Original Article

The Role of Zileuton in Indomethacin-Induced Gastric Ulceration in Pyloric-Ligated Diabetic Rats

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ABSTRACT

Zileuton is an active inhibitor of 5-lipoxygenase, it also inhibits leukotrienes (LTB4, LTC4, LTD4, and LTE4) formation. It has antioxidant and anti-inflammatory properties which renders it an attractive candidate for protection against peptic ulcer. Therefore, this study was performed to assess the protective property of this agent against indomethacin (IND)-induced gastric ulceration in diabetic rats. A total of 36 adult male albino rats were used in the study and divided into 6 groups (6 rats each) into the following groups: normal control group, diabetic control group, diabetic IND group (single intraperitoneal injection of 30 mg/kg), diabetic IND/omeprazole-treated group (40 mg/kg/day), diabetic IND/zileuton-treated group (25 mg/kg/day), diabetic IND/omeprazole/zileuton-treated group. The treated drugs were administered 14 days before pyloric ligation and IND administration to the diabetic rats. Zileuton pretreatment significantly attenuated the gastric mucosal lesions induced by IND administration, decreased the total gastric acidity, and pepsin activity with marked attenuation of the gastric mucosal lipid peroxidation and serum tumor necrosis factor alpha. In addition, zileuton pretreatment significantly increased the activity of both catalase and superoxide dismutase, gastric mucosal levels of nitric oxide and the concentration of mucin in gastric juice in comparison to the diabetic indomethacin-treated rats. Furthermore, zileuton pretreatment had antiapoptotic effect as evident by immunological study of caspase 3. In conclusion, zileuton can be considered a potential therapeutic agent to protect against the major clinical challenge of gastric injury in diabetic rats.

Key Words: Diabetic rats; gastric ulceration; indomethacin; zileuton.

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1. INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder manifesting with chronic hyperglycemia resulting from insulin deficiency or defect in its action, or both. This causes several organs, mainly the kidneys, heart, nerves and blood vessels to be affected on the long run (Vador et al., 2012). DM is characterized by increased reactive oxygen species (ROS) and decreased antioxidant levels in the body (Hadi and Al Suwaidi, 2007), which may increase the prevalence of GIT symptoms (Bytzer et al., 2002), and make it prone to ulcerogenic agents as ethanol, ischemia/reperfusion, stress, and nonsteroidal anti-inflammatory drugs (NSAIDs) (Takehara et al., 1997; Suleyman et al., 2002; Brzozowska et al., 2004). Despite all this, low attention has been paid to the incidence rate of peptic ulcer in diabetes (Vador et al., 2012).

Peptic ulcer is a multifactorial disorder that is frequently seen nowadays. Gastric ulcer is an erosion in the wall of the GIT due to shedding of inflammatory necrotic tissue (Ejam et al., 2015). Gastric lesion is considered a result of disturbed balance between gastroprotective factors such as mucus, bicarbonate and prostaglandins, and gastro-destructive substances. Peptic ulcer is caused by stress, Helicobacter pylori bacteria, long term NSAIDs therapy, and hereditary factor (Fahmy et al., 2015).

Indomethacin (IND) and other NSAIDs cause impairment of the protective factors in humans and experimental animals. Their use is accompanied with a significant risk of hemorrhage, erosions, and perforation of gastric ulcers (Fukushima et al., 2014). The molecular basis for the gastric toxicity of NSAIDs is mainly due to reduction in prostaglandins synthesis due to their non-selective inhibition of cyclooxygenase (Laine et al., 2008). It has been reported that NSAIDs cause injurious effects on the gastric mucosa through ROS activation with consequent ROS-mediated oxidation of lipids, proteins, and DNA (Suzuki et al., 2017).
2011). Zileuton is an active inhibitor of 5-lipoxygenase, it also inhibits leukotrienes (LTB4, LTC4, LTD4, and LTE4) formation. It is an anti-inflammatory agent most commonly used in the treatment of bronchial asthma (Wenzel and Kamada, 1996). Among the inflammatory mediators, leukotrienes, mainly LTB4, is an activator factor and a potent chemotactic for leukocytes. Elevated levels of leukotrienes reflect the severity of the disease (Lewis and Austen, 1984). This study aimed to investigate the effect of zileuton on gastric ulcer and its role in gastroprotection in diabetic rats.

