Research Article

Gastroprotective effect of sitagliptin in experimentally-induced peptic ulcer in rats

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Abstract
Sitagliptin one of the anti-diabetic drugs. We aimed to evaluate the gastroprotective effect of sitagliptin in indomethacin-induced peptic ulcer in rats. Adult male albino rats were divided to four groups; control, sitagliptin (SIT), indomethacin (IND) and IND+SIT group. In each group, ulcer index, gastric mucosal malondialdehyde (MDA), total nitrites (NOx) and Tumor necrosis factor-alpha (TNF-α) were determined. In IND+SIT group, ulcer index, MDA and TNF-α were reduced, as compared to IND group. At the same time, NOx was increased in IND+SIT group, as compared to IND group. In conclusion, sitagliptin showed gastroprotective effect in indomethacin induced peptic ulcer in rats mostly through anti-oxidant and anti-inflammatory actions.
Key words: Sitagliptin, indomethacin, peptic ulcer.

Introduction
One of the common gastrointestinal disorder is peptic ulcer which occurred as a consequence of increasing of aggressive and/or reduction in defensive factors of the gastric mucosa. Oxidative stress and inflammation are linked to aggravation of gastric mucosal damage and peptic ulcer occurrence [1,2].

One of the most commonly prescribed drugs in the world is Non-steroidal anti-inflammatory drugs (NSAIDs). Due to their anti-inflammatory, anti-platelet and analgesic effects, NSAIDs are used in either treatment or prophylaxis of osteoarthritis, collagen disease, rheumatoid arthritis, and ischemic cardiovascular or cerebrovascular disease[3,4].

Diabetes increases morbidity and mortality of gastric ulceration. Diabetes may also influence the outcome of complicated peptic ulcer disease, due to angiopathy, blurring of symptoms, and increased risk of sepsis[5]. Sitagliptin (SIT) is an inhibitor of dipeptidyl peptidase-4 commonly used in treating type II diabetes mellitus with antioxidant, anti-inflammatory and anti-apoptotic characters[6].
In the current study, we aimed to evaluate the possible gastroprotective effect of SIT in indomethacin-induced peptic ulcer.

Material and methods
Ethics
Handling, treatments, and scarification of rats were done according to the guidelines for the care of experimental animals. This was according to the Institutional Ethical Committee, Faculty of Medicine, Minia University, Egypt. This approval is in accordance with the NIH Guide for taking care and use of laboratory animals (Council No. 132:12/2018).

Animals
Male wistar rats weighing 150-200 gm were used in the present study. Rats were purchased from the National Research Center, Cairo, Egypt.

Chemicals
Sitagliptin was purchased from Multipharma, Egypt, indomethacin (IND) was purchased from Nile co. for pharmaceuticals. Tumor necrosis factor-alpha (TNF-α) was measured by ELISA kit (Elabscience, USA). All other chemicals were of analytical grade and were obtained from commercial sources.

Experimental design
Experimental Procedures
Animals were fed a standard diet of commercial rat chow and tap water (El-Nile Company,

Egypt) and left to accommodate to the environment for at least one week before the start of the experiments. Prior to the study, rats were fasted for 24 hours and to avoid variations due to diurnal rhythms of putative regulators of gastric functions, all experiments were performed at the same time of the day.

The animals were randomly divided into 4 experimental groups of 6 animals each. Group 1; control group, group 2; received SIT (5 mg/kg; i.p.)[7], group 3; received IND (40 mg/kg; i.p.)[7], group 4; received SIT one hour before IND administration.

The stomach was washed with ice-cold saline and scored for macroscopic gross mucosal lesions and stored at -80°C until used for assessment of biochemical parameters.

Assessment of gastric mucosal lesions

Ulcer index (UI) was used to show the severity of gastric mucosal lesions and graded as follows; 0 for no lesions; 1 for petechae; 2 for erosions less than 1 mm; 3 for erosions of 1-2 mm; 4 for erosions of 2-4 mm and 5 for erosions greater than 4 mm in length. The partial scores were then summated to obtain the UI of the animal examined. The UI for each group taken as the mean lesion score of all the rats in that group[9].

Preparation of Tissue Homogenates

Gastric mucosa was scraped and homogenized separately in potassium phosphate buffer 10 mM pH (7.4). The homogenates were centrifuged at 5000 rpm for 10 min at 4°C. The resulting supernatant was used for determination of Malondialdehyde (MDA) level, total nitrite/nitrate (NOx) and TNF-α.

Malondialdehyde, a measure of lipid peroxidation, was evaluated by a method that depends on the reaction between MDA with thiobarbituric acid[10]. Gastric mucosal NOx, the stable oxidation end products of nitric oxide, served as an index of nitric oxide level and was measured by reduction of nitrate into nitrite using activated cadmium granules, followed by color development with Griess reagent in acidic medium[11]. TNF-α were measured using ELISA kits according to the manufacturer's instructions.

