Protective effects of nebivolol on acetic acid-induced ulcerative colitis in rats

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Background
Ulcerative colitis (UC) is a chronic inflammatory disease of large intestine. Overproduction of free radicals, lowered antioxidant capacity and abnormal apoptosis are involved in pathogenesis. Nebivolol, a β blocker with vasodilatory, antioxidant, anti-inflammatory effects, can play future role in therapies for UC.
Aim of the study
The purpose of this study was to evaluate the protective effect of nebivolol against acetic acid (AA)-induced UC in rats.
Methods
Male wistar rats were pre-treated orally with nebivolol 5 mg/kg/d, 10 mg/kg/day, for seven days, before and 3 days after induction of colitis (by intra-rectal administration of 2 ml of 4% AA). Colonic macroscopic scoring and histopathological examination were done. Colonic content of thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), superoxide dismutase (SOD) and myeloperoxidase (MPO) activities were assessed. Apoptosis was monitored by determining caspase-3 gene expression. Serum levels of interleukin (IL)1-β, SOD, TBARS and tumour necrosis factor (TNF)-α were measured.
Results
In AA- group, serum levels of TBARS, TNF-α and IL-1β were significantly increased. SOD activity was significantly reduced. Caspase 3 protein expression was upregulated. Colonic content of TBARS and the activity of MPO were elevated. GSH concentration and activity of SOD were significantly reduced, compared to control group. In nebivolol pretreated groups (5 and 10 mg/kg/d) and sulfasalazine group, all parameters were near normal. Nebivolol significantly decreased colonic macroscopic scoring and wet colon weight compared to AA group. The coloprotective effect of nebivolol was confirmed by histopathological examination.
Conclusion
Nebivolol has a protective effect against AA- induced colitis, through its anti-inflammatory, anti-oxidant and anti-apoptotic effects.

Keywords:
nebivolol, ulcerative colitis, rats, anti-oxidant, anti-apoptotic

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Introduction

Ulcerative colitis (UC) is a disease of the gastrointestinal tract with periods of relapses and remission [1]. Its pathogenesis includes immunological, genetic, and environmental factors [2]. The overwhelming levels of pro-oxidants over antioxidants [3] cause cell membrane lipid peroxidation and severe colonic inflammation [4,5], characterized by migration of neutrophils, basophils with the release of tumor necrosis factor (TNF), interleukins (ILs), and interferons [6], causing increased apoptosis at the sites of acute inflammation [7]. There is accelerated neonate epithelial cell apoptosis and decreased inflammatory cell apoptosis. This causes colonic tissue injury and disturbed intestinal functions [8,9].

High expression of the Fas ligand in active UC and activation of the Fas receptor activate caspase 8, which in turn activates caspase 3, leading to apoptosis [10,11]. Caspase 3 can also be activated by another pathway regulated by the B-cell lymphoma 2 (*Bcl-2*) family [12,13]. The *Bcl-2* gene is an antiapoptotic gene [14]. Apoptosis regulator Bax is a protein that forms a dimer with *Bcl-2* to inhibit its function, and therefore acts as an apoptotic activator [15]. When the expression of Bax dominates, apoptosis occurs, and when the expression of *Bcl-2* increases cells continue to thrive [16].

Antioxidant enzymes such as superoxide dismutase (SOD) and sulfydryl groups play a major role in

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defense against free radicals overproduction. Antioxidants may be therapeutic potentials in managing UC [17].

Nebivolol, a long-acting, third-generation, selective β1-adrenoceptor antagonist promotes nitric oxide (NO)-induced vasodilatation through endothelial nitric oxide synthase (eNOS)-mediated β3-adrenoceptor activation [18]. It exhibits antioxidant effects by decreasing superoxide production through inhibition of NADPH oxidase and prevention of eNOS uncoupling [19]. Consequently, nebivolol inhibits nuclear factor-κB activation, leading to decreased proinflammatory cytokines [20]. Nebivolol shows a potent antiapoptotic property through its NO-regulating effect [21]. It is an attractive candidate for protection against UC. Despite this, it is still not well investigated in UC. In this study, the effects of nebivolol on a rat experimental model of UC were investigated.

