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Gastroprotective effects of nebivolol and simvastatin against cold restraint stress-induced gastric ulcer in rats

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Abstract: Gastric ulcer is one of the most serious diseases. Nebivolol (Neb), a β1-blocker, exhibits vasodilator and anti-oxidative properties, simvastatin (Sim) antihyperlipidemic drug, exhibits anti-oxidative, anti-inflammatory properties and promote endogenous nitric oxide (NO) production. The aim of this study was to evaluate the gastroprotective effects of Neb and Sim against cold restraint stress (CRS)-induced gastric ulcer in rats. Rats were restrained, and maintained at 4°C for 3 hours. Animals were divided into six groups; control group, CRS group, and four treatment groups received ranitidine (Ran), Neb, Sim and both Neb and Sim. Treatments were given orally on a daily basis for 7 days prior to CRS. The gastroprotective effects of Neb and Sim were assessed biochemically by measuring variations in prostaglandins E2, NO, reduced glutathione and malondialdehyde, and functionally by estimating force of contractions of isolated rat fundus in the studied groups in response to acetylecholine stimulation and morphologically using hematoxylin and eosin staining, periodic acid Schiff's reaction and immunohistochemistry for cyclooxygenase 2 in gastric mucosa. CRS caused significant ulcerogenic effect. Alternatively, pretreatment with Ran, Neb, and Sim significantly corrected biochemical findings, pharmacological and histological studies.

Key words: Gastric ulcer, Cold restraint stress, Ranitidine, Nebivolol, Simvastatin

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Introduction

Gastric ulcer is an illness that affects a considerable number of people worldwide [1]. It occurs at a site where the mucosa epithelium is exposed to aggressive factors such as acid and pepsin [2, 3]. The pathophysiology of gastric ulcer has generally focused on imbalance between aggressive and protective factors in the stomach [4], such as acid-pepsin secretion, parietal cell, mucosal barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents; prostaglandins (PGs) and epidermal growth factors [5]. Under normal conditions, the integrity of the stomach mucosal barrier is maintained by equilibrium between irritation and defensive factors [6].

The major causes of peptic ulcer disease are - stress, chronic use of non-steroidal anti-inflammatory drugs, alcohol, cigarette smoking, genetic predisposition, diet, and Helicobacter pylori [5]. The contribution of gastric neutro-

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phil accumulation, inflammatory cytokine production, free radical production, decreased antioxidants and decreased mucosal blood flow have been reported to be involved in the pathogenesis of stress-induced gastric lesions [7].

It is well established that nitric oxide (NO) exerts gastroprotective activity mostly due to the maintenance of blood flow around the ulcer [8]. The beneficial effects of NO on wound repair may be attributed to its functional influences on angiogenesis and inflammation [9].

Nebivolol (Neb), a third-generation highly selective β1-adrenergic receptor antagonist, causes vasodilatation by stimulating the production of NO via rapid activation of endothelial and neuronal NO synthase and exhibits anti-oxidant effects by reducing nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-induced superoxide generation [10, 11]. Neb is well tolerated and does not appear to have adverse effects on lipid metabolism and insulin sensitivity like traditional β-blockers [12].

Statins are widely used clinically for lowering hypercholesterolemia [13]. Besides the therapeutic use in hyperlipidemia, the antioxidant, anti-inflammatory and immunomodulatory benefits of statins have been reported in many studies [14]. They promote endogenous NO production [15] decrease platelet aggregation and inhibit thromboxane formation [16]. Simvastatin (Sim) which has tested in this study is a commonly prescribed statin with antioxidant and anti-inflammatory effects [17].

The present study was conducted to elucidate the possible gastroprotective effect of Neb and Sim pretreatment on stress-induced gastric ulcer in rats and the mechanisms underlying this protection.

Materials and Methods

The experimental steps, animal handling, sampling and scarification were done according to the *Guide for the Care and Use of Laboratory Animals*, Eighth edition [18] and were approved by the Ethics and scientific Committee guidelines for animal care and use, Department of Pharmacology, Kasr Al Ainy Faculty of Medicine, Cairo University, Egypt.

Drugs and chemicals

Sim was obtained from AstraZeneca, England. Neb was obtained from Chemipharma, Egypt and ranitidine (Ran) was obtained from Glaxo, England. All drug solutions and suspensions were freshly prepared.

Animals and study design

A total of 36 male albino Wistar adult rats with an average weight of 200–250 g were housed in a temperature-controlled room (22°C±1°C) with a 12 hours light/dark cycle. The animals were divided into 6 groups (each consisting of 6 rats):

Control group: In which the animals received distilled water orally for 7 days. The control group was kept at room temperature without any stress.

Cold restraint stress: Rats were restrained by fixing the four limbs to a wooden board using a quartz-pasted tape and placed in a refrigerator at 4°C for 3 hours. The door of the refrigerator was opened every 0.5 hours for inspection and follow-up [19] and maintained for 3 hours [20].

Ranitidine pretreated group: In which animals were pretreated with Ran 50 mg/kg orally daily by a gastric tube for 7 days then, gastric ulceration was induced by cold restraint stress (CRS) [21].

Simvastatin pretreated group: In which animals were pretreated with 40 mg/kg Sim orally for 7 days then, gastric ulceration was induced by CRS [22].

