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Ameliorative Effects of Sitagliptin against Indomethacin-Induced Gastric Ulcer in Rats

Amr A. Fouad [1]*, Moataz Mohamedalhasan Ali [2], Heba M. Hafez [3]

[1] Department of Pharmacology, Faculty of Medicine, Al-Baha University, Al-Baha, Saudi Arabia
afouad@bu.edu.sa
[2] Department of Pathology, Faculty of Medicine, Al-Baha University, Al-Baha, Saudi Arabia & Department of Pathology, Faculty of Medicine, University of Elimam Elmahdi, Sudan
moataz.m@bu.edu.sa
[3] Department of Pharmacology, Faculty of Medicine, Minia University, El-Minia, Egypt
hebahafez100@yahoo.com

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Abstract

The present study investigated the potential protective effect of sitagliptin against indomethacin-induced gastric ulcer in rats. Sitagliptin was administered in two doses (5 and 10 mg/kg/day, p.o.) for 14 days, then gastric ulcer was induced by a single dose of indomethacin (50 mg/kg, p.o.). In another set of experiments, the effects of sitagliptin on gastric secretion were tested in pylorus-ligated rats. Sitagliptin significantly decreased the ulcer index, malondialdehyde, tumor necrosis factor-α, and cleaved caspase-3, and significantly increased the reduced glutathione, superoxide dismutase and prostaglandin E_2 in gastric mucosa of indomethacin-challenged rats. Sitagliptin also reduced the volume of gastric secretion, total acid concentration, and pepsin activity in pylorus-ligated rats. Histopathological examination showed that sitagliptin markedly minimized the gastric mucosal injuries caused by indomethacin. Both sitagliptin doses significantly protected against indomethacin-induced gastric lesions in rats, most probably by inhibiting oxidative stress, inflammation, and apoptosis.

Keywords: gastric ulcer, indomethacin, oxidative stress, rats, sitagliptin.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used anti-inflammatory, analgesic, and antipyretic medications. However, gastric erosions, ulceration, and bleeding are common adverse effects, which may limit the usefulness of NSAIDs. Inhibition of prostaglandin E_2 synthesis, and disruption of the equilibrium between aggressive and defensive mechanisms in the gastric mucosa are responsible for NSAID-induced gastric ulceration [1]. In addition, strong evidence suggests that oxidative stress, inflammation, and apoptosis are implicated in the pathogenesis of this adverse effect. Increased production of reactive oxygen species (ROS), depletion of endogenous antioxidants, and enhanced membrane lipid peroxidation were demonstrated in gastric lesions caused by NSAIDs [2]. Besides, increased generation of inflammatory cytokines as tumor necrosis factor-α, and apoptotic factors as caspase-3 was observed in this adverse impact [3].

Sitagliptin (SGN) is a dipeptidyl peptidase-4 inhibitor commonly used for treatment of type 2 diabetes mellitus. It was also reported in the literature that SGN significantly provided antioxidant, anti-inflammatory, and antiapoptotic effects. Previous studies showed that SGN ameliorated diabetes-induced cardiac injury in rats [4], protected rat kidneys from adenine-induced injury [5], prevented aflatoxin B_1-induced hepatotoxicity in rats [6], and minimized testicular ischemia/reperfusion damage in rats [7]. However, the protective effect of SGN against gastric ulcer induced by NSAIDs was not yet investigated. Therefore, this study was conducted to assess the possible gastroprotective effect of SGN in indomethacin (IND)-induced ulcer in rats, and to reveal the mechanisms underlying this effect.

2. Materials And Methods

Drugs and chemicals

SGN and indomethacin IND were purchased from Sigma-Aldrich, USA. SGN and IND were prepared in carboxymethylcellulose (CMC), 0.5% solution. The doses of SGN and IND used in the current work were selected from previous studies [8,9].