Materials and methods

Chemicals

Acetic acid (AA) from CID Pharmaceutical Co. (Giza, Egypt), nebivolol and sulfasalazine from Sigma-Aldrich Company (St Louis, Missouri, USA), and myeloperoxidase (MPO) from eBioscience (San Diego, California, USA), were purchased. IL-1β and TNF-α enzyme-linked immunosorbent assay kits from R&D Systems (Minneapolis, MN, USA), thiobarbituric acid reactive substances (TBARS) assay kit from ZeptoMetrix Inc. (NY, USA), and reduced glutathione (GSH) kit from Biodiagnostic (Giza, Egypt), were used for analyses. All chemicals were of analytical grade and obtained from commercial sources.

Animals

Adult, healthy, mature male Wistar-albino rats, weighing 150–180 g, matched for age and weight, were used after 1 week of acclimatization to the conditions of the animal house at the Ophthalmology Institute. Animals were maintained at a constant temperature (24±1°C), with 55% humidity, on a12-h light–dark cycle with free access to standard rat chow and water. Animals were handled according to the guidelines for animal care approved by the Ethical Committee of the Ophthalmology Institute.

Induction of experimental colitis

UC was induced by intrarectal administration of AA, as described by Mascolo *et al*. [22]. After an overnight fast, rats were lightly anesthetized with ether. A (2.7 mm) soft pediatric catheter was lubricated with gel and inserted into the rat's colonic lumen through the anus. Two milliliters (4% v/v) of AA in 0.9% saline was slowly infused into the distal colon, and rats were maintained in the Trendelenburg position for 30 s to limit AA leakage. Rats in the normal control group were treated similarly, but instead of 4% AA, they received an equal volume of 0.9% saline by intrarectal infusion.

Experimental design

A total of 30 healthy rats were used for this study. They were randomly allocated into the following five groups (six animals in each). Group I, the normal negative control group, received distilled water for 11 days. Group II, the AA-ulcerated positive control group, received AA intrarectally on eighth day and were sacrificed 3 days later. Group III, the nebivolol-treated group, received nebivolol (5 mg/kg/day) [23] +AA (intrarectally). Group IV, the nebivolol-treated group, received nebivolol (10 mg/kg/day) [24]+AA (intrarectally). Group III and IV received nebivolol for 7 days before induction of colitis. On the eighth day, colitis was induced. Next, nebivolol treatment in either dose was continued till the 11th day. Group V, the sulfasalazine-treated group, received sulfasalazine, 500 mg/kg/day [25]+AA (intrarectally), was our reference drug group. Sulfasalazine was not given as pretreatment. It was given on the eighth day (of colitis induction) and continued for 3 days after that. Drugs were freshly prepared daily, dissolved in distilled water, and administered by oral gavage. On the 11th day, blood samples were collected from animals. Serum was separated and stored at −80°C until analyzed to measure serum levels of TBARS, SOD, IL-1β, and TNF-α. Subsequently, on the same day, animals were killed under deep anesthesia [22,26]. The colon was dissected from each rat, and the specimens were maintained in 10% formalin for histopathological studies. The remaining colonic tissues were maintained at −80°C (ultra-low freezer, Environmental Equipment, Cincinnati, Ohio, USA). Tissue samples were homogenized in 10-mmol Tris-HCl buffer for measuring TBARS, MPO, SOD, GSH, and caspase 3.

Assessment of colitis

Macroscopic colonic damage scoring

Mucosal damage was assessed macroscopically by the scoring system of Millar *et al*. [27]. Colonic samples were obtained from the distal 5 cm. Macroscopic inflammation scores were assigned using a scale ranging from 0 to 4: 0 indicates no macroscopic changes, 1 indicates mucosal erythema only, 2 indicates mild mucosal edema, slight bleeding, or small erosions, 3 indicates moderate edema, bleeding

ulcers, or erosions, and 4 indicates severe ulceration, erosions, edema, and tissue necrosis.

Colonic wet weight-to-body weight ratio

Weight of the distal 8 cm of the colon was measured. The ratio of wet colonic weight to body weight was calculated (mg/g). It was used as a parameter to assess the degree of edema and severity of colitis [28].