Nebivolol pretreated group: In which animals were pretreated with 5 mg/kg Neb orally daily for 7 days then, gastric ulceration was induced by CRS [23].

Simvastatin+nebivolol pretreated group: In which animals were pretreated with both Neb orally daily for 7 days then, gastric ulceration was induced by CRS.

Rats were deprived of food for 24 hours prior to the experiment in mesh-bo ttomed cages to minimize coprophagia but allowed free access to water except for the last hour before the experiment. All experiments were performed during the same time of the day to avoid variations due to diurnal rhythms of putative regulators of gastric functions.

All groups received equivalent volumes of the above used vehicles.

In vitro (Isolated rat fundus)

Rats were euthanized by decapitation and exsanguinated. The stomach was dissected out and strips were cut according to the method described by Vane [24]. The strips were cut up in an organ bath maintained at 37°C containing Tyrode solution and bubbled with a gas mixture containing 95% oxygen-5% carbon dioxide.

The abdomen was opened along the midline and the gastric fundus was excised and put into a dissecting dish filled with Tyrode solution. The fundus was at first flattened by

two parallel incision along the great curvature. According to the method of Vane [24], several parallel sections at 1.5 mm intervals with the great curvature were made in the fundus producing very long and narrow isolated preparations (40 mm in length and 1.5 mm in width) containing all the layers of the gastric wall. One end of the preparation was attached to the bottom of the bath, and the other to the lever of an isotonic transducer (T3 isotonic transducer; Palm Bio Science, Los Angeles, CA, USA). All strips were loaded with 1.0 g weight Vane [24].

The experiment was designed to test the effect of different doses of acetylcholine 2, 4, and 8 μcg on the stomach fundus of all rat groups.

Biochemical analysis of gastric mucosa

Determination of oxidative stress markers

The gastric mucosa was weighed, minced with scissors, and homogenized using 0.1 M phosphate buffer (pH 7.4) (0.000005 m^3 for each g of tissue) in homogenizer. After centrifugation at 2,000–3,000 rpm for 20 minutes, the supernatant was extracted and frizzed in –80°C for later use. Oxidative stress markers were detected in the resultant supernatant of gastric mucosal homogenate. The appropriate kits (Biodiagnostic kits, Biodiagnostic, Giza, Egypt) were used for the determination of malondialdehyde (MDA), the end product of lipid peroxidation [25]. A reaction mixture containing 8.1% sodium dodecyl sulfate, 20% acetate buffer (pH 3.5) and 0.8% thiobarbituric acid (TBA) was mixed well with 0.2 ml of stomach tissue homogenate for 3 minutes and then incubated at 95°C for 60 minutes. After cooling with running water, the TBA-reactive substance (MDA) was extracted with 1 ml of H_2O and 2.5 ml of n-butanol: Pyridine mixture (15:1, v/v). The upper organic layer containing the MDA, which was produced by lipid peroxidation, was measured at 532 nm.

Reduced glutathione (GSH) level activity was estimated based on the method of Ellman [26] and Habig et al. [27]. The precipitated tissue homogenate was treated with 5,5'-dithiobis-(2-nitrobenzoic acid) reagent. The absorbance was read at 412 nm and the amount of GSH is expressed as mmol/mg mucosal tissue.

Determination of prostaglandin E2 and nitric oxide

The supernatant was used for determination of prostaglandin E_2 (PGE_2) level by ELISA using PGE_2 immunoassay kit (R&D systems, Minneapolis, MN, USA). It was assessed according to the method of Hamberg and Samuelsson [28]. Based on the competitive binding technique in which PGE_2 present in a sample competes with a fixed amount of horseradish peroxidase-labeled PGE_2 for sites on a monoclonal antibody.

NO measurement is difficult because of its brief half-life. Therefore, nitrate/nitrite levels, which are stable degradation products of NO metabolism, were used as markers. It was quantified in gastric mucosal homogenate using commercial kits (Biodiagnostic) according to the method described by Miranda et al. [29]. A 100 l of the supernatant was mixed with 100 l Griess reagent (0.1% N-(1-naphthyl) ethylenediamide dihydrochloride, 1% sulfanilamide in 5% phosphoric acid) and after 10 minutes the absorbance was measured at 540 nm. The standard curve was obtained by using sodium nitrite.

Histopathological evaluation

The tissue specimens from the fundic region of the stomach were fixed in 10% formalin solution for 24 hours. Paraffin blocks were processed and serial 5 μm-thick sections were obtained and subjected to hematoxylin and eosin (H&E) for demonstration of structural morphology, and periodic acid Schiff's (PAS) reaction to evaluate the gastric mucosal glycoprotein synthesis.

Immunohistochemistry

Immunohistochemical staining for cyclooxygenase 2 (COX-2) was performed as it is considered a pivotal mediator for gastric mucosal healing [30]. Rabbit polycolonal anti-COX-2 antibody (Ab) (Thermo scientific cat. # RB-9072-R7; Thermo Scientific, Waltham, MA, USA) was used. Sections of 5 μm thickness were cut from paraffin blocks, deparaffinized, rehydrated and microwaved in citrate buffer, pH 6.0 for 10 minute followed by cooling at room temperature for 20 minutes for antigen retrieval. Quenching of endogenous peroxidase activity was performed. The sections were incubated with the primary Ab against COX-2 (1:200 dilution) and stained as regard to the avidin-biotin complex method using a UltraVision LP Detection. The sections were counterstained with Meyer's haematoxylin to visualize the nucleus.

