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Losartan versus Enalapril in the Protection of the Gastric Mucosa against Aspirin-Induced Gastric Mucosal Injury in Rats

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Abstract Different mechanisms have been suggested for the development of nonsteroidal anti-inflammatory drugs (NSAIDs) induced gastropathy. Angiotensin receptor blockers (ARBs) and angiotensin-converting enzyme inhibitors (ACEIs) have been suggested to have gastroprotective effects, sothe present work aims to elucidate the protective effect of the renin-angiotensin system (RAS) inhibitors through their effect on prostaglandins (PGs) and oxidative stress against peptic ulcer induced by aspirin in rats and to compare the efficacy of ACEIs and ARBs in the treatment of peptic ulcer. Thirty-six adult male Wistar rats weighing 180-200 g were randomly divided into 6 groups, 6 animals each. Groups 1, 2, and 3 were received saline (normal control), losartan (3mg/kg/day) and enalapril (10 mg/kg/day) i.p respectively for 4 weeks. Groups 4, 5, and 6 were pretreated with saline (aspirin group), losartan (3mg/kg/day) and enalapril (10 mg/kg/day) i.p respectively for 4 weeks duration. On 29th day, rats of group 4, 5 and 6 were submitted to gastric ulcer by single oral administration of 300 mg/kg aspirin then animals of all groups were sacrificed, stomachs were excised for gross and microscopic examination and determination of the mucosal levels of prostaglandin E_2 (PGE_2), superoxide dismutase (SOD), nitric oxide (NO) and catalase (CAT).Treatment of rats with aspirin produced a significant decrease in gastric PGE_2, SOD, NO, and CAT levels and produced deep gastric ulcer compared to normal control group. Pretreatment of rats with losartan or enalapril decreased the aspirin-induced alterations in gastric PGE_2.SOD and NO levels, but only losartan caused a significant increase in CAT activity while enalapril caused an insignificant increase. Also, pretreatment with losartan or enalapril ameliorated the severe deep gastric ulcer induced by aspirin to shallow erosions and inflamed gastric mucosa compared to changes in the aspirin group.In conclusions, the prophylactic use of losartan and enalapril ameliorated aspirin-induced gastric ulcer, but losartan has better influence as it has an additional effect on CAT.

Keywords: *losartan, enalapril, aspirin, gastric ulcer, PGE_2, SOD, catalase*

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1. Introduction

Angiotensin II (ANG II), the main effector of the renin-angiotensin system (RAS), is generated from the precursor angiotensinogen by the actions of renin, angiotensin-converting enzyme (ACE), chymase and various peptidases [1]. ANG II not only regulates blood pressure through its action on vascular tone in resistance arteries and body fluid homeostasis [2] but also constricts the gastric vasculature through angiotensin$_1$ (AT1) receptor stimulation [3]. In addition, ANG II generates reactive oxygen species (ROS) with cellular damage and inflammation [4]. Oxidative stress with the generation of (ROS), mucosal vasoconstriction, and proinflammatory effects of ANG II could contribute to the production of gastric ulcers [5].

Non-steroidal anti-inflammatory drugs (NSAIDs), with their broad analgesic, anti-inflammatory and antipyretic effects are among the most frequently used drugs, approximately 30 million people worldwide are prescribed NSAIDs daily [6]. Gastroduodenal ulceration and bleeding are the major limitations to the use of NSAIDs. These drugs as aspirin can cause damage to the gastroduodenal mucosa via several mechanisms, including the topical irritant effect of these drugs on the epithelium, suppression of gastric PG synthesis, and reduction of gastric mucosal blood flow [7].

Inhibitorsof RAS include angiotensin converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs). Possible association between peptic ulcer and ACEIs and ARBs through their effects on ANG II is proved but their involvement in the mechanism of the protection of gastric mucosa has not been extensively studied [8].So, this study was carried out in an attempt to find a new method to minimize gastric mucosal damage due to aspirin. We investigate the effects of enalapril, as one ofthe ACEIs, and losartan, as one of the ARBs, on the protection of gastric lesions induced by aspirin through its

effects on the levels of prostaglandin E_2 (PGE_2), superoxide dismutase (SOD), nitric oxide (NO) and catalase (CAT) and to compare the efficacy of both drugs.