Laboratory animals

Male Sprague-Dawley rats weighing 220 ± 10 g were obtained from National Research Centre, Giza, Egypt. Rats were kept at standard housing facilities (24°C, 50% humidity, and 12 h light/dark cycle), and supplied with laboratory chew and tap water *ad libitum*. The research protocol was approved by the Research Ethics Committee, Minia University, Egypt (approval number: 227-42020). The international guidelines for care and use of laboratory animals were followed.

Study design

The study included two sets of experiments. The first set investigated the effects of SGN on gastric ulcer induced by IND in rats, and the second set investigated the effects of SGN on gastric secretion in pylorus-ligated rats.

i. Gastric ulcer induction group

Rats were randomly divided into 5 equal groups (n = 8) as follows:

- Group 1 (negative control) received daily CMC (as a vehicle), p.o., for 14 days.
- Group 2 (positive control) received SGN (10 mg/kg/day), p.o., for 14 days.
- Group 3 received a single dose IND (50 mg/kg, p.o.), on 14th day of the study.
- Group 4 received SGN (5 mg/kg/day, p.o.) for 14 days, then at the last day of the study IND was administered.
- Group 5 received SGN (10 mg/kg/day, p.o.) for 14 days, then IND was administered 1 h later at the last day.

Four hours after IND administration, rats were euthanized by thiopental (70 mg/kg, i.p.). The stomach was dissected, opened, and washed with saline to calculate the ulcer index for the visible lesions as previously described [10].

The gastric mucosa was scrapped and homogenized in cold potassium phosphate buffer (pH 7.5, 0.5 N). The homogenate was used for estimation of malondialdehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD) using colorimetric kits (Biodiagnostic, Egypt). Activity of cleaved caspase-3 was also determined by a colorimetric kit (R&D Systems, USA). Additionally, ELISA kits were used to measure tumor necrosis factor-α (TNF-α) and prostaglandin E_2 (PGE_2) (R&D Systems, USA).

ii. Pylorus-ligated group

Rats were randomly divided into 3 equal groups (n = 6), and received CMC, SGN (5 mg/kg/day), and SGN (10 mg/kg/day), p.o., respectively, for 14 days. The rats were fasted for 24 h before the end of experimental period to ensure complete emptying of the stomach and water was allowed *ad libitum*.

Rats were anaesthetized and the abdomen was opened and pylorus ligation was carried out. Then, the stomach was replaced carefully and the abdominal wall was closed with interrupted sutures. After 4 h, each stomach was dissected out and cut open along the greater curvature. After dissection and removal of the stomach, gastric juice was collected. Then, it was centrifuged for 10 min at 3000 rpm, and the supernatant was separated to measure the gastric juice volume. Additionally, total acid concentration and pepsin concentration were determined in the supernatant as previously described [11,12].

Histopathological examination

Parts of gastric tissues were fixed in 10% formalin, dehydrated in ascending grades of alcohol, and embedded in paraffin. Sections of 5-µm thickness were cut and stained with hematoxylin and eosin (H&E). The slides were examined under light microscope by a pathologist unaware of the treatment protocol.

Statistical analysis

Data analysis was done by GraphPad Prism Software Program (version 6.01) using one-way ANOVA test followed by Tukey test for *post hoc* comparisons. Results were expressed as mean ± S.E.M., and significance level was at $P < 0.05$.

3. Results

Results of gastric mucosa analyses

Fig. 1 shows the gross lesions in gastric mucosa induced by a single dose of IND (50 mg/kg, p.o.) and the effects of prior treatment with SGN (5 and 10 mg/kg/day, p.o.) for 14 days.