Morphometry analysis

Using 'Leica Qwin 500 C' image analyzer (Cambridge, UK), assessment of the area percent (%) of PAS positive reaction and COX-2 positive (+ve) immunostaining were accom-

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plished. The measures were taken in 10 non overlapping high power fields (×400)/rat.

Statistical analysis

Data were coded and entered using the IBM SPSS Statistics for Windows, Version 21.0 (IBM Co., Armonk, NY, USA). Data was summarized using mean and standard deviation. Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc Benferoni test. The *P*-values≤0.05 were considered as statistically significant [31].

Results

Concentration response of isolated rat fundus to acetylcholine

As shown in Fig. 1, 2: there is significant decrease of acetyl choline induced gastric contraction in CRS compared to control group. Pretreatment with Ran, Sim, nebivolo or combined Sim and Neb significantly increased the force of gastric contraction compared to CRS group. Pretreatment with Ran or combined Sim and Neb achieved the best results which reflect good healing and viability of gastric tissue, however none of the given drugs could increase the force of gastric contraction to normal value measured in the control group.

Biochemical measurement

Table 1 demonstrates the following: there is significant increase (*P*-value≤0.05) in MDA in CRS group compared to control group. Pretreatment with Ran, Sim, nebivolo or combined Sim and Neb significantly decreased gastric MDA compared to CRS group, however with all the given drugs gastric MDA was significantly increased (*P*-value≤0.05) compared to control group.

CRS group showed significant decrease (*P*-value≤0.05) in gastric GSH, PGE_2, and NO compared to control group. Pretreatment with Ran, Sim, nebivolo or combined Sim and Neb significantly increased the gastric content for GSH, PGE_2 and NO compared to CRS group.

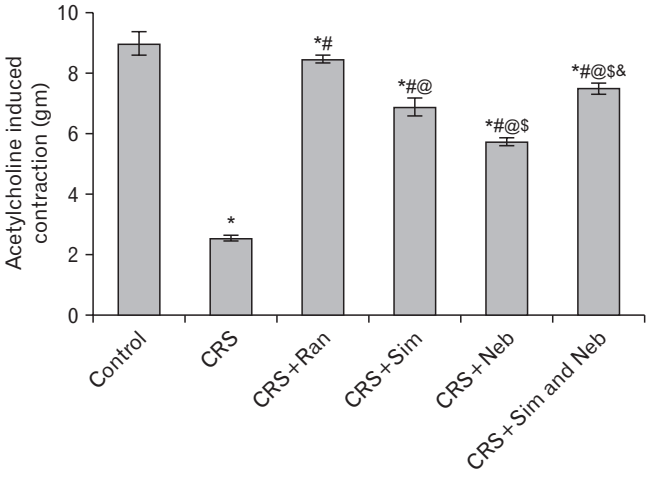

Fig. 2. Comparison of Mean±SD of contraction force generated by acetyle choline stimulation in different studied groups. CRS, cold restraint stress; Neb, nebivolol; Ran, ranitidine; Sim, simvastatin. *Significant compared to Control group at *P*-value≤0.05; #Significant compared to CRS group at *P*-value≤0.05; @Significant compared to CRS+Ran group at *P*-value≤0.05; $Significant compared to CRS+Sim group at *P*-value≤0.05; &Significant compared to CRS+Neb group at *P*-value≤0.05.

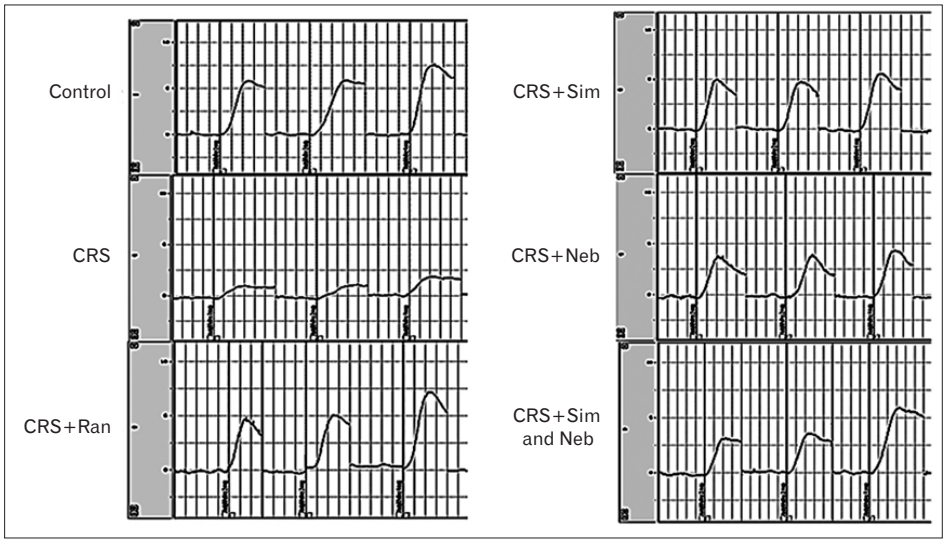

Fig. 1. Force of contractions in the studied groups in response to acetylecholine stimulation. CRS, cold restraint stress; Neb, nebivolol; Ran, ranitidine; Sim, simvastatin.