Histopathological study

Two-centimeter portions of the injured mucosal segment were evaluated by light microscopy. Cross-sections of colonic tissues were fixed in 10% formaldehyde, embedded in paraffin wax blocks, and cut using a microtome. Samples were collected on glass slides, stained with hematoxylin and eosin (H&E), mounted, and observed microscopically by a pathologist in a blinded manner. Additional sections from the paraffin wax blocks were stained with Alcian blue dye. Histopathological slides were examined for destruction of the epithelium and glands, dilatation of glandular crypts, depletion and loss of goblet cells, inflammatory cells infiltration, edema, hemorrhagic mucosa, and crypt abscesses using parameters scored from 0 to 3. The colitis score of each rat represents the sum of the subscores of different parameters [29].

Biochemical studies

Estimation of colonic TBARS and GSH levels: The colonic content of the lipid peroxide product malondialdehyde was measured using the assay kit of TBARS as described by Parlakpinar *et al.* [30], whereas GSH was measured as described by Sahna *et al.* [31]. The results are expressed in nmol/g.

Measurement of colonic MPO levels: MPO activity was measured as described by Krawisz *et al.* [32]. The results are expressed in ng/g.

Estimation of colonic SOD activity: The enzymatic activity of SOD was measured in the postmitochondrial supernatant of the colon homogenate as described by Kono [33]. The results are expressed in U/g.

Gene expression of caspase 3: RNA was isolated from 100 mg of tissue, using a RNA extraction kit (Qiagene, USA) according to the manufacturer's instructions. First-strand complementary DNA was synthesized from 2 μg total RNA using the superscript first-strand synthesis system (Invitrogen Inc., Carlsbad, California, USA). After denaturing the template RNA and primers (25 pmol of each reverse oligonucleotide primer) at 70°C for 10 min, 40 U reverse transcriptase was added in the presence of RT buffer, 4 μl dNTP mix (250 μmol/l each), 40 U RNase inhibitor, and RNase-free water to achieve the final volume. The reaction mixture (50 μl) was incubated at 43°C for 1 h, then stopped at 4°C, and used immediately for PCR or kept at −80°C until use. Reactions were carried out in triplicate. The reaction conditions were as follows: an initial 15 min at 95°C, followed by 40 cycles of 15 s at 94°C, 30 s at 55–60°C, and 30 s at 72°C. Real-time PCR was carried out in an ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, California, USA). Relative gene expression was calculated by using the fx1 method, where CT is the threshold cycle [34].

Measurement of serum levels of IL-1β and TNF-α: serum levels of IL-1β and TNF-α were evaluated using murine enzyme-linked immunosorbent assay kits according to the manufacturer's instructions and the values were expressed as pg/ml.

Measurement of serum levels of SOD: serum levels of SOD were determined using a SOD assay kit. It contained all reagents required for determining SOD. Values are expressed in U/ml.

Measurement of serum levels of TBARS: TBARS serum levels were estimated as described by Dubovskiy *et al.* [35]. The results are expressed in nmol/ml.

Statistical analysis

Data were statistically described in terms of mean±SD and compared using the Mann–Whitney *U*-test for independent samples. *P* values less than 0.05 were considered to be statistically significant, whereas *P* values less than 0.01 were considered to be highly significant. All statistical calculations were performed using the computer program SPSS (Statistical Package for the Social Sciences; SPSS Inc., Chicago, Illinois, USA) release 15 for Microsoft Windows (2006).

Results

Effects on macroscopic examination

AA caused severe edematous inflammation in the colon, with significantly higher macroscopic scoring of colonic damage compared with the control group. The mucosa appeared ulcerated, edematous, and hemorrhagic (P=0.002). Nebivolol (5 and 10 mg/kg/day) and sulfasalazine (500 mg/kg/day) significantly reduced the severity of gross lesion score compared with the AA group (P=0.01, 0.003, and 0.002, respectively). There was a significant difference between results with the administration of nebivolol

5 mg/kg/day and nebivolol 10 mg/kg/day (P=0.02). In addition, a highly significant difference was found between the sulfasalazine and nebivolol 5 mg/kg/day groups (P=0.002). Meanwhile, no significant difference was found between treatment by sulfasalazine and treatment by nebivolol 10 mg/kg/day (P=0.6) (Table 1 and Fig. 1).

Effects on colon weight per body weight (mg/g)

AA significantly increased the colon weight/body weight compared with the control group (P=0.004). On the other hand, treatment with nebivolol (5 and 10 mg/kg/day) and sulfasalazine significantly reduced colon weights compared with the AA group (P=0.004). However, the reduction by sulfasalazine was highly significant compared with nebivolol 5 and 10 mg/kg/day (P=0.004). The results of the nebivolol 5 mg/kg/day and nebivolol 10 mg/kg/day groups showed a highly significant difference (P=0.004) (Table 2 and Fig. 2).