2. MATERIALS AND METHODS

2.1. Drugs:

Indomethacin, alloxan monohydrate were purchased from Sigma Company, (USA), omeprazole was purchased from Gulf pharmaceutical industries, UAE., zileuton was purchased from Abbott Laboratories pharmaceutical company, USA. All other chemicals were obtained from commercial sources and were of analytical grade.

2.2. Animals:

A total of 36 adult male Albino (Sprague dawley strain) rats, weighing 170-190 gm, were purchased from the National Research Center, Cairo, Egypt. They were accommodated to the atmosphere of the animal house (45± 5% humidity, 12 h lighting cycle and 25±2 °C temperature) for a whole week before the experiment and allowed free intake of water and standard rodent chow. Procedures involving animals' care and welfare followed the protocols of the Research Advisory Ethical Committee of Faculty of Medicine, Minia University, Egypt.

2.3. Experimental design:

2.3.1. Induction of Diabetes in Rats

Induction of diabetes in rats followed the method described by Lenzen, 2008. Rats were fasted overnight before a single intraperitoneal injection of alloxan (100 mg /Kg body weight). Alloxan monohydrate (2% solution) was freshly prepared by dissolving 100 mg alloxan in 5 ml saline. The used dose also matched the dose used in our pilot experiment. Three days later, fasting basal blood glucose level (BGL) of each animal was determined and only rats with BGL of 200 mg/100 ml or more were included in the study. Blood samples used for determination of BGL were collected from the rats' tail veins.

2.3.2. Determination of OGTT:

Two days before the day of sacrifice, an oral glucose tolerance test (OGTT) using 2 g/Kg of glucose solution was performed after a 12h fasting period. Blood glucose concentration from the tail vein was measured using the ACCU-CHEK Active blood glucose meter (Roche, Mannheim, Germany) at 0, 30, 60, 90 and 120 min (Whiting et al., 1990).

2.3.3. Animal grouping:

Two weeks after alloxan injection, BGL was remeasured and rats were divided into six groups (six rats in each group):

- Normal control group: Rats received 2 ml of distilled water orally and a single i.p. injection of 1% of Tween 80.
- Diabetic control group: A single I.P dose of 100mg/kg alloxan was given to rats to induce diabetes.
- Diabetic IND group: a single i.p dose of 30 mg/kg IND in 1% Tween 80 was given to the diabetic rats to induce gastric ulcer (Khatab et al., 2001).
- Diabetic IND /omeprazole- treated group: rats pretreated with 40 mg/kg omeprazole orally, two weeks before IND-induction in diabetic rats (Almasaudi et al., 2015).
- Diabetic IND /zileuton- treated group: rats pretreated with 25 mg/kg, zileuton, orally two weeks before IND-induction in diabetic rats (Chen et al., 2013).
- Diabetic IND/ omeprazole/ zileuton- treated group: rats pretreated with 40 mg/kg omeprazole and 25 mg/kg zileuton, orally two weeks before IND-induction in diabetic rats.

2.3.4. Pyloric ligation:

Pyloric ligation was done before IND administration to allow for collection of gastric juice. Rats were fasted for 24 hours and kept in mesh-based cages, to decrease faecal consumption. They were given water, freely, except for the last hour before the experiment (Bregonzio et al., 2003). Performing the experiment at a fixed time of the day excludes diurnal variations in gastric function regulation. a midline incision of the rat abdomen was done under ether anaesthesia. The pyloric sphincter was ligated with a silk ligature, to collect the gastric juice, trying to keep intact the blood supply. The incision was sutured and the animals recovered from light anaesthesia (Alumets et al., 1982).