Combined Sim and Neb had the best outcome in all measured parameters in the stomach and it normalized the level of NO.

Histopathological results

H&E stained gastric mucosal sections of cold restrain group demonstrated multiple mucosal abrasions, hemorrhagic ulcers, and disturbed glandular arrangement. Ran-pre-treatment provided complete protection of the gastric mucosa. Administration of either Sim or Neb alone ameliorated the mucosal injury. Meanwhile, combined Sim and Neb pretreatment substantially protected the gastric mucosa against aberration and ulceration provided with intact mucosa and surface mucus layer covering the gastric pits (Fig. 3).

PAS stained mucosal sections of CRS group displayed areas of absent or little PAS+ve reaction. Ran+CRS exhibited continuous strong PAS+ve reaction. Pretreatment with Sim or Neb provided partial increase in the mucosal PAS+ve reaction provided with significant increase in the area % of PAS+ve reaction compared to CRS group. Sim+Neb+CRS augmented the mucosal PAS+ve reaction which was comparable to control (Fig. 4, 5).

Table 1. Biochemical parameters measured in the stomach of the studied groups

Parameter	Control	CRS	CRS+Ran	CRS+Sim	CRS+Neb	CRS+Sim and Neb
MDA (nmol/mg protein)	1.25±0.0498	12.73±0.35*	5.173±0.102*#	4.235±0.079*#@	3.608±0.075*#@$	2.197±0.069*#@$&
GSH (mmol/mg protein)	58.8±1.544	20.54±0.845*	31.92±0.8264*#	43.81±1.529*#@	44.067±1.344*#@	49.43±1.801*#@$&
PGE_2 (pg/mg protein)	380.75±7.574	124.31±3.401*	260±6.164*#	188.33±4.633*#@	186.31±3.237*#@	192.5±5.683*#@
NO (mmol/mg protein)	2.32±0.047	0.475±0.018*	2.025±0.058*#	2.255±0.050*#@	2.193±0.025*@	2.335±0.045#@&

Values are presented as mean±SD. CRS, cold restraint stress; Ran, ranitidine; Sim, simvastatin; Neb, nebivolol; MDA, malondialdehyde; GSH, glutathione; PGE_2, prostaglandin E2; NO, nitric oxide. *Significant compared to Control group at *P*-value≤0.05; #Significant compared to CRS group at *P*-value≤0.05; @Significant compared to CRS+Ran group at *P*-value≤0.05; $Significant compared to CRS+Sim group at *P*-value≤0.05; &Significant compared to CRS+Neb group at *P*-value≤0.05.