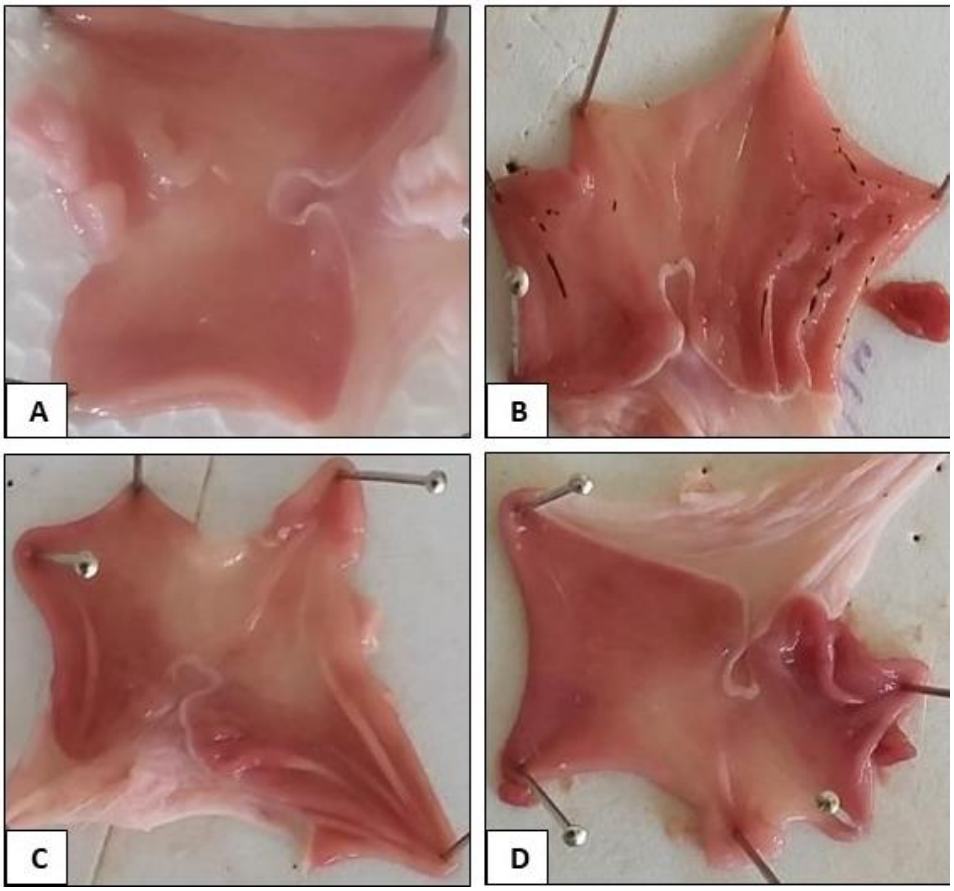
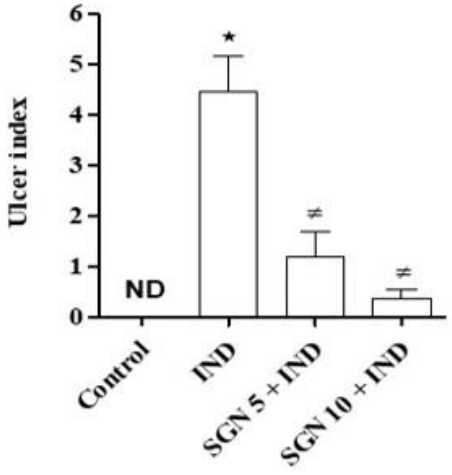