2.4. Gastric mucosal lesions assessment:

Three hours after pyloric ligation, rats were sacrificed using diethyl ether. Their stomachs were removed, incised along the greater curvature, and collection of the gastric content was done. The stomachs were washed with ice-cold saline. Examination of the gastric mucosal lesions was done by the pathologist. Ulcer index (U.I.) was an expression of the gastric mucosal lesions (Robet al., 1968). The length of the lesions correlated to the severity factor. It was measured from 0 to 3 according to this score: 0;
means no petichae or erosion, 1; means only petichae or erosion <1 mm, 2; means lesion size is 1-5 mm or 3; means lesion >5 mm. The U.I. for each group was measured as the mean score of the lesion of all the rats in that group. The preventive index (P.I.) of a given drug was calculated according to the equation of Hano et al., 1975:

Preventive index (%) = UI Control – UI Treated/UI Control × 100

2.5. Gastric juice analysis:

Gastric juice from each rat was collected, centrifuged at 1000 rpm for 10 min and then we measured the volume of the supernatant. It was then used to measure pepsin activity, mucin and total acid concentrations:

2.5.1. Pepsin activity determination:

Pepsin activity is the major factor involved in gastric secretion proteolytic activity. It was determined by a modified spectrophotometric method (Sanyal et al., 1971). It was represented by the amount of liberated tyrosine in micro mole per 1 ml of gastric juice per minute using 1:100 diluted gastric juice and 2% bovine serum albumin in 0.01 NHCL as substrate.

2.5.2. Determination of total gastric acidity:

Total gastric acidity was determined according to the method described by Hara et al., 1991. Total acidity was determined by titrating 1.0 ml gastric juice with 0.01 N sodium hydroxide (NaOH) using phenolphthalein which serves as the indicator and was expressed as mEq/3h.

2.5.3. Mucin concentration determination:

Mucin concentration was determined according to the method described by Winzler, 1955. It depends on determination of the hexose component of mucin, based on the reaction of carbohydrate in concentrated sulfuric acid with orcinol to give a colored product measured colorimetrically.

2.6. Biochemical study:

- Malondialdehyde (MDA) level of gastric mucosa was measured according to the thiobarbituric acid method, previously described by Uchiyama and Miura 1978.
- NO (nitric oxide) content was measured as total nitrite/nitrate, which are the stable degradation products of nitric oxide; this was by using copperized cadmium for reduction of nitrate into nitrite, finally, a colour develops with Griess reagent in acidic medium (Sastry et al., 2002)
- Catalase (CAT) (Biodiagnostic, cairo, Egypt) activity was estimated based on the colorimetric method of Aebi, 1984.
- Serum tumor necrosis factor (TNF-α) was measured by enzyme linked rat TNF-α ELISA kit (Ray-Biotech, Inc., GA, USA) according to supplier’s instructions.
- SOD (superoxide dismutase) activity was estimated using SOD colorimetric kits (Biodiagnostic, cairo, Egypt).

2.7. Histological Examination:

The gastric Specimens were fixed in 10% neutral-buffered formalin and embedded in paraffin wax. Tissue sections 5-6μm thickness were obtained and deparaffinized. Some sections were stained with hematoxylin and eosin (H&E) (Bancroft and Gamble, 2008)

The immunohistochemical detection of caspase 3 expression was determined according to the manufacturer’s protocol (Lab Vision Corporation, USA). Sections were de-paraffinized with xylene, hydrated with ethanol, and then treated with 3% hydrogen peroxide for 30 min to inactivate endogenous peroxides. It was then washed in phosphate-buffered saline (PBS) solution. Perform heat mediated antigen retrieval with citrate buffer pH 6.0 for 20 min in microwave and then allow cooling. After rinsing in PBS, primary antibody was incubated overnight at 40°C using caspase-3 primary antibody (Polyclonal rabbit antibody), then washed with PBS before applying the biotinylated secondary antibody for 30 min and incubated with the streptavidin-biotin complex reagent for 30 min. A brown color was developed with 3, 3-diaminobenzidine tetra-hydrochloride for 5 min, then washed in distilled water, counterstained using haematoxylin, dehydrated, cleared in xylene, mounted, and covered slip was put. Each staining batch included both positive and negative control sections. In negative control, primary antibody was omitted and replaced by PBS (Côté et al., 1993).

Caspase-3 semi-quantitative scoring was done by determining immunoreactivity under light microscope magnification 40x; 200x for each group. The intensity of the staining was grouped as (0= negative), (1=weak), (2=moderate) and (3=strong). Staining extent was scored as 0 (0%), 1 (1–25%), 2 (26–50%), 3 (51–75%), and 4 (76–100%) according to the percentage of cells staining positive for active caspase 3. The sum of the intensity and extent of scores was calculated to estimate the final average staining scores (0–7). This scoring system was according to Kafa and co-workers, 2009. A positive control consists of one of our tissue specimens treated with Stauroporine to induce apoptosis.
2.8. Photography

We photographed slides using an Olympus digital camera. We used an Olympus (U.TV0.5XC-3) light microscopy. Images were processed using Adobe Photoshop.