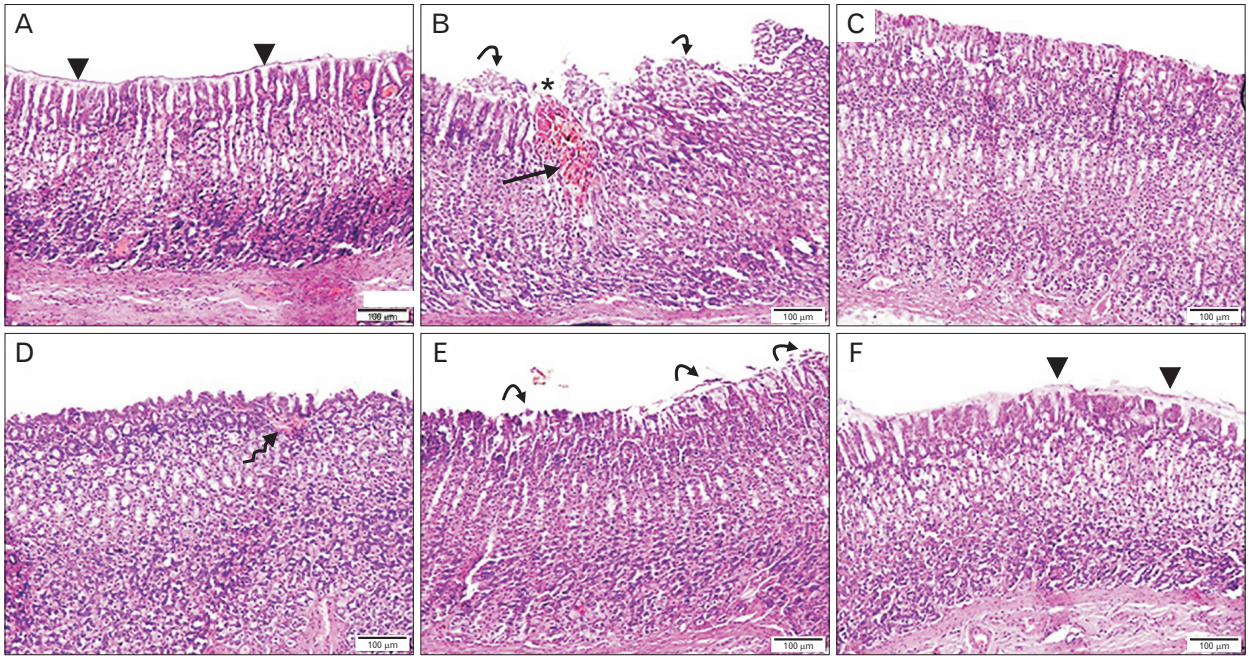

Fig. 3. Photomicrograph of H&E stained sections in gastric mucosa (×100) showing: (A) Control group: intact mucosa with surface mucus layer (arrowheads) covering the gastric pits, gastric glands. (B) CRS group: diffuse mucosal abrasions, hemorrhage (asterisk), hemosiderin precipitation (arrow), patches of sloughed mucosal epithelium (curved arrows) and disturbed glands. (C) Ran+CRS group: intact gastric mucosa with intact surface epithelium. (D) Sim+CRS group: intact gastric mucosa with mild congestion in the lamina propria (curved arrow). (E) Neb+CRS group: ameliorated mucosal erosion with slight disturbance in the surface and cell desquamation (curved arrows). (F) Sim+Neb+CRS group: intact gastric mucosa with surface mucus layer (arrowheads) covering the gastric pits. CRS, cold restraint stress; Neb, nebivolol; Ran, ranitidine; RS, cold restraint stress; Sim, simvastatin. Scale bars=100 μm (A–F).

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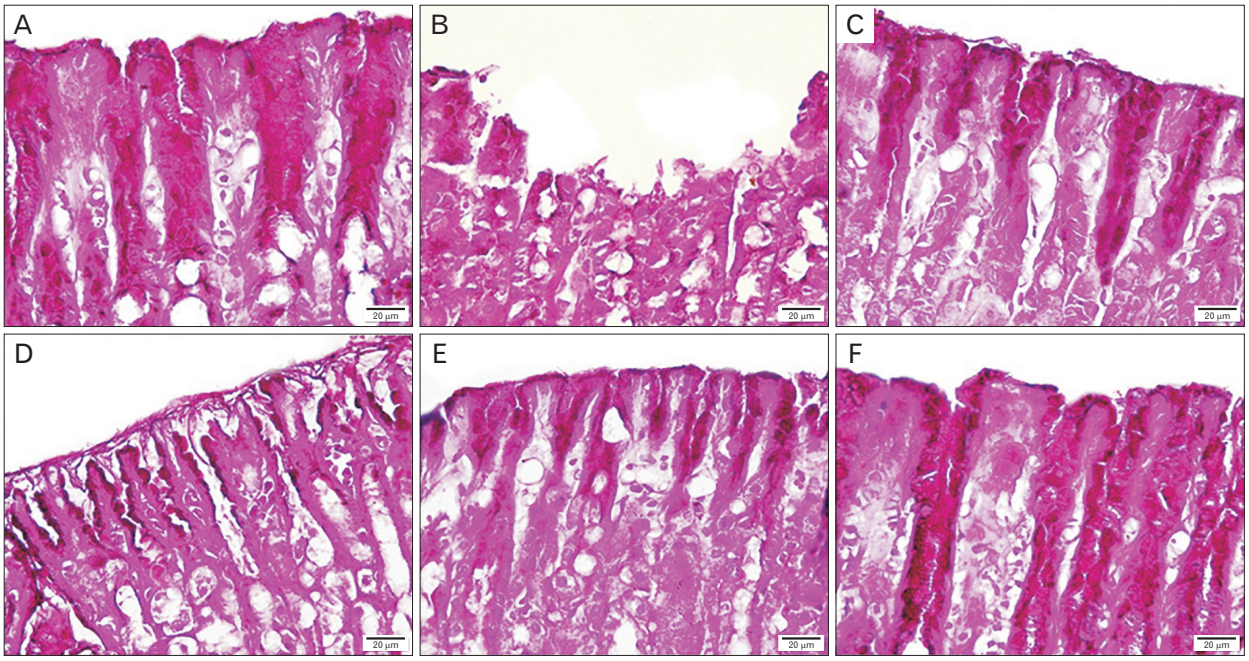

Fig. 4. Photomicrograph of PAS stained sections in gastric mucosa (×400) showing: (A) Control group: continuous strong PAS+ve reaction in the surface and neck region of the gastric glands. (B) CRS: mucosal abrasion with alternating absent to few PAS+ve reaction. (C) Ran+CRS: continuous strong PAS+ve reaction. (D) Sim+CRS: preserved PAS+ve reaction. (E) Neb+CRS: preserved PAS+ve reaction. (F) Sim+Neb+CRS: marked increase in the mucosal PAS+ve reaction. CRS, cold restraint stress; Neb, nebivolol; PAS, periodic acid Schiff's; Ran, ranitidine; Sim, simvastatin. Scale bars=20 μm (A–F).