2. Materials and Methods

2.1. Drugs and Chemicals

Losartan potassium salt powder, enalapril maleate salt powder and kits for determination of PGE_2 level by enzyme-linked immunosorbent assay (ELISA) were obtained from Sigma-Aldrich (USA). Kits for determination of antioxidant parameters: (SOD, NO, CAT) were obtained from a Biodiagnostic company, Egypt.

2.2. Animals

Thirty-six male adult Wistar rats weighing 180-200g have been used. Animals were purchased from the animal house, Faculty of Medicine, Assiut University Egypt. The animals were kept at standard housing place, with room temperature being maintained at 22-24 °C. They were fed on a commercial pellet diet and kept under 12 hours light / dark cycle. Animals were given a free access to food and water. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the Sohag University.

2.3. Experimental Design

The rats were fasted for 24 hours prior to the experiment in mesh-bottomed cages to minimize coprophagia. The animals had free access to water except for the last hour before the experiment. Rats were divided into 6 groups, 6 animals each, group 1(normal control): rats were treated with 2 ml/kg/day saline i.p for 4 weeks. Group 2: rats were treated with 3 mg/kg/day losartan i.p dissolved in saline for 4 weeks [9]. Group 3: rats were treated with 10 mg/kg/day enalaprili.p dissolved in saline for 4 weeks [10]. Groups 4, 5, and 6: rats were pretreated with 2 ml/kg/day saline, 3 mg/kg/day losartan, and 10 mg/kg/day enalapril respectively all i.p. for 4 weeks duration. At the end of experiment rats of groups 4, 5, and 6 were treated with 300 mg/kg aspirin orally by gastric tube suspended in 3 ml of 1% carboxymethyl cellulose as a single dose for induction of gastric ulcer [11]. 3 hours after aspirin treatment, animals were sacrificed by decapitation.

2.4. Sample Collection

Immediately after scarification, the animals of all groups were dissected. The stomachs were removed and opened along the greater curvature; the lumen was rinsed with saline. The stomach tissues were divided into two parts and weighted. First part at which gastric mucosa scrapped, homogenized in 2 ml normal saline containing 0.1 M dithiothreitol, for determination of PGE_2 level in gastric tissues, the second part was homogenized in 2ml cold potassium phosphate buffer (0.05 M, pH 7.4) for measurement of SOD and CAT enzyme activities and NO level. All samples were centrifuged at 2000 rpm for 10 minutes at 4°C. the supernatant was kept at -80°C for subsequent measurement.

2.5. Histopathological Examination

Specimens of the stomach were examined macroscopically for the presence of gross changes, photographed by a digital camera (Fujifilm A100 China). Gastric tissue samples were fixed in 10% formalin, embedded in paraffin wax, sectioned and stained with hematoxylin and eosin for histopathological examination using light microscopy.

2.6. Biochemical Analysis

2.6.1. Determination of Gastric Prostaglandin E_2 (PGE_2)

This assay employed the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with a goat-anti-rabbit antibody. Standards or samples are added to the appropriate microtiter plate wells with an antibody specific for PGE_2 and Horseradish Peroxidase (HRP) conjugated PGE_2 and incubated, then substrate solutions are added to each well. The reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm [12].

2.6.2. Determination of Gastric Superoxide Dismutase (SOD) Enzymes Activities

The activity of SOD in gastric tissue homogenate was determined by a colorimetric method using commercially available kits. The method was described by Nishikimi et al. [13]. This assay relies on the ability of the enzyme to inhibit the phenazinemethosulphate-mediated reduction of nitrobluetetrazolium (NBT) dye. The change in the absorbance was measured at 560 nm for control and sample at 25°C. SOD activity was expressed in U/g tissues.