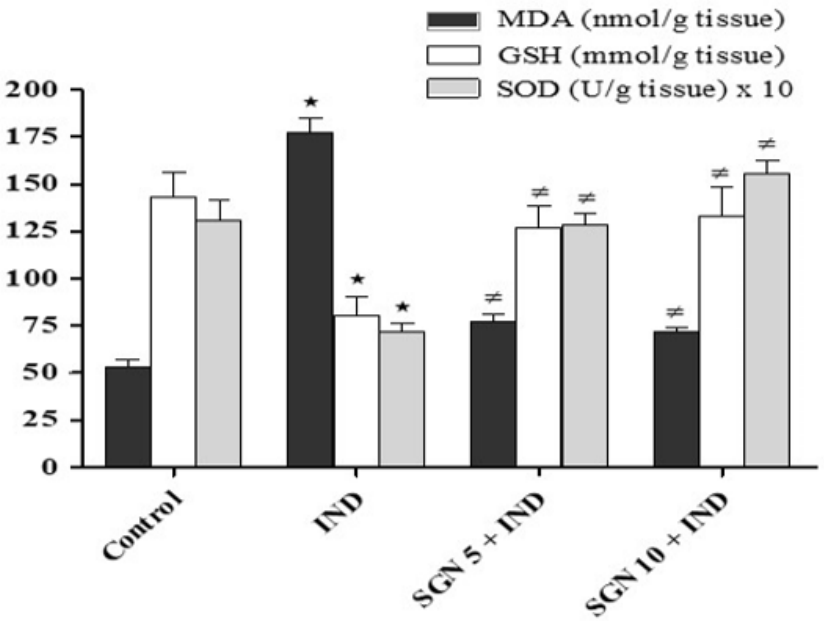
Fig. 1. Gross pictures of rat gastric mucosa from: (A) control showing normal gastric mucosa; (B) indomethacin-challenged rats showing multiple gastric mucosallesions in the form of petechial hemorrhages, erosions, and ulcerations; (C and D) sitagliptin-treated rats (5 and 10 mg/kg, respectively) showing marked reduction of the gastric mucosal lesions, particularly with the higher sitagliptin dose.

In addition, IND administration caused significant elevations ($P < 0.05$) of ulcer index, MDA, TNF-α, and cleaved caspase-3, and significant reductions ($P < 0.05$) of GSH, SOD, and PGE_2 in gastric mucosa of rats (Figs. 2A-2D). Treatment with SGN (5 and 10 mg/kg/day) significantly decreased ($P < 0.05$) ulcer index, MDA, TNF-α, and cleaved caspase-3, and significantly increased ($P < 0.05$) GSH, SOD, and PGE_2 in gastric mucosa of IND-challenged rats (Figs. 2A-2D).

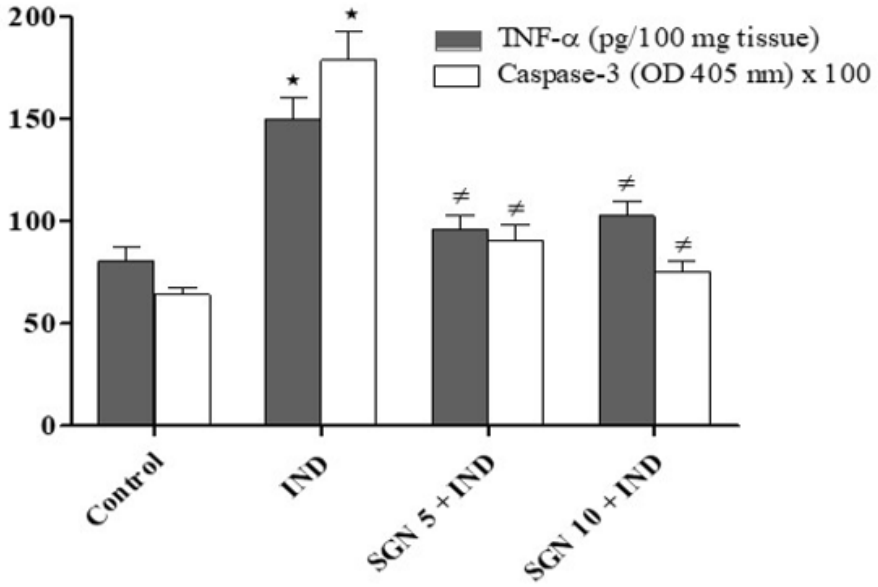
2A


Ulcer index
6
5
4
3
2
1
0
ND
Control
IND
SGN 5 + IND
SGN 10 + IND

2B


MDA (nmol/g tissue)
GSH (mmol/g tissue)
SOD (U/g tissue) x 10
200
175
150
125
100
75
50
25
0
Control
IND
SGN 5 + IND
SGN 10 + IND