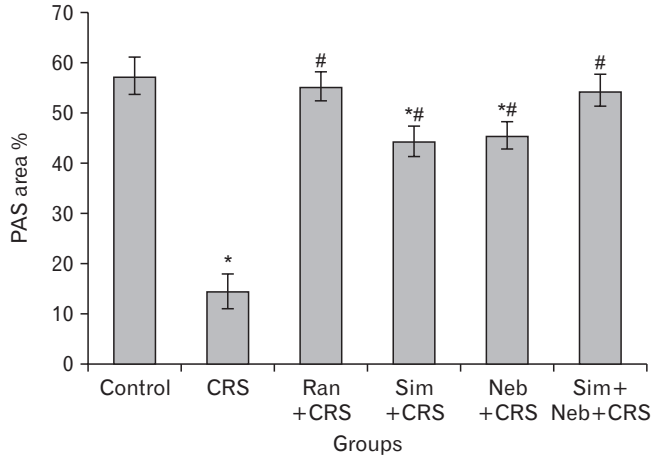

Fig. 5. Quantification analysis of the mean PAS+ve area %. CRS, cold restraint stress; Neb, nebivolol; PAS, periodic acid Schiff's; Ran, ranitidine; Sim, simvastatin. *Significant decrease (P<0.05) when compared with control. #Significant increase (P<0.05) when compared with CRS.

Immunohistochemical evaluation of COX-2 in gastric mucosa

Minimal detection of COX-2+ve immunostaining was observed in the control gastric mucosa. In CRS, minimal COX-2+ve immunostaining was detected at the edge of the ulcer with negative immunostaining at the base of the ulcer. Ran pretreatment induced significant augmentation of COX-2+ve immunostaining in gastric mucosa as compared to CRS group. Pretreatment with Sim or Neb illustrated moderate COX-2+ve immunostaining. However, Sim+Neb+CRS displayed substantial increase in COX-2+ve immunostaining in gastric mucosa as compared to CRS, Sim+CRS and Neb+CRS groups (Fig. 6, 7).

Discussion

The results of the present study showed that exposure to CRS in rats resulted in severe mucosal ulceration associated with significant increase in MDA and, significant reduction in gastric mucosal GSH, NO, and PGE_2.

Kwiecien et al. [32] and Brzozowski et al. [33] reported that the cold-restraint stress model in rats mimics clinical acute gastric lesions, that may appear in the gastric mucosa as a consequence of major trauma, surgery or sepsis and it is widely accepted for studying the mechanism of stress induced gastric lesions.

In the present study, pretreatment with Neb and Sim

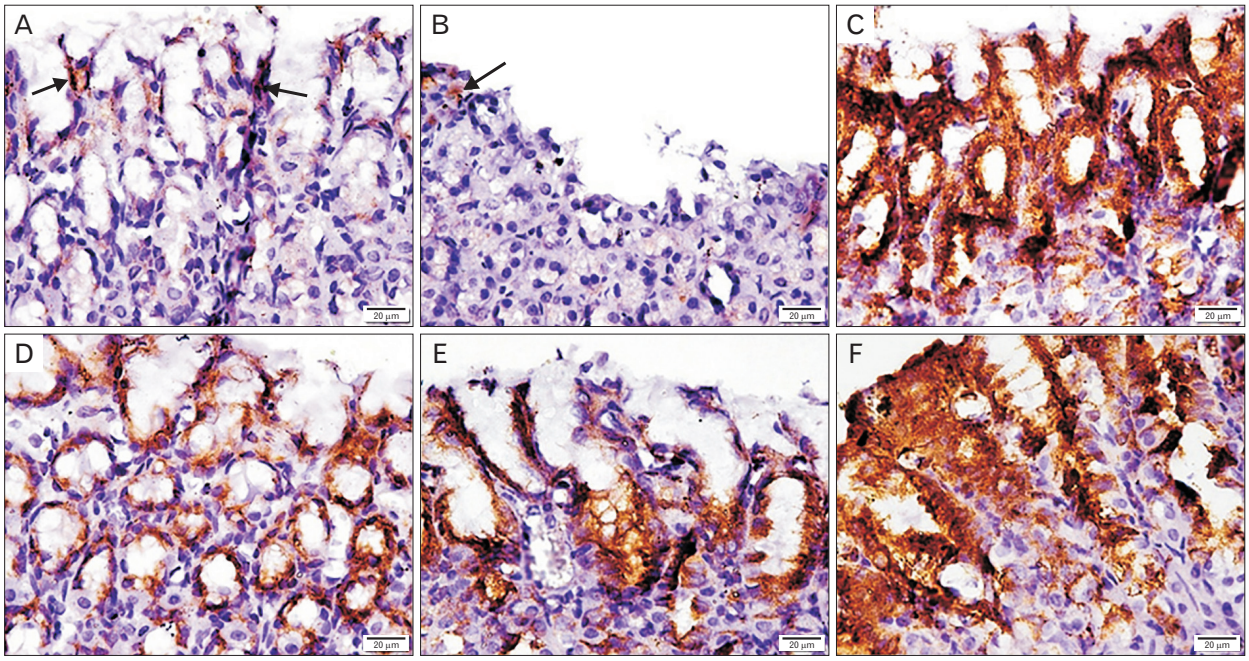

Fig. 6. Photomicrograph of COX-2 immunostaining (×400) showing: (A) Control group: minimal COX-2+ve immunostaining in gastric mucosa. (B) CRS: minimal+ve immunostaining at the edge of the ulcer. (C) Ran+CRS: enhanced COX-2+ve immunostaining in the glandular epithelium and stromal cells. (D) Sim+CRS: moderate COX-2+ve immunostaining in the gastric mucosa. (E) Neb+CRS: moderate COX-2+ve immunostaining in the gastric mucosa. (F) Sim+Neb+CRS: marked increase COX-2+ve immunostaining in gastric mucosa. COX-2, cyclooxygenase 2; CRS, cold restraint stress; Neb, nebivolol; PAS, periodic acid Schiff's; Ran, ranitidine; Sim, simvastatin. Scale bars=20 μm (A–F).