2.6.3. Determination of Gastric Catalase (CAT) Enzymes Activities

The enzyme activity was determined by a colorimetric method using commercially available kits, as described by Aebi [14]. This assay relies on the reaction of CAT with a known quantity of H_2O_2. The reaction is stopped after exactly one minute with CAT inhibitor. In the presence of peroxidase, the remaining H_2O_2 reacts with 3, 5-Dichloro-2-hydroxybenzene sulfonic acid (DHBS) and 4-Amino-phenazone (AAP) to form a chromophore. The change in the absorbance was measured at 510 nm. CAT activity was expressed in U/g tissues.

2.6.4. Determination of Gastric Nitric Oxide (NO) Level

The level of NO was determined by a colorimetric method using commercially available kits according to the method of Montgomery and Dymock [15]. This assay depends on that, in acid medium and in the presence of nitrite the formed nitrous acid diazotize sulphanilamide the product is coupled with N-(1-naphthyl) ethylenediamine. The resulting azo dye has a bright reddish- purple color which can be measured at 540 nm. The level of NO was expressed in nmol/g tissue.

2.7. Statistical Analysis

Data are expressed as mean± S.E.M., with a value of P< 0.05 considered statistically significant. Statistical evaluation was performed by one-way analysis of variance (ANOVA) followed by post hoc Tukey testfor multiple comparisons of means. All analysis was performed with SPSS software package (version 17).

3. Results

3.1. Effect of Losartan or Enalapril on Gastric Mucosa and on the Levels of Gastric mucosal PGE_2, SOD, CAT and NO in Male Wistar Rats

As shown in Figure (1,2, and 4) administration of losartan in a dose of 3mg/kg/day orenalapril in a dose of 10mg/kg/day for 4 weeks duration led to significant elevation ($p<0.05$) in PGE_2, SOD, and NO levels compared to control groups. However, only losartan treatment led to significant elevation ($p<0.05$) in CAT activity but enalapril caused an insignificant alteration in CAT activity compared to control group (Figure 3).

The macroscopic picture showed that saline, losartan, and enalapril administration produced no pathological changes in gastric mucosa and the incidence of ulceration was zero% (Table 1 and Figure 5A, B, and C).

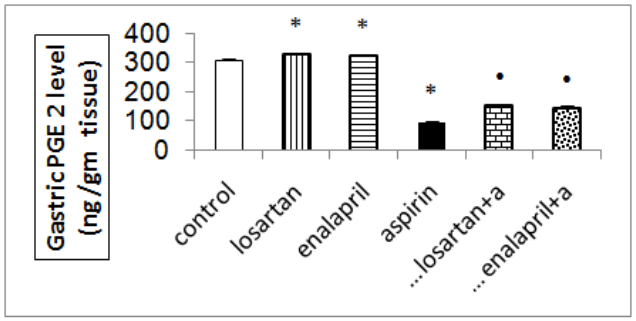

Figure 1. Effect of i.p. injection of losartan (3mg/kg/day)orenalapril (10 mg/kg/day) for 4 weeks duration on mucosal prostaglandin E_2 (PGE_2) level (ng/g wet tissue) in aspirin-induced gastric ulcer in rats. n=6 rats in each group. * Significant difference at P< 0.05 compared to control group.● Significant difference at P< 0.05 compared toaspirin group

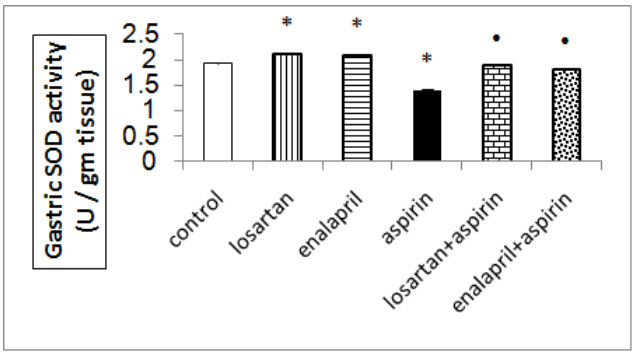

Figure 2. Effect of i.p. injection of losartan (3mg/kg/day)or enalapril (10 mg/kg/day) for 4 weeks duration on mucosal superoxide dismutase (SOD) activity (U/g wet tissue) in aspirin- induced gastric ulcer in rats. n= 6 rats in each group. * Significant difference at P< 0.05 compared to control group.● Significant difference at P< 0.05 compared to aspirin group