2.9. Statistical analysis

Data were represented as means ± standard errors of the mean (SEM). Graph pad Prism 5 software was used to perform the statistical analysis and we used one-way analysis of variance (ANOVA) test to do the significant difference between different groups, followed by Tukey-Kramer post hoc test for multiple comparisons with a value of P ≤ 0.05 considered statistically significant.

3. RESULTS

3.1. Effect of omeprazole, zileuton either solely or in combination on OGTT:

Alloxan caused a significant increase in blood glucose level, compared to the normal control group. Zileuton, omeprazole, either solely or in combination caused significant decrease in blood glucose level in comparison to the diabetic control group at 0, 30, 60, 90 and 120 minutes showing evident improvement in OGTT (Figure 1).

3.2. Effect of omeprazole, zileuton either solely or in combination on ulcer index and preventive index

Diabetic control group showed significant increase in ulcer index, compared to the normal control group. IND administration induced a high ulcer index when compared to the normal and diabetic control group. Additionally, omeprazole, zileuton either solely or in combination showed significant protection against IND-induced gastric ulcer in diabetic rats (Table 1).

3.3. Effect of omeprazole, zileuton either solely or in combination on total acidity of the gastric secretion, pepsin activity and mucin concentration

Zileuton, omeprazole and their combination significantly attenuated the gastric mucosal lesions induced by IND administration, which was accompanied by significant reduction of the gastric total acidity, decrease in pepsin activity with significant increase in the gastric juice mucin concentration (Table 2).

3.4. Effect of omeprazole, zileuton either solely or in combination on tissue oxidative stress parameters (MDA, CAT, SOD and NO)

Indomethacin and diabetic control rats showed significant elevation in gastric MDA with reduction in gastric CAT, SOD and NO levels when compared to normal control group. Meanwhile, zileuton solely or in combination with omeprazole significantly improved these parameters when compared with diabetic control and diabetic IND- treated groups (Table 3) (Figure 2A).

3.5. Effect of omeprazole, zileuton either solely or in combination on serum TNFα

Zileuton, omeprazole and their combination significantly decreased the level of TNFα as compared to diabetic control and diabetic IND- treated groups (Figure 2B).

3.6. Effect of omeprazole, zileuton either solely or in combination on gastric mucosal histological changes:

Sections of the normal control group showed the normal appearance of the gastric mucosa with short pits lined by pale columnar mucus secreting cells leading into long glands which contain bright pink partial cell (Figure 3A). However, diabetic control group showed infiltration by neutrophil cells with necrosis and shedding of the superficial mucosal layer forming erosion with dilated congested blood vessels (Figure 3B). Examination of gastric mucosa of the diabetic IND-treated group showed shedding of the mucosa forming sharply demarcated ulcer with wide area of the hemorrhage and infiltration of acute inflammatory cells in the form of neutrophil in all layers of the gastric wall (Figure3C).

On the other hand, microscopic picture of either zileuton or omeprazole -treated groups showed mild degree of the inflammation and infiltration by acute inflammatory cells in the form of neutrophil and macrophage as compared with IND- treated group (Figure 3D, 3E). In addition, zileuton in combination with omeprazole-treated group appeared near normal control group with non-significant minimal inflammatory infiltration (Figure 3F).