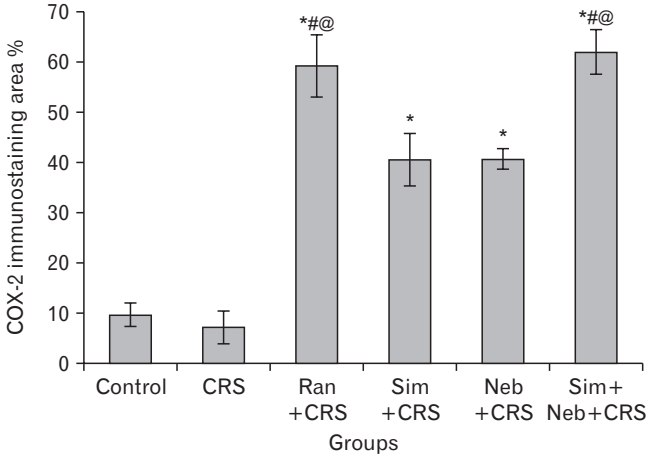

Fig. 7. Quantification analysis of the mean COX-2+ve immunostaining area %. COX-2, cyclooxygenase 2; CRS, cold restraint stress; Neb, nebivolol; Ran, ranitidine; Sim, simvastatin. *Significant increase (P<0.05) when compared with control and CRS groups. #Significant increase (P<0.05) when compared with Sim+CRS, @Significant increase (P<0.05) when compared with Neb+CRS groups.

significantly protected rats from CRS-induced gastric ulceration; they significantly reduced gastric mucosal MDA level with concomitant increase in the GSH and PGE_2 and NO.

The results of the present study are in accordance with Haendeler et al. [34] who showed that Sim pretreatment caused significant reduction in MDA level in both ulcer models as compared to non-treated control groups and it was reported that Sim possesses free radicals scavenger activity. This suggests that Sim afforded part of its gastroprotective effect in both ulcer models via antioxidant activity. Tariq et al. [35] also demonstrated that oral administration of Sim in doses of 20, 40, 60 mg/kg for 7 days significantly and dose dependently reduced gastric lesions induced by indomethacin and ethanol in rats, an effect accompanied by reduction in total acidity and volume of gastric juice.

Moreover, Matsui et al. [36] have shown that rats pretreated with Sim showed significant increase in PGE_2 level. PGs also play an essential role in gastric mucosal defense. This effect is dependent on the PG-induced stimulation of bicarbonate and mucous secretion, inhibition of gastric acid secretion, and regulation of maintaining epithelial cell restitution and mucosal blood flow. Furthermore, Bjarnason et al. [37] reported marked elevation of nitrite level in CRS while Sim was able to normalize the nitrite levels, it deceased NO level in CRS group. Stress ulcer increases the formation of reactive

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oxygen metabolites and promotes inhibition of PG synthesis, leading to alterations in gastric NO levels.

NO is a double-edged weapon exerting either protective or destructive effects depending on the extent of NO synthesis Tariq et al. [35]. It has been reported that NO generated from constitutive NOS (cNOS) plays an important role in gastric ulcer formation and healing [38] and considered to be beneficial in maintaining the mucosal integrity [39], whereas NO generated from inducible nitric oxide synthase (iNOS) participates in ulcer formation through the production of oxygen derived radical and their cytotoxic actions [40]. This suggests that NO production from iNOS may play a detrimental role in stress-induced gastric injuries. The present work showed a marked reduction in nitrite levels (an iNOS) in the simvatstin pre-treated group, a change not observed in the Ran pre-treated group. These findings can be attributed to the ability of simvatstin to downregulate the iNOS mRNA expression leading to decreased production of gastric mucosal NO. The nitrite levels is markedly elevated, which could be occurring due to stimulation of iNOS, which reacts with superoxide to form peroxynitrite, a potent cytotoxic oxidant causing gastric damage [41].

In this study, Neb-induced a significant increase in GSH which is in accordance with the findings of Goel et al. [42] who reported that chronic administration of Neb shows significant increase in brain GSH level. Ceron et al. [43] also demonstrated that Neb attenuated the vascular remodeling associated with increased oxidative stress in hypertensive rats. Moreover, Rizzi et al. [44] found evidence that Neb as a selective β1-blocker with antioxidant properties attenuates hypertensioninduced left ventricular hypertrophy and cardiac collagen deposition in association with significant cardiac antioxidant effects in rats.

Furthermore, Dursun et al. [45] reported that Neb showed an antioxidant effect besides its low density lipoprotein lowering effect in a rat model of N-nitroL-arginine methyl ester-induced oxidative stress, vascular inflammation, and arteriosclerosis.

Uzar et al. [46] reported that Neb was shown to prevent oxidative stress in rats with ischemia-induced cerebral injury. Whaley-Connell et al. [47] also have shown that Neb may reduce oxidative stress in skeletal muscle tissue by reducing both NADPH oxidase and mitochondrial generation of ROS.