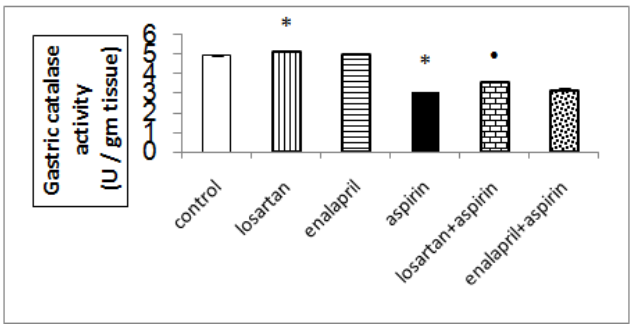

Figure 3. Effect of i.p. injection of losartan (3mg/kg/day)or enalapril (10 mg/kg/day) for 4 weeks duration on mucosal catalase (CAT) activity (U/g wet tissue) in aspirin-induced gastric ulcer in rats. n=6 rats in each group. * Significant difference at P< 0.05 compared to control group.● Significant difference at P< 0.05 compared to aspirin group

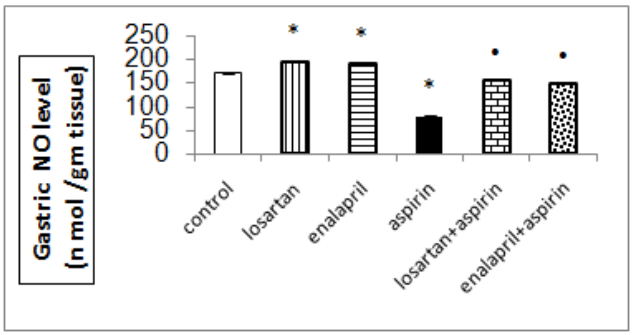

Figure 4. Effect of i.p. injection of losartan (3mg/kg/day)orenalapril (10 mg/kg/day) for 4 weeks duration on mucosal nitric oxide (NO) level (nmol/g wet tissue) in aspirin- induced gastric ulcer in rats.n=6 rats in each group. * Significant difference at P< 0.05 compared to control group.● Significant difference at P< 0.05 compared toaspirin group

3.2. Effect of Aspirin on Gastric Mucosa and on the Levels of Gastric Mucosal PGE_2, SOD, CAT and NO in Male Wistar Rats

Administration of aspirin once in a dose of 300 mg /kg led to significant reduction ($p<0.05$) in all measured gastric mucosal parameters; PGE_2,SOD, CAT and NO concentrations compared to control groups, (Figure 1, Figure 2, Figure 3 and Figure 4). The macroscopic picture showed that aspirin produced ulceration surrounded by an area of severe inflammation in gastric mucosa and the incidence of ulceration was increased to reach 83% compared to control group (Table 1 and Figure 5 D).

3.3. Effect of Pretreatment with Losartan or Enalapril on Gastric Mucosa and on the Levels of Gastric Mucosal PGE_2, SOD, CAT and NO in Aspirin- Induced Gastric Ulcer in Male Wistar Rats

The pretreatment of experimental animals with 3mg/kg/day losartan or10 mg/kg/day enalaprili.p for 4 weeks duration before induction of ulcer by single dose of 300 mg/kg aspirin, led to significant increase ($P<0.05$) not only in mucosal $PGE_2$and in gastric mucosal SOD activitybut also in NO level all compared to aspirin ulcer group (Figure 1, Figure 2 and Figure 4). On the other hand, only losartan led to a significant increase ($P<0.05$) in the gastric mucosal activity of CAT but enalapril led to insignificant changes in this enzyme activity compared to aspirin ulcer

group (Figure 3). Macroscopically, pretreatment with losartan or enalapril before induction of ulcer by aspirin produced area of gastric mucosal inflammation with a decrease in the incidence of ulceration to become 17% compared to aspirin ulcer group (Table 1 and Figures 5 E and F).