Effects on histopathological changes

The macroscopic findings of all groups were emphasized by their histopathological examinations (Fig. 3). The histopathological scoring of all groups is illustrated in Table 3. The histopathological scoring of the AA group was significantly increased compared with the control group (P=0.002), whereas that of the nebivolol-treated (10 and 5 mg/kg/day) and sulfasalazine-treated groups were significantly decreased compared with the AA group (P=0.003).

Table 1 Effects on macroscopic scoring in AA-induced ulcerative colitis in albino rats

Groups	Macroscopic scoring
Group 1: normal negative control	0.0±0.0
Group 2: AA, positive control	3.7±0.5^{**a}
Group 3: nebivolol (5 mg/kg/day)+AA	2.5±0.5^{*bc}
Group 4: nebivolol (10 mg/kg/day)+AA	1.5±0.5^{**b}
Group 5: sulfasalazine+AA	1.00±0.0^{**bd}

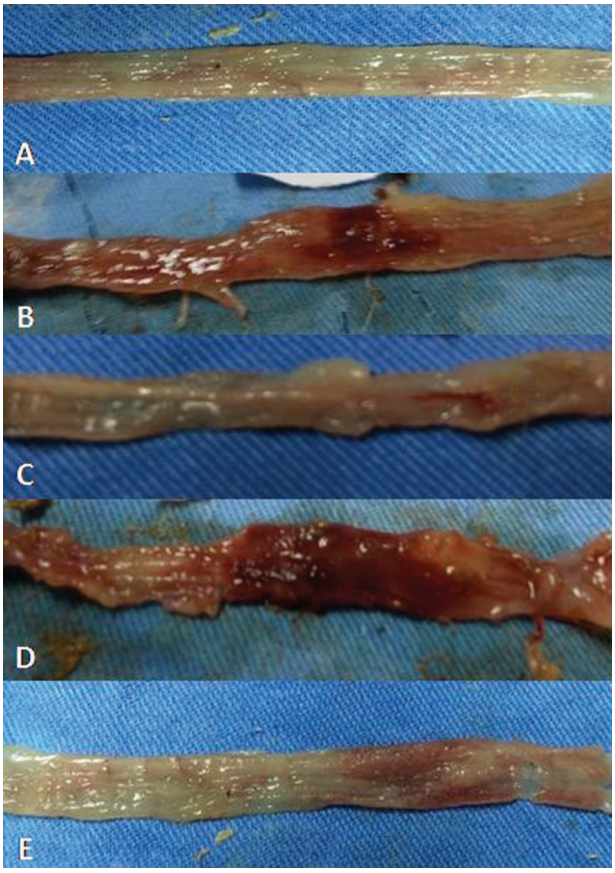
n=6, data are expressed as mean±SD. AA, acetic acid. [a]Compared with the control group. [b]Compared with the AA group. [c]Compared with nebivolol (10 mg/kg/day) group. [d]Compared with nebivolol (5 mg/kg/day) group. $^{*}P<0.05$, significant. $^{**}P<0.01$, highly significant.

Table 2 Effects on colon weight/body weight (mg/g) in rats of different groups of AA-induced colitis

Groups	Colonic weight/body weight ratio (mg/g)
Group 1: normal, negative control	2.2±0.1
Group 2: AA, positive control	11.3±0.4^{**a}
Group 3: nebivolol (5 mg/kg/day)+AA	9.7±0.4^{**bc}
Group 4: nebivolol (10 mg/kg/day)+AA	4.31±0.04^{**bd}
Group 5: sulfasalazine+AA	3.8±0.06^{**be}

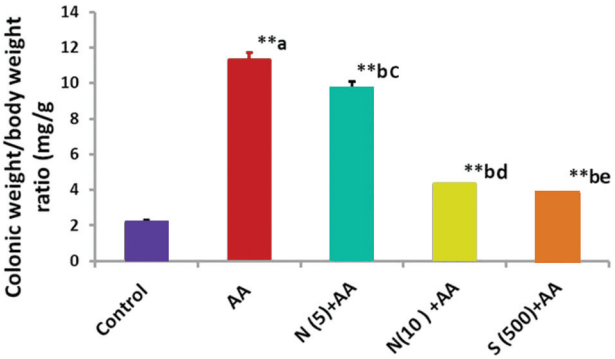
n=6, data are expressed as mean±SD. AA, acetic acid. [a]Compared with the control group. [b]Compared with the AA group. [c]Compared with nebivolol (10 mg/kg/day) group. [d]Compared with sulfasalazine group. [e]Compared with nebivolol (5 mg/kg/day) group. $^{**}P<0.01$, highly significant.

