setting.

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