Table 1. Effect of Pretreatment with Losartan (3mg/kg/day) or Enalapril (10mg/kg/day) i.p for 4 weeks Duration on the Incidence of Aspirin- Induced Gastric Ulceration (Gross Picture) in Rats

Groups	Incidence of ulceration (Gross picture)
Control	(0/6) 0%
Losartan	(0/6) 0%
Enalapril	(0/6) 0%
Aspirin	(5/6) 83%
Losartan+ aspirin	(1/6) 17%
Enalapril+ aspirin	(1/6) 17%

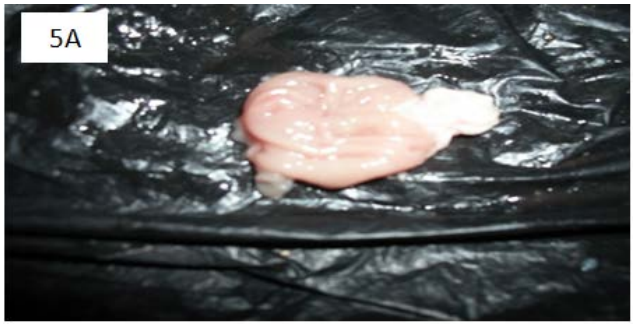

Figure 5 A. Gastric mucosa of rat from control group showing smooth gastric mucosa with no inflammation. The incidence of ulceration was zero%

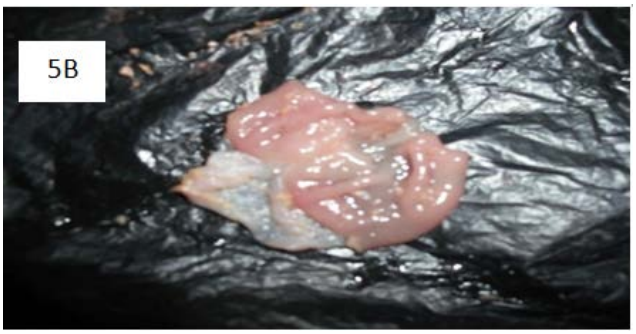

Figure 5 B. Gastric mucosa from rat received losartan for 4 weeks duration showing smooth gastric mucosa with no inflammation. The incidence of ulceration was zero%

Figure 5 C. Gastric mucosa of rat received enalapril for 4 weeks duration, smooth gastric mucosa with no inflammation. The incidence of ulceration was zero%

Figure 5 D. Gastric mucosa of aspirin–induced ulcer rat showing ulcer (arrow) surrounded by area of severe inflammation, the incidence of ulceration was 83%

Figure 5E. Gastric mucosa of rat pretreated with losartan 3mg/kg/day for 4 weeks duration before induction of ulcer by aspirin showing a decrease in the severity of inflammation area with a decrease in the incidence of ulceration to become 17%

Figure 5F. Gastric mucosa of rat pretreated with enalapril 10 mg/kg/day for 4 weeks duration before induction of ulcer by aspirin showing a decrease in the severity of inflammation with a decrease in the incidence of ulceration to become17%

3.4. Histopathological Changes

3.4.1. Light Microscopic Picture of Gastric Tissues after i.p. Administration of Losartan 3 mg/kg/day or Enalapril 10 mg/kg/day for 4 weeks

In the control group, light microscopic examination of gastric tissues showed normal gastric biopsy with intact mucosal surface and no significant inflammatory cell infiltration or edema. Light microscopic examination of gastric tissues after administration of losartan and enalapril also showed normal gastric biopsy with an intact mucosal surface (Figures 6A, B, and C).