Figure 1



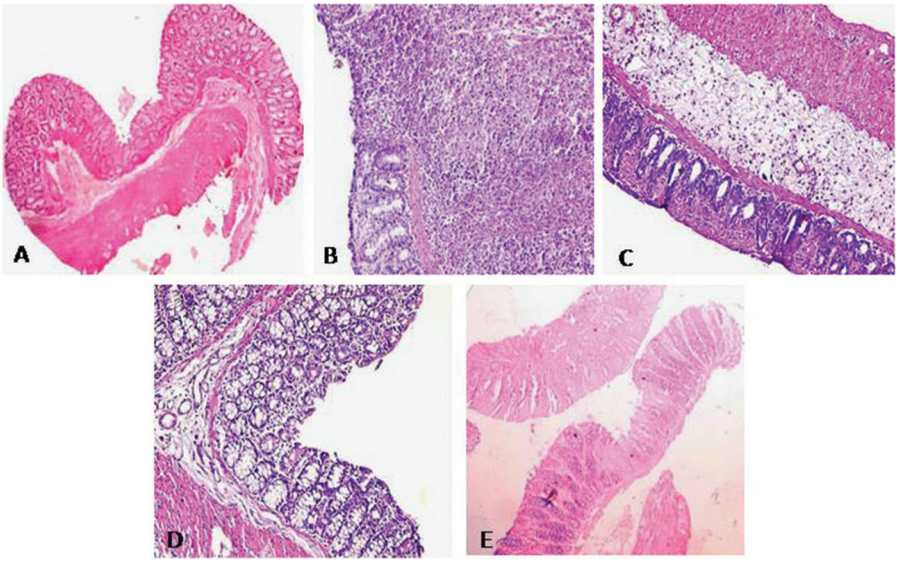
Macroscopic appearance of the colonic mucosa: (a) normal negative control group, (b) AA positive control group, (c) nebivolol 5 mg/kg/day pretreated group, (d) nebivolol 10 mg/kg/day pretreated group, and (e) sulfasalazine 500 mg/kg/day treated group. AA, acetic acid.

Figure 2



Effects on colonic weight/body weight ratio in AA-induced ulcerative colitis in rats. Values are represented as mean±SD for a group of six rats each. $^{*}P<0.05$ (significant), $^{**}P<0.01$ (highly significant), (a) compared with the control group, (b) compared with the AA group, (c) compared with N(10), (d) compared with S, and (e) compared with N5. AA, acetic acid; N, nebivolol; and S, sulfasalazine.

Figure 3



Histopathological sections of colons from rats stained with H&E (a–e): (a) normal colonic tissue stained with H&E (×50), (b) AA-induced colitis stained with H&E (×200), (c) nebivolol-treated group (10 mg/kg/day) stained with H&E (×100), (d) sulfasalazine-treated group stained with H&E (×100), and (e) nebivolol-treated group (5 mg/kg/day) stained with Alcian blue (×50). AA, acetic acid.

Table 3 Effects on histopathological changes of colonic tissues of rats with AA-induced UC

Groups	Histopathological scoring
Group 1: normal, control	0.0±0.0
Group 2: AA, positive control	17.3±0.8^{**a}
Group 3: nebivolol (5 mg/kg/day)+AA	9.5±0.5 b^{**bc}
Group 4: nebivolol (10 mg/kg/day)+AA	2.2±0.4^{**b}
Group 5: sulfasalazine+AA	2.0±0.6^{**bd}

n=6, data are expressed as mean±SD. AA, acetic acid; UC, ulcerative colitis. [a]Compared with the control group. [b]Compared with the AA group. [c]Compared with nebivolol (10 mg/kg/day) group. [d]Compared with nebivolol (5 mg/kg/day) group. $^{**}P<0.01$, highly significant.