3.7. Effect of omeprazole, zileuton either solely or in combination on gastric mucosal caspase 3 expression

The caspase 3 immunohistochemical-stained sections of the control group showed negative expression in gastric mucosa (Figure 4A). Meanwhile diabetic control group showed mild to moderate expression (Figure 4B). In the IND -treated group, moderate to strong expression was noticed in gastric mucosa (Figure 4C). The caspase 3 immunohistochemical- stained sections of zileuton, omeprazole and their combination-treated groups showed mild expression (Figure 4D, 4E, 4F).
Table (1): Effect of omeprazole, zileuton either solely or in combination on ulcer index and preventive index:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcer index (mm)</th>
<th>Preventive index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control group</td>
<td>0.60 ± 0.42</td>
<td>97.2%</td>
</tr>
<tr>
<td>Diabetic control group</td>
<td>10.60 ± 0.67a</td>
<td>50.9%</td>
</tr>
<tr>
<td>Diabetic IND group</td>
<td>21.60 ± 0.51ab</td>
<td>0</td>
</tr>
<tr>
<td>Diabetic IND/omeprazole- treated group</td>
<td>1.4± 0.24bc</td>
<td>93.5%</td>
</tr>
<tr>
<td>Diabetic IND/zileuton -treated group</td>
<td>2.1 ± 0.40bc</td>
<td>90.3%</td>
</tr>
<tr>
<td>Diabetic IND/omeprazole/zileuton -treated group</td>
<td>0.90 ± 0.42bc</td>
<td>95.8%</td>
</tr>
</tbody>
</table>

Results represent the mean ± S.E. (n= 6).  
*significant difference from normal control group (P < 0.05),  
*significant difference from diabetic control group (P < 0.05),  
*significant difference from diabetic IND group (P < 0.05); (IND: Indomethacin).

Table (2): Effect of omeprazole, zileuton either solely or in combination on total acidity of the gastric secretion, pepsin activity and mucin content:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total acidity (mEq/3h)</th>
<th>Pepsin activity (μg/ml tyrosine)</th>
<th>Mucin content (mg % hexose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control group</td>
<td>48.67 ± 3.32</td>
<td>119.50 ± 4.26</td>
<td>105.17 ± 2.63</td>
</tr>
<tr>
<td>Diabetic control group</td>
<td>70.09± 1.98a</td>
<td>167.45± 2.09a</td>
<td>76.34 ± 1.89a</td>
</tr>
<tr>
<td>Diabetic IND group</td>
<td>96.00 ± 3.37ab</td>
<td>194.50 ± 4.31ab</td>
<td>44.33 ± 3.19ab</td>
</tr>
<tr>
<td>Diabetic IND/omeprazole- treated group</td>
<td>55.67± 4.16bc</td>
<td>128.17 ± 4.39bc</td>
<td>92.33± 2.46bc</td>
</tr>
<tr>
<td>Diabetic IND/zileuton -treated group</td>
<td>60.67 ± 3.42abc</td>
<td>143.67 ± 6.20abc</td>
<td>89.67 ± 3.18abc</td>
</tr>
<tr>
<td>Diabetic IND/omeprazole/zileuton -treated group</td>
<td>49.00 ± 4.40bc</td>
<td>130.71 ± 5.67bc</td>
<td>97.5± 5.37bc</td>
</tr>
</tbody>
</table>

Results represent the mean ± S.E. (n= 6).  
*significant difference from normal control group (P<0.05),  
*significant difference from diabetic control group (P<0.05),  
*significant difference from diabetic IND group (P<0.05); (IND: Indomethacin).

Table (3): Effect of omeprazole, zileuton either solely or in combination on gastric mucosal MDA, CAT, SOD activities:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MDA (nmol/gwet tissue)</th>
<th>CAT (nmol H₂O₂ consumed/g tissue/min)</th>
<th>SOD (μg /mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control group</td>
<td>22.83 ± 2.10</td>
<td>53.33 ± 3.13</td>
<td>182.67± 3.94</td>
</tr>
<tr>
<td>Diabetic control group</td>
<td>74.89±3.09a</td>
<td>31.56± 1.98a</td>
<td>101.05± 4.87a</td>
</tr>
<tr>
<td>Diabetic IND group</td>
<td>88.67 ± 5.46a</td>
<td>28.50 ± 2.70a</td>
<td>90.00± 8.56a</td>
</tr>
<tr>
<td>Diabetic IND/omeprazole- treated group</td>
<td>33.00 ± 2.85bc</td>
<td>49.67± 3.04bc</td>
<td>160.09 ± 13.17bc</td>
</tr>
<tr>
<td>Diabetic IND/zileuton -treated group</td>
<td>34.67 ± 2.16bc</td>
<td>43.16 ± 3.77bc</td>
<td>168.33 ± 6.85bc</td>
</tr>
<tr>
<td>Diabetic IND/omeprazole/zileuton -treated group</td>
<td>29.33 ± 2.37bc</td>
<td>50.62 ± 3.05bc</td>
<td>173.33 ±6.40bc</td>
</tr>
</tbody>
</table>