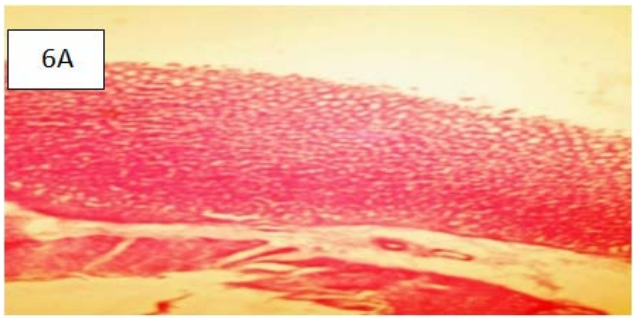

Figure 6A. Gastric biopsy from a rat in control group shows intact mucosal surface HandE x40

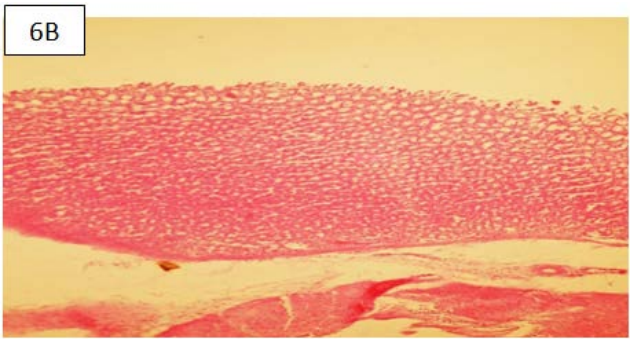

Figure 6B. Gastric biopsy from a rat treated with losartan in a dose of 3 mg/kg/day for 4 weeks duration showing intact mucosal surface HandEx40

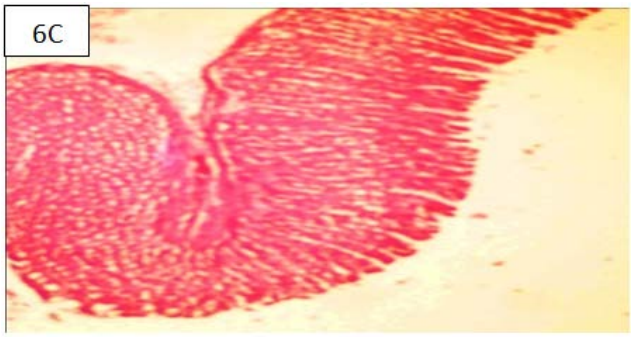

Figure 6C. Gastric biopsy from a rat treated with enalapril in a dose of 10 mg/kg/day for 4 weeks duration showing intact mucosal surface HandEx40

3.4.2. Light Microscopic Picture of Gastric Tissues Pretreated with Losartan 3 mg/kg/day orEnalapril 10 mg/kg/day for 4 Weeks Duration in Aspirin-Induced Gastric Ulceration:

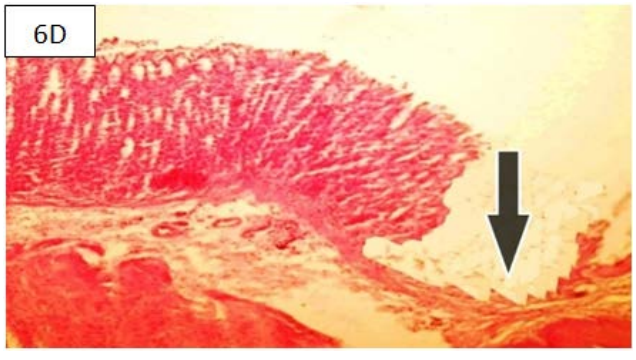

Figure 6D. Gastric biopsy from aspirin-induced ulcer rat, showing epithelial damage, necrotic damage of mid and lower mucosa and congestion of submucosa HandEx100