The results of the nebivolol 5 mg/kg/day and nebivolol 10 mg/kg/day groups showed a highly significant difference (P=0.002). In addition, a highly significant difference was found between the sulfasalazine and the nebivolol 5 mg/kg/day groups (P=0.003). Meanwhile, no significant difference was found between the results of the sulfasalazine and the nebivolol 10 mg/kg/day groups (P=0.6).

Effects on biochemical studies

Effects on tissue glutathione content

Colonic GSH content was significantly inhibited in the colon tissues of the AA group compared with the normal control group (P=0.004). Treatment of rats with both nebivolol doses (5 and 10 mg/kg) and sulfasalazine improved the reduced levels of GSH compared with the AA group (P=0.004). However, the improvement by sulfasalazine was highly significant compared with nebivolol 5 and 10 mg/kg/day (P=0.004). The results of the nebivolol 5 mg/kg/day and nebivolol 10 mg/kg/day groups showed a highly significant difference (P=0.004) (Fig. 4a).

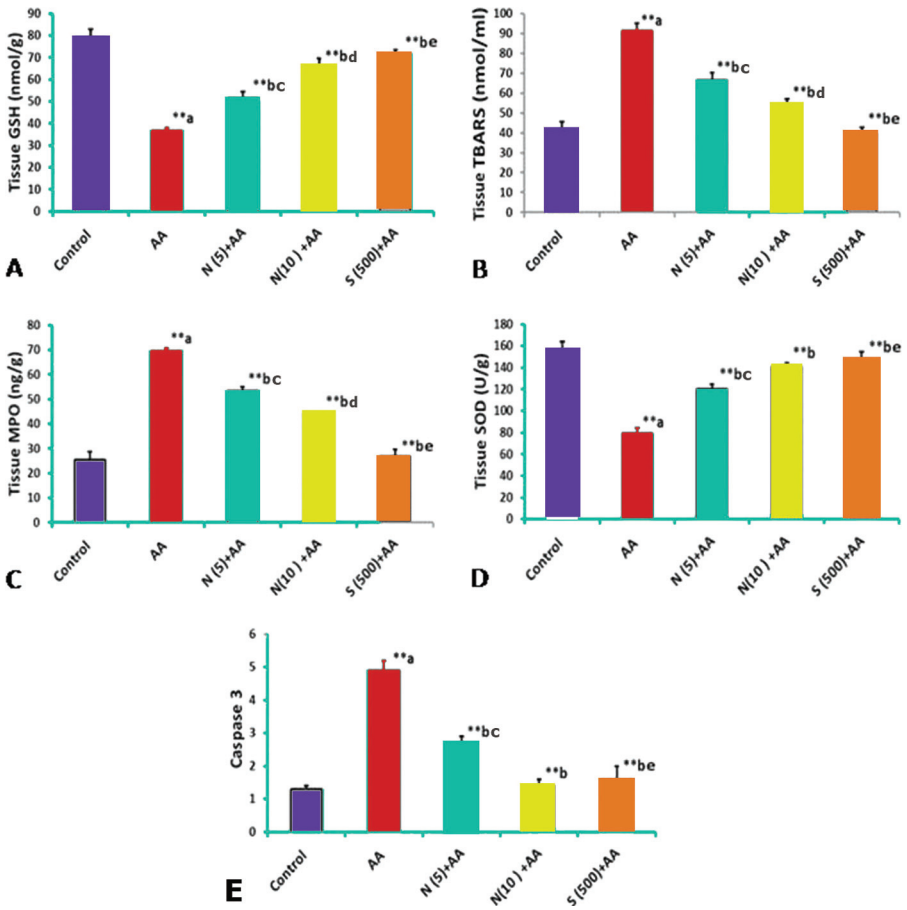
Effects on tissue thiobarbituric acid reactive substances concentration

Colonic levels of TBARS were found to be significantly increased in the AA group compared with the control group (P=0.004). Pretreatment of rats with nebivolol (5 and 10 mg/kg) and sulfasalazine significantly inhibited elevation of malondialdehyde compared with the AA group (P=0.004). However, the inhibition by sulfasalazine was highly significant compared with nebivolol 5 and 10 mg/kg/day (P=0.004). The results of the nebivolol 5 mg/kg/day and nebivolol 10 mg/kg/day groups showed a highly significant difference (P=0.004) (Fig. 4b).