2C


TNF-α (pg/100 mg tissue)
Caspase-3 (OD 405 nm) x 100
200
150
100
50
0
Control
IND
SGN 5 + IND
SGN 10 + IND

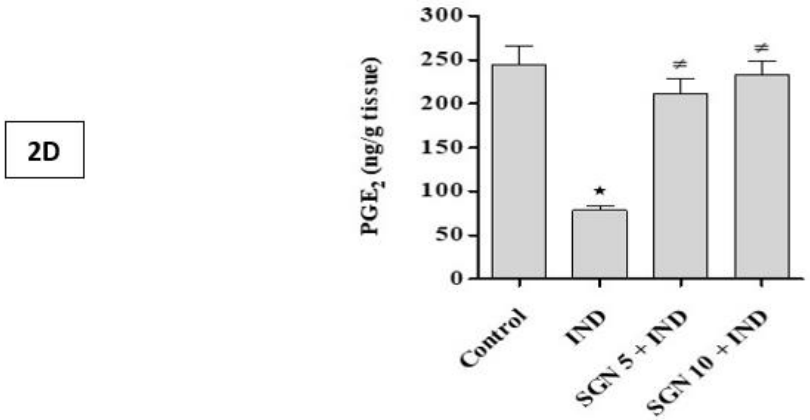


Fig. 2. Effects of siatagliptin (SGN) treatment (5 and 10 mg/kg) on: (A) ulcer index; (B) malondiadehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD); (C) tumor necrosis facor-α (TNF-α) and cleaved caspase-3; (D) prostaglandin E_2 (PGE_2) in gastric mucosa of indomethacin (IND)-challenged rats. $^{*}P < 0.05$ vs. control group, $^{\neq}P < 0.05$ vs. IND group.

Results of gastric secretion analyses

In the second set of experiments, both doses of SGN (5 and 10 mg/kg) significantly reduced ($P < 0.05$) the volume of gastric secretion, total acid concentration, and pepsin activity in the pylorus-ligated rats (Figs. 3A and 3B).