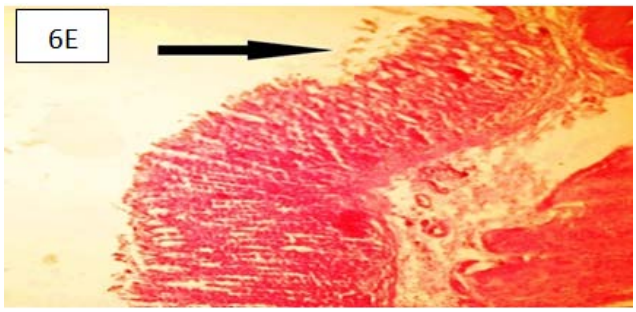

Figure 6E. Gastric biopsy from rat pretreated with losartan in a dose of 3 mg/kg/day for 4 weeks duration before induction of ulcer by aspirin showing superficial gastric epithelial erosion HandEx100

Light microscopic examination of gastric tissues after induction of ulcer by aspirin showed epithelial damage and congestion of submucosa (Figure 6D). Comparing the effect of losartan and enalapril on the histopathological changes in gastric tissue, it was found that the pretreatment with losartan orenalapril was showing superficial gastric epithelial erosion with no edema of the submucosal layer (Figures 6 E and F).

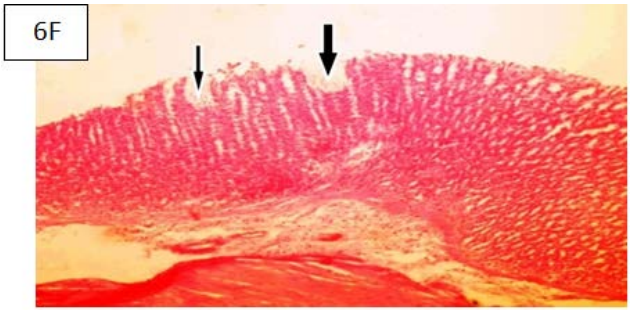

Figure 6F. Gastric biopsy from rat pretreated with enalapril in a dose of 10 mg/kg/day for 4 weeks duration before induction of ulcer by aspirin, showing superficial gastric mucosal erosions. HandEx100

4. Discussion

Studies have previously shown that co-treatment with ARBs or ACEIs seemed to reduce peptic ulcer among patients taking low-dose aspirin [16].

Prostaglandins are believed to play a pivotal role in the ulcer healing process as they are involved in improving gastric mucin synthesis, triggering mucosal cell proliferation, promoting angiogenesis, and inducing several other functions which modulate and regulate gastric mucosal integrity and gastric acid secretion [17].

In our study, oral administration of single dose of aspirin caused significant reduction of the PGE_2 level compared to the control group. This observation is in agreement with other previous reports done on aspirin-induced gastric ulceration [18,19].

The role of PGE_2 in mediating the gastroprotective effect of losartan was investigated. The results of the present study showed a significant increase in PGE_2 level following administration of losartan in comparison with an aspirin-induced peptic ulcer. These findings are in disagreement with a similar study done by Morsy et al. [20] which investigated the gastroprotective effects of telmisartan on experimentally induced gastric ulcers in rats and mentioned that telmisartan has no effect on gastric mucosal PGE_2 level and the difference in the

results could be attributed to the difference of the drugs used in both studies.

Also, enalapril has significant positive effect on the PGE_2 level when compared to aspirin-induced ulcer model with an insignificant difference from losartan results.

These results are in contrast to a study by Matloub [21] done to compare the protective effect of captopril and enalapril against NSAID -induced gastric mucosal injury in rats and stated that enalapril has no effect on the PGE_2 level and mentioned that the differences observed between captopril and enalapril in their ability to protect the gastric mucosa in that experiment could be partly explained on the basis of the pharmacokinetic profile of these two ACEIs, where captopril is active by itself and has a rapid onset of action while enalapril is a prodrug with a delayed onset of action that requires absorption after de-esterification by the liver to the active enalaprilat. Therefore, the time which was allowed for enalapril to produce its effects in his study was rather shorter than our study.

In our study, the increase in mucosal generation of PGE_2 observed after treatment with losartan and enalaprilis probably mediated, at least in part, by NO. Thus, the gastroprotection afforded by both drugs could be attributed to the interaction of nitric oxide synthase (NOs) and Cyclooxygenase (COX) enzymes and their products, NO and PGE_2 in gastric mucosa. A mutual interaction between NOs and COX enzymes has been confirmed by many investigators [22,23].