Results represent the mean ± S.E. (n= 6).  
*significant difference from normal control group (P<0.05),  
*significant difference from diabetic control group (P<0.05),  
*significant difference from diabetic IND group (P<0.05); IND: Indomethacin; MDA: malondialdehyde; CAT: catalase; SOD: superoxide dismutase.
The Role of Zileuton in Indomethacin-Induced Gastric Ulceration in Pyloric-Ligated Diabetic Rats

Figure 1: Effect of omeprazole, zileuton either solely or in combination on OGTT in diabetic rats.
Results represent the mean ± S.E. (n= 6). *Significant difference from normal control group (P < 0.05), †significant difference from diabetic control group (P < 0.05). ‡significant difference from diabetic IND group (P < 0.05); (IND: Indomethacin); OGTT: oral glucose tolerance test.
Diabetic control group and diabetic IND group are significant from normal control group; diabetic IND/omeprazole, diabetic IND/zileuton and diabetic IND/omeprazole/zileuton-treated groups are significant from diabetic control group and diabetic IND group.

Figure 2 A: Effect of omeprazole, zileuton either solely or in combination on gastric mucosal NO in indomethacin-induced gastric ulceration in diabetic rats.
Results represent the mean ± S.E. (n= 6).
*significant difference from normal control group (P<0.05), †significant difference from diabetic control group (P < 0.05), ‡significant difference from diabetic IND group (P < 0.05); (IND: Indomethacin); NO: nitric oxide.
Figure 2B: Effect of omeprazole, zileuton either solely or in combination on serum TNFα in indomethacin –induced gastric ulceration in diabetic rats.
Results represent the mean ± S.E. (n = 6). a significant difference from normal control group (P < 0.05), b significant difference from diabetic control group (P < 0.05), c significant difference from diabetic IND group (P < 0.05); (IND: Indomethacin); TNFα: tumour necrosis factor alpha

Figure 3: A photomicrograph of gastric tissue of: Normal control group (A), Diabetic control group (B), Diabetic IND group (C), Diabetic IND / omeprazole group (D), Diabetic IND / zileuton group (E), Diabetic IND / omeprazole/zileuton group (F) stained by H&E showing (A): Normal appearance of the gastric mucosa with short pits lining by pale columnar mucus secreting cells leading into long glands which contain bright pink partial cell. (B): Infiltration by neutrophil cells with necrosis and shedding of the superficial mucosal layer forming erosion with dilated congested blood vessels. (C): Shedding of the mucosa forming sharply demarcated ulcer with wide area of hemorrhage and infiltration by acute inflammatory cells in the form of neutrophil in all layers of the gastric wall. (D, E): Mild degree of inflammation and infiltration by acute inflammatory cells in the form of neutrophil and macrophage. (F): Near normal control group with non significant minimal inflammatory infiltration. (IND: Indomethacin) (Magnification x40;x200 for each group).
The Role of Zileuton in Indomethacin-Induced Gastric Ulceration in Pyloric-Ligated Diabetic Rats

**Figure 4:** A photomicrograph of gastric tissue of: Normal control group (A), Diabetic control group (B), Diabetic IND group (C), Diabetic IND / omeprazole group (D), Diabetic IND / zileuton group (E), Diabetic IND / omeprazole/zileuton group (F) stained for caspase 3 showed (A); negative expression in gastric mucosa. (B); mild to moderate expression. (C); moderate to strong expression. (D, E, F); mild expression in gastric mucosa. (IND: Indomethacin) (Magnification x40; x200 for each group).

4. DISCUSSION

D.M. is widely spread metabolic disease with numerous common complications like intestinal enteropathy and gastric paresis. Much attention should be paid to the incidence of gastric ulceration in diabetic patients. These ulcers may be severe leading to gastrointestinal bleeding (Pradeepkumar et al., 2011). Peptic ulcer is considered as an inflammatory debilitating illness characterized by a high rate of recurrence which creates a load on the patient himself and on the economy of the society (Russo and Brutti, 2007).