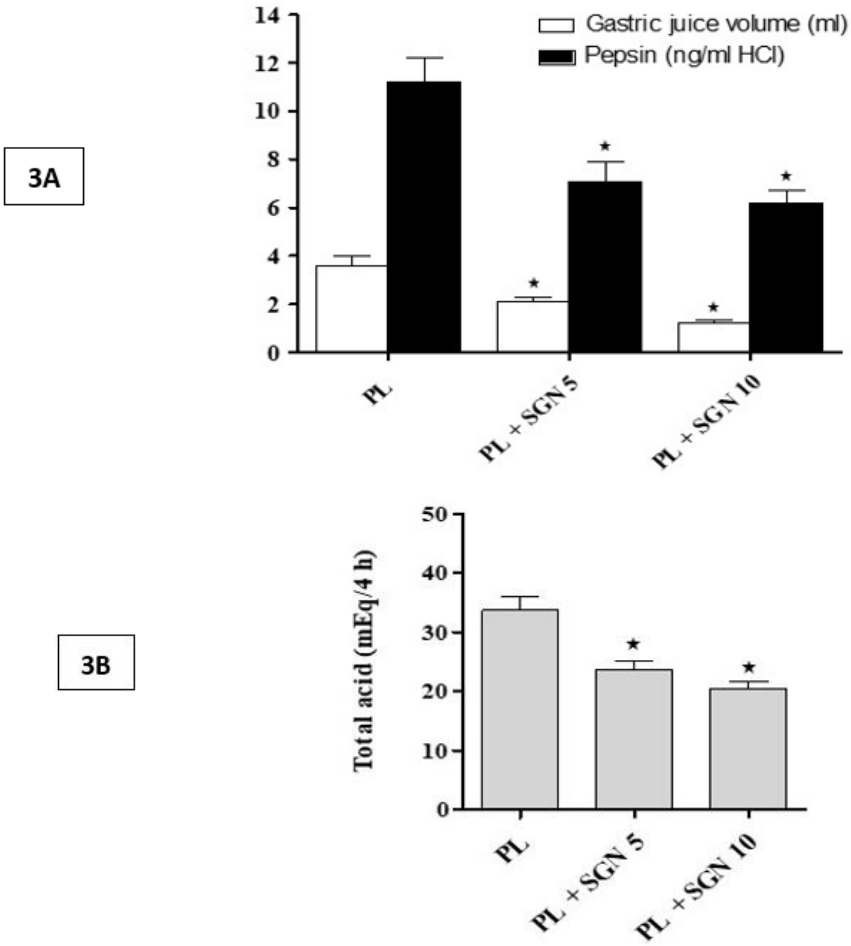


Fig. 3. Effects of siatagliptin (SGN) treatment (5 and 10 mg/kg) on: (A) volume of gastric secretion and pepsin activity; (B) total acid concentration in the pylorus-ligated (PL) rats. $^{*}P < 0.05$ vs. control group.

Histopathological results

Administration of a single dose of IND (50 mg/kg, p.o.) caused multiple ulcerative lesions, necrosis of gastric glands, and inflammatory cell infiltration in the gastric mucosa of rats. However, SGN treatment in both doses (5 and 10 mg/kg, p.o.) resulted in marked improvement in the histological picture of gastric mucosa with minimum lesions, preservation of gastric glands, and reduction of inflammatory cell infiltration (Fig. 4).