Several studies have demonstrated the importance of endogenous NO in the protection of gastric mucosa [24], [25]. NO formed by endothelial NOs plays an important role in the modulation of gastric mucosal integrity by interacting with sensory neuropeptides and endogenous PGs [26].

In the present study, aspirin significantly reduced gastric mucosal NO level compared to control group. These findings are in accordance with Tripp and Tepperman [27] who reporteda decrease in NO biosynthesis, as a result of decreased NOs activity that was associated with an increase in the extent of the damage. Treatment with losartan significantly increased mucosal NO level when compared to aspirin ulcers induced groups.

Also, enalapril has a similar effect on attenuating the observed aspirin-induced changes in gastric mucosal NO with no significant difference from losartan; these results were in harmony with a study on the effects of losartan and enalapril therapies on the levels of NO, in patients with essential hypertension. Both enalapril and losartan produced a significant increase in plasma NO in exhaled air after chronic therapy. Initial values of plasma nitrate levels in patient groups were similar to the control group and increased significantly at the end of treatment period [28].

On the other hand, our results were in disagreement with the result of a previous study which stated that enalapril has no effect on NO, but the difference in the results may be attributed to the difference in the dose or the duration where the dose in our study was larger and administered for a longer duration [21].

Any imbalance in the activity of various antioxidant enzymes like CAT, SOD, and glutathione peroxidase leads to faulty disposal of free radicals and its accumulation [29]. Antioxidants seemed to have a protective role in gastric ulcers and carcinomas [30]. Induction of experimental gastric ulcer with aspirin in the present study is associated with low SOD and CAT activities and this was in harmony with a previous study by Pohle et al. [31] and Ajaikumar et al.[32].

In our study, the administration of losartan or enalaprilto rats with aspirin- induced ulcer led to an elevation in SOD activity compared to aspirin group. Thisresult was in agreement with a study done on the effects of losartan on the oxidative stress in spontaneously hypertensive rat tissues [33]. Also, other study revealed that enalapril administration led to increasing in SOD and glutathione peroxidase activities in mouse liver [34].

In our study, the administration of losartan will cause a significant rise in catalase level in comparison with aspirin-induced ulcer group this was in harmony with a study by Khaperand Singal [35].The administration of enalapril caused insignificant rise in CAT level in comparison with aspirin-induced ulcer group, these results were in agreement with the results of a study byDe Cavanagh et al.[34] which was done to investigate the antioxidant effect of captopril and enalapril and revealed that SOD and glutathione peroxidase activities are increased by enalapril and captopril in mice liver while CAT activity was not affected.

In contrast to a study by Santos et al. [36] on normotensive rats fed with standard or hyperlipidemic diets that showed an increase in CAT level where the difference in results could be explained by the variation in duration of both studies.

Losartan, a selective AT1 receptor antagonist, was proved effective in reducing the generation of ROS and proinflammatory mediators. The antioxidant and anti-inflammatory effects of losartan are related to the ability of its metabolite EXP3179 to prevent the activation of nuclear factor- signaling pathway which promotes the transcription of NADPH oxidase, tumor necrosis factor-alpha (TNF-α) and inducible NOs genes [37]. Other mechanisms independent of AT1 receptor blockade are the drug acts as a partial agonist at the nuclear peroxisome proliferator-activated receptor [38].

5. Conclusions

Losartanand enalapril protected the gastric mucosa from aspirin-induced gastric ulceration. The mechanisms of gastroprotection include its ability to increase gastric mucosal PGE_2, SOD and NO levels andlosartan had an additional effect on CAT level.

Losartan or enalapril can be used in combination with low-dose aspirin where the later usedfor prevention of thrombotic events in cardiovascular diseases, due to losartan and enalapril provide gastroprotective effect and losartan is better than enalapril due to its potent antioxidant influence.

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