Omaima and Abeer [48] found that Neb induced significant decrease in iNOS expression and mucosal nitrite/nitrate levels compared to indomethacin administered rats, this may be attributed in part to the increased consumption of NO in free radical scavenging, hindering lipid peroxidation and to the stimulation of cNOS in gastric tissue which produce a low concentration of cytoprotective NO. These data depict the involvement of NO pathway in anti-ulcerogenic potential of Neb. Several reports have demonstrated the NO-mediated beneficial effects of Neb on different tissues. Neb has been shown to produce NO-mediated relaxation of the rat mesenteric vascular bed and increase cNOS activity in cultured bovine coronary postcapillary endothelial cells [49]. Neb has also been found to induce relaxation of rat renal glomerular vasculature by increasing NO release [50]. Another study by Zhou et al., [51] demonstrated that the vasodilatory and anti-oxidant properties of Neb are likely through reduction in NADPH oxidase activity and enhancement of endothelial NO synthase activity. Besides, it has been reported that Neb treatment leads to reductions in NADPH oxidase activity in the heart and vascular tissue [11]. A study by Manrique et al. [52], has highlighted that Neb induced improvement in insulin metabolic signaling and enhancement in bioavailable NO in skeletal muscle. Other than the important role of NO in maintenance of blood flow, it may protect against indomethacin-induced gastric damage by promotion of PGs synthesis. PGs, especially PGE_2, modulate a number of components of mucosal defense as they stimulate mucus and bicarbonate secretion, increase mucosal blood flow, increase the resistance of epithelial cells to cytotoxins-induced injury and suppress the recruitment of leukocytes into gastric mucosa. PGs can also down regulate the release of other inflammatory mediators that may contribute to the generation of gastric ulcer. PGE_2 has been shown to be a potent inhibitor of TNF-α and IL-1 release from macrophages and of leukotriene B4 and IL-8 release from neutrophils [53].

Histological analysis of gastric mucosa of CRS group revealed mucosal erosions and hemorrhagic ulcers with significant decrease in the mucus production indicating affection of the mucosal defense of the stomach. Similar results were documented by Vaseem et al. [54]. Many factors have been postulated in the development of stress peptic ulcer, as inadequate blood microcirculation with following tissue hypoxemia and oxidative stress [23] and increasing the acid secretion with reduction in mucus secretion [55].

Combined administration of Sim and Neb pretreatment substantially protected the gastric mucosa and augmented the mucosal PAS+ve reaction as compared to Ran. However, pretreatment with either Sim or Neb alone provided signifi-

cant protection of gastric mucosa, but it was less comparable to Ran. Sim, a commonly prescribed statin, was documented as anti-inflammatory, antioxidant effect and immunomodulatory agent [56]. Additional benefits of Sim included gastroprotective effects and amelioration of peptic ulcer. It was found to enhance gastric mucosal defense against indomethacin-induced gastric ulceration via increasing NO and PGE_2 levels, promoting gastric mucin release and providing antioxidant activity [57]. Recent evidences concluded that statin mitigate Helicobacter pylori-associated pathogenesis [58] and protect against peptic ulcer in patients [59]. Neb has pleiotropic effects as being vasodilator and anti-oxidative which provides preferable protection from gastric ulcer [23]. It has been reported that Neb significantly reduces MDA, tumor necrosis factor-α, and interleukin-1beta in indomethacin-induced gastric ulcer due to its anti-oxidant activity [48].

The present study showed minimal detection of COX-2+ve immunostaining, in gastric mucosa of CRS group at the gastric ulcer margin. Meanwhile, combined Sim and Neb pretreatment exhibited marked COX-2+ve immunostaining in the surface and glandular gastric epithelium. COX enzyme plays pivotal role in PG synthesis. It exists in 2 isoforms: COX-1 and COX-2. COX-1 is constitutively expressed in most of tissues and mediates PG synthesis needed for physiological functions. COX-2 and PG can induce angiogenesis and heavy infiltration of macrophages and reconstruct the inflammatory microenvironment [60].

COX-2 is normally undetectable in most tissues but substantially inducible as a compensatory mechanism to enhance PG synthesis during pathological conditions [23]. It promotes cell survival, cell proliferation and angiogenesis [61]. It was postulated that COX-2 expression at the ulcer margins is a trial for healing; however continuous inhibition of COX-2 activity delays the healing process [62]. Statins was documented to up-regulate the expression of COX-2 in human vascular smooth muscle cells. Moreover, Neb pretreatment was found to increase the level of NO which increases the activity of COX-2 enzyme. Several studies have reported cross talk between NO and COX-2 in contribution to gastric defense mechanisms [63].

The combination of beta blockers as Neb and statins as Sim has revealed a beneficial effect in previous study by Rizos et al. [64]. The Neb decrease the serum high sensitivity C-reactive protein and increase the homocysteine level while insulin levels and the homeostatic model assessment index were reduced. Adding statins is useful as protective against the stress effect as it decrease level of cholesterol, Low density lipoprotein and apoproteins. Moreover, homocysteine levels and C-reactive protein were also reduced [64].

Our work showed that Neb and Sim reversed all the deleterious effects induced by CRS on gastric mucosa.

In conclusion, it could be concluded from the results of the present study that both Neb and Sim have gastroprotective effect against CRS induced gastric ulcer in rats. The mechanism could be attributed to their NO releasing property and inhibitory effect on oxidative stress as evident from their antioxidant activity.

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Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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