The aim of our study was to evaluate the gastroprotective effect of each of omeprazole and zileuton, alone and combined against indomethacin-induced gastric ulcer in diabetic rats.

In our study, A model of gastric ulcer was induced in rats by intraperitoneal injection of IND. Our study showed that IND caused remarkable increase in total gastric acidity, pepsin activity, and ulcer index. This may be attributed to its effect on increased ROS production; stimulation of lipid peroxidation; induction of apoptotic cascade and inhibition of prostaglandin synthesis following the increased free radicals, which harm the cellular constituent and impair prostaglandin production (Bech et al., 2000). Decreased prostaglandin synthesis causes ulcer deterioration and perforation due to acid production and impaired gastroprotection (Inas et al., 2011; Nasadyuk and Sklyarov, 2013).

In our results, IND caused elevation of MDA levels, and decreased enzymatic activity of catalase and SOD enzymes. Catalase, SOD and other anti-oxidant...
enzymes are affected by the overproduction of free radicals (El-Missiry et al., 2001). This is because the rates of free radical formation and scavenging capacity are in equilibrium. Any disproportion between them results in oxidative stress and in turn cellular functions disruption (Chattopadhyay et al., 2006). Thus, any agent which prevents the oxygen free radical damage should act as a mucosal shield (Hawkins and Hanks, 2000).

It was found also in our results that IND caused decreased mucin concentrations. Similarly, Naito et al., 1995 reported that IND decreased the mucin secretion, causing back diffusion of hydrogen ions and decreasing the protective capacity of the mucosal membrane against physical damage. Mucin is the gastric mucus coat thought to be important in protecting gastric mucosa against ulcerogens and in facilitating the repair of the damaged gastric epithelium. Thus, by several mechanisms; it acts as a barrier protecting against hydrogen ions back diffusion. During peristalsis, it decreases the gastric wall friction (Sevak et al., 2002). On the other hand, mucus acts as an antioxidant reducing the mucosal injurious effect of oxygen free radicals (Repetto and Liesuy, 2002). The protective effect of the mucus arises from its gelatinous consistency and its thickness covering the mucosal shell (Penissi and Pizzi, 1999).

Data of the current study showed that IND decreased gastric mucosal NO level significantly, when compared to the control group. This finding was in accordance with that of Lanas et al., 2000 and Cadirci et al., 2007. Nitric oxide is an endogenous protective factor through its adjustment of alkaline secretion, gastric motility and preservation of mucus content (Samini et al., 2002). Besides, NO can protect against lipid peroxide products (Hogg and Kalyanaraman, 1999), modulate gastric acid levels, and maintain blood flow in gastrointestinal tissues (Martín et al., 2001). Additionally, it has a role in prostaglandin synthesis and therefore can protect gastric tissues from NSAID damage (Salvemini et al., 1993). A mutual interaction was found to exist between NOS and cyclooxygenase (COX) enzymes. NO donors were shown to enhance COX activity whereas NOS inhibitors blocked PGE2 production. This may explain the decrease in mucosal NO by IND administration as observed in our study.

In this study, IND significantly increased serum TNF-α, when compared to the control group. This coincided with the finding of Katary and Salahuddin, 2017 who reported that gastric tissue levels of proinflammatory cytokines such TNF-α, IL-1β and IL-6 increased as well as gastric level of anti-inflammatory cytokine IL-10 decreased by IND. On the other hand, the up-regulatory action of IND to serum TNF-α may be responsible for the decrease in mucosal NO. This finding was in agreement with the reported results of Bauer et al., 1997 who recorded that TNF-α strongly inhibited constitutive NO, which mostly performed its protective effect on the stomach through cytokine production modulation. Tumour necrosis factor is a proinflammatory cytokine released by macrophages whose levels rise in ulcerative stress (Hamaguchi et al., 2001). It increases gastric mucosal infiltration by neutrophils (Wei et al., 2003) and the expression of inducible nitric oxide (Calatayud et al., 2001). TNF-α levels are directly proportionate to incidence of gastric ulceration (Sugimoto et al., 2007) and vice versa (Kwiecien et al., 2002).