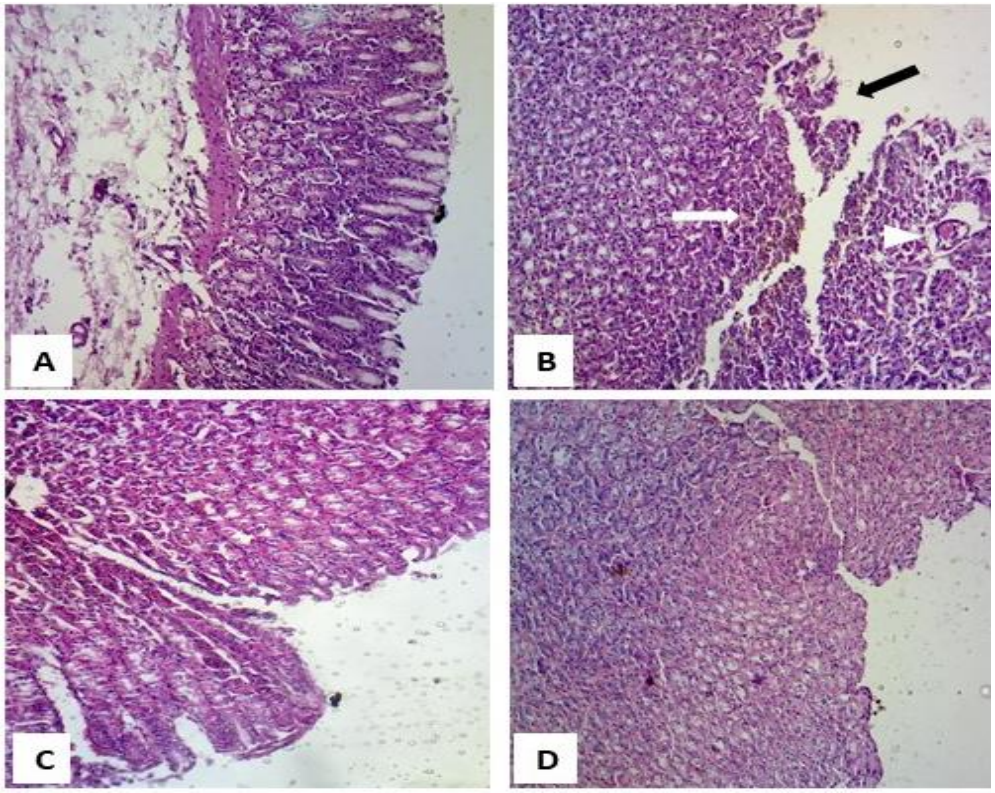


Fig. 4. Histopathological examination (H&E, 200×) of rat gastric mucosa from: (A) control rats showing normal gastric mucosa; (B) indomethacin (IND)-challenged rats showing multiple ulcerative lesions (black arrow), inflammatory cell infiltration (white arrow), and degenerated gastric glands (white head); (C and D) IND + sitagliptin (SGN) 5 and IND + SGN 10, respectively, showing marked improvement in the histological picture of gastric mucosa with minimum lesions, normal appearance of gastric glands, and reduction of inflammatory cell infiltration.

4. Discussion

Previous investigations, in accordance with the present one, showed oxidative stress, increased generation of ROS, depletion of endogenous antioxidants, and increased lipid peroxidation of biomembranes are implicated in development of NSAID-induced gastric erosions and ulcerations.[2] The current investigation showed that SGN treatment at both doses (5 and 10 mg/kg, p.o.) significantly inhibited the gastric mucosal lesions induced by IND in rats. SGN provided significant antioxidant effect evidenced by decreased MDA production and preservation of endogenous antioxidants, GSH and SOD. This can be related to the ability of SGN to suppress NADPH oxidase, which is the main source of ROS during oxidative stress [13]. Similarly, it was shown that SGN preserved the endogenous antioxidants, GSH and SOD, and inhibited the production of MDA [14].

Additionally, previous investigations revealed that oxidative stress up-regulated the inflammatory cascades, and increased the production of inflammatory cytokines, as TNF-α in IND-induced gastric ulceration [3,15]. Prior studies also demonstrated that SGN provided significant anti-inflammatory properties, and reduced the levels of inflammatory cytokines [16,17]. This is in accordance with the present results which demonstrated that SGN significantly reduced the inflammatory cytokine, TNF-α in the gastric mucosa of IND-challenged rats.

It is well-known that PGE_2 plays an important role as gastroprotective agent against erosion and ulceration. It reduces gastric acidity and pepsin, increases bicarbonate production, and enhances gastric mucosal blood flow [18]. Previous investigations, in accordance with the present one, showed that inhibition of PGE_2 production was responsible for NSAID-induced gastric injury [3,19]. The current study also revealed that SGN preserved PGE_2 level in the gastric mucosa of IND-challenged rats. This is most probably due to the antioxidant and anti-inflammatory effects of SGN which preserved the gastric defense mechanisms against ulcerogenic effect of IND.

Additionally, prior investigations showed that cell apoptosis is involved in NSAID-induced gastric mucosal ulcerogenesis [3,20]. Other studies also revealed that SGN ameliorated apoptotic cell death by inhibiting the generation of cleaved caspase-3, the executioner of cell apoptosis. This was related to the antioxidant and anti-inflammatory effects of SGN with the resultant reduction of the generation of ROS and inflammatory cytokines [4,21] This is consistent with the present results which showed that SGN inhibited cleaved caspase-3 production and prevented apoptosis gastric mucosal cells in IND-challenged rats.

Besides, studies on the pylorus-ligated rats demonstrated that SGN reduced gastric acid and pepsin production. This can be attributed to the preservative effect of SGN on PGE_2 level in gastric mucosa of pylorus-ligated rats. Moreover, histopathlogical examination confirmed the protective effect of SGN of the gastric mucosa as evidenced by reduction of the ulcerative lesions, and gastric gland necrosis.

5. Conclusion

The current study revealed that SGN significantly protected the gastric mucosa in rats challenged with IND. The gastroprotective effect of SGN can be attributed to its antioxidant, anti-inflammatory, and antiapoptotic properties.

Conflicts of interest

There are no conflicts of interest.

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