Statistical analysis

Results were expressed as means ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by the Tukey post analysis test was used to analyze the results for statistically significant difference. p values less than 0.05 were considered significant. Graph Pad Prism was used for statistical calculations (version 5.03 for Windows, Graphpad Software, San Diego California USA, www.graphpad.com).

Results

Effect of sitagliptin on ulcer index.

There was no significant difference between SIT group and control group in UI. In IND+SIT group, there was a significant reduction in UI, as compared to IND group [figure1].

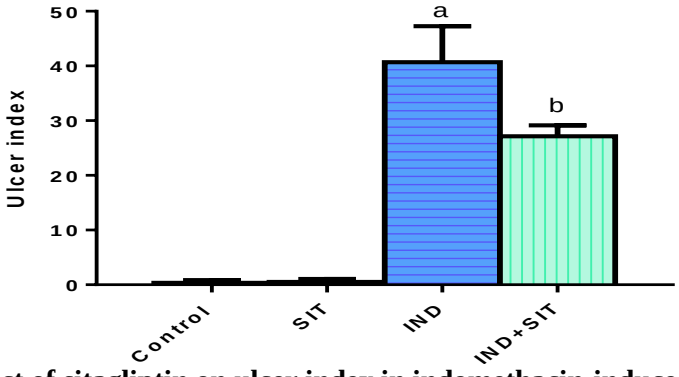

Figure (1): Effect of sitagliptin on ulcer index in indomethacin-induced peptic ulcer.

Data represent mean ± SD (n=6). SIT; sitagliptin, IND; indomethacin. [a]significance from control group, [b]significance from IND group. Significance is at $P < 0.05$.

Effect of sitagliptin on Malondialdehyde and Total Nitrites.

There was no significant difference between SIT group and control group in MDA and NOx. In IND+SIT group, there was a significant reduction in MDA and significant increase in NOx, as compared to IND group [table 1].

Table (1): Effect of sitagliptin on malondialdehyde and total nitrites in gastric mucosa in indomethacin-induced peptic ulcer

Group	Malondialdehyde (nmol/g tissue)	Total nitrites (nmol/g tissue)
Control	31.2 ± 6.68	174 ± 12.9
SIT	29.8 ± 6.57	171 ± 8.39
IND	65.2 ± 14.9[a]	119 ± 15.4[a]
SIT+IND	39.7 ± 8.28[b]	166 ± 23.4[b]

Data represent mean ± SD (n=6). SIT; sitagliptin, IND; indomethacin. [a]significance from control group, [b]significance from IND group. Significance is at $P < 0.05$.

Effect of sitagliptin on tumor necrosis factor-α.

There was no significant difference between SIT group and control group in TNF-α. In IND+SIT group, there was a significant reduction in TNF-α, as compared to IND group [table 2].

Table (2): Effect of sitagliptin on Tumor necrosis factor-α in gastric mucosa in indomethacin-induced peptic ulcer

Group	Tumor necrosis factor-α (pg/g tissue)
Control	15.4 ± 2.69
SIT	16.6 ± 4.51
IND	33.5 ± 5.53[a]
SIT+IND	25.5 ± 3.01[b]

Data represent mean ± SD (n=6). SIT; sitagliptin, IND; indomethacin. [a]significance from control group, [b]significance from IND group. Significance is at $P < 0.05$.

Discussion

Peptic ulcer has been identified as the main disease of the 21st century due to profound changes in life habits, mainly feeding habits[12]. There is a link between peptic ulcer morbidity and mortality and diabetes mellitus[5]. SIT is one of the commonly used antidiabetics commonly. It was also reported that it show a protective effects against different models of tissue injury[6,7].

The most common proposed mechanism for the ulcerogenic effect of IND is inhibition of COX pathway of arachidonic acid metabolism resulting in the inhibition of the gastric prostaglandins. Such reduction in Prostaglandins results in induction of oxidative stress and inflammation in gastric mucosa, which reduce mucosal resistance and increase aggressive factors such as acid and pepsin secretion[13,14,15]. The results of this study indicate that SIT displays an antiulcer effect as it significantly reduced IND-induced gastric ulcer index. SIT antagonized oxidative stress induced by IND, which evidenced by significant reduction in MDA level together with significant increase in total nitrites in gastric mucosa. It also antagonized inflammation which is evidenced by reduction in TNF-α. These findings are in agreement with previous studies, which

reported the anti-oxidative stress and anti-inflammatory actions of SIT in different models of cell injury[16,17].

In conclusion, SIT has a gastroprotective effect through antagonizing oxidative stress and inflammation induced by indomethacin in rats.

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