In this study, omeprazole was used as a standard drug for treatment of ulcer in diabetic rats as it is a drug of choice in treatment of peptic ulcer. Our study proved that omeprazole treatment showed protection against indomethacin- induced gastric ulcer in diabetic rats, as seen from its effect on decreasing ulcer index, significant reduction in the gastric total acidity, and pepsin activity with significant increase in the gastric mucin concentration. Moreover, it significantly improved tissue oxidative stress parameters (MDA, CAT, SOD, NO), compared to the non-treated ulcerated -diabetic rats. And significantly decreased the level of serum TNF. These results were coincident with results of other published studies which proved its gastroprotective effect (Ketuly et al., 2013; Sidahmed et al., 2013; Almasaudi et al., 2015).

Our current data proved that zileuton improved the ulcer index, gastric juice total acidity and pepsin activity, improved mucin concentration, oxidative stress, NO and TNF-α significantly in ulcerated diabetic rats. This was most probably related to its antioxidant and anti-inflammatory effects. The current study was in line with Hadi et al., 2013 who reported that zileuton retarded the progression of atherosclerosis via down regulation of the inflammatory and oxidative pathways. This was also similar to the results of Isikdemir et al., 2014 who found that zileuton treatment prevented the augmentation in MDA levels in a torsion detorsion injury model in rats. Similarly,

Alabbassi, 2015 showed that zileuton reduced the levels of interleukin-1β and TNF-α. This was reported to decrease oxidative stress in amiodarone induced pulmonary fibrosis in rats. Moreover, Genovese et al., 2008 stated that treatment of mice with zileuton reduced the spinal cord inflammation and tissue injury, neutrophil infiltration, TNF-α. On the other hand, zileuton decreased the activity of NF-kB and reduced the expression of iNOS. Also, NF-kB regulated the expression of iNOS and other inflammatory mediators, attenuation of iNOS expression and NO production demonstrated a protective role (Tu et al., 2010).
In our findings, both omeprazole and zileuton combined together succeeded to achieve a gastroprotective effect against IND-induced ulcer in diabetic rats as shown from decreased gastric acidity, reduced pepsin activity, increased mucin concentration, decreased ulcer index, improved oxidative parameters, reduced TNF-α levels, and amelioration of the histological findings towards normal.

Histological studies of the gastric mucosa revealed that IND induced a mucosal ulceration, leukocytic infiltration, congested blood vessels, necrotic epithelium and denuded basal lamina. This effect on mucosal oxidative stress and histological derangement was in accordance with the reports of Valcheva-Kuzmanova et al., 2007. Zileuton either alone or in combination with omeprazole, had protective effect against IND-induced inflammatory infiltration and congestion at the ulcer sites. Its gastroprotective potential is most probably through the scavenging of the free radicals, inhibition of the lipid peroxidation and increased prostaglandin synthesis as previously stated by Hadi et al., 2013.

Gastric ulcer healing is a complex process, which involves cell proliferation and apoptosis (Sánchez-Fidalgo et al., 2004). We examined caspase-3 activation, which is taken as an index of apoptotic cell death, in ulcerated tissues. In IND-treated group, there was an increase in caspase-3 activation. However, either zileuton or omeprazole pretreatment or their combination in ulcerated-diabetic rats showed reduction in the caspase-3 expression. This was coincident with the results of Shi et al., 2013 who suggested that zileuton reduced brain damage and neuronal apoptosis by inhibition of caspase-1 and the regulation of caspase-3.

What seemed interesting was that zileuton, either solely or in combination with omeprazole also caused significant decrease in blood glucose level as shown by the results of OGTT, in comparison to the diabetic control group which showed a significant increase in blood glucose level. May be the reason behind this was its anti-oxidant and anti-inflammatory effects which may have helped in amelioration of blood glucose level.

5. CONCLUSION

Zileuton proved to have a gastroprotective effect against indomethacin-induced gastric ulcer in diabetic rats. The gastroprotective role of zileuton may be attributed to its enhancement of the gastric mucosal barrier and decrease in acid secretory parameters. In addition, it was found to increase the mucosal nitrite level, anti-inflammatory, antioxidant and anti-apoptotic activities. Zileuton may be of future benefit in peptic ulcer diseases which proved to be more prevalent in diabetic patients. Moreover, Further studies can go into the details of the effect of zileuton on lowering blood glucose level and study the exact mechanisms behind these findings.

Conflict of Interests

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