Effects on tissue myeloperoxidase concentrations

In the AA group, there was increased colonic MPO concentration compared with the control group (P=0.004). Treatment with nebivolol (5 and 10 mg/kg/day) and sulfasalazine significantly inhibited the elevation of MPO activity compared with the AA group (P=0.004). However, the inhibition by sulfasalazine was highly significant compared with nebivolol 5 and 10 mg/kg/day (P=0.004). The results of the nebivolol 5 mg/kg/day and nebivolol 10 mg/kg/day groups showed a highly significant difference (P=0.004) (Fig. 4c).

Figure 4



Mean±SD of colonic tissue GSH (a), TBARS (b), MPO (c), SOD (d), and caspase 3 (e) between the study groups (n=6) [$^{*}P<0.05$ (significant), $^{**}P<0.01$ (highly significant)] (a) compared with the control group, (b) compared with the AA group, (c) compared with N(10), (d) compared with S, (e) compared with N5. AA, acetic acid; GSH, glutathione; MPO, myeloperoxidase; N, nebivolol; S, sulfasalazine; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substance.

Effects on tissue superoxide dismutase activity

SOD activities were significantly decreased in the AA group compared with control animals (P=0.004). Treatment with nebivolol (5 and 10 mg/kg/day) and sulfasalazine showed a significant increase in SOD activity compared with the AA group (P=0.004). The results of the nebivolol 5 mg/kg/day and nebivolol 10 mg/kg/day groups showed a highly significant difference (P=0.004). In addition, a highly significant difference was found between the sulfasalazine group and the nebivolol 5 mg/kg/day group (P=0.004). Meanwhile, the results of the sulfasalazine group and the nebivolol 10 mg/kg/day group were comparable (P=0.1) (Fig. 4d).

Effects on caspase 3 gene expression

Caspase 3 proteins were highly upregulated in the AA group compared with the control group (P=0.004). This upregulation was significantly decreased by nebivolol (5 and 10 mg/kg/day) and sulfasalazine compared with the AA group (P=0.004). The results of the nebivolol 5 mg/kg/day and nebivolol 10 mg/kg/day groups showed a highly significant difference (P=0.003). In addition, a highly significant difference was found between the sulfasalazine group and the nebivolol 5 mg/kg/day group (P=0.004). Meanwhile, no significant difference was found between treatment by sulfasalazine and treatment by nebivolol 10 mg/kg/day (P=0.4) (Fig. 4e).

Effects on serum tumor necrosis factor-α

Serum TNF-α levels in the AA group were significantly increased compared with the control group (P=0.004). However, they were significantly lower in the nebivolol-treated (5 and 10 mg/kg) and sulfasalazine-treated groups compared with the AA-induced colitis group (P=0.004). The results of nebivolol 5 mg/kg/day and nebivolol 10 mg/kg/day groups showed a highly significant difference (P=0.004). In addition, a highly significant difference was found between the sulfasalazine and the nebivolol 5 mg/kg/day groups (P=0.004). Meanwhile, the results of the sulfasalazine and

nebivolol 10 mg/kg/day groups were comparable (P=0.5) (Fig. 5a).

Effects on serum interleukin-1β

Serum IL-1β levels of the AA group were significantly increased compared with the control group (P=0.004). However, they were significantly lower in the nebivolol-treated (5 and 10 mg/kg) and sulfasalazine-treated groups, respectively, compared with the AA-induced colitis group (P=0.004). Nevertheless, the inhibition by sulfasalazine was highly significant compared with nebivolol 5 and 10 mg/kg/day (P=0.004). The results of the nebivolol 5 mg/kg/day and nebivolol 10 mg/kg/day groups showed a highly significant difference (P=0.004) (Fig. 5b).

Effects on serum superoxide dismutase

Serum SOD levels in the AA group were significantly decreased compared with the control group (P=0.004). This decrease in SOD was significantly prevented by nebivolol (5 and 10 mg/kg) and sulfasalazine compared with the AA group (P=0.004). However, the effect by sulfasalazine was highly significant compared with nebivolol 5 and 10 mg/kg/day (P=0.004). The results of the nebivolol 5 mg/kg/day and nebivolol 10 mg/kg/day groups showed a highly significant difference (P=0.004) (Fig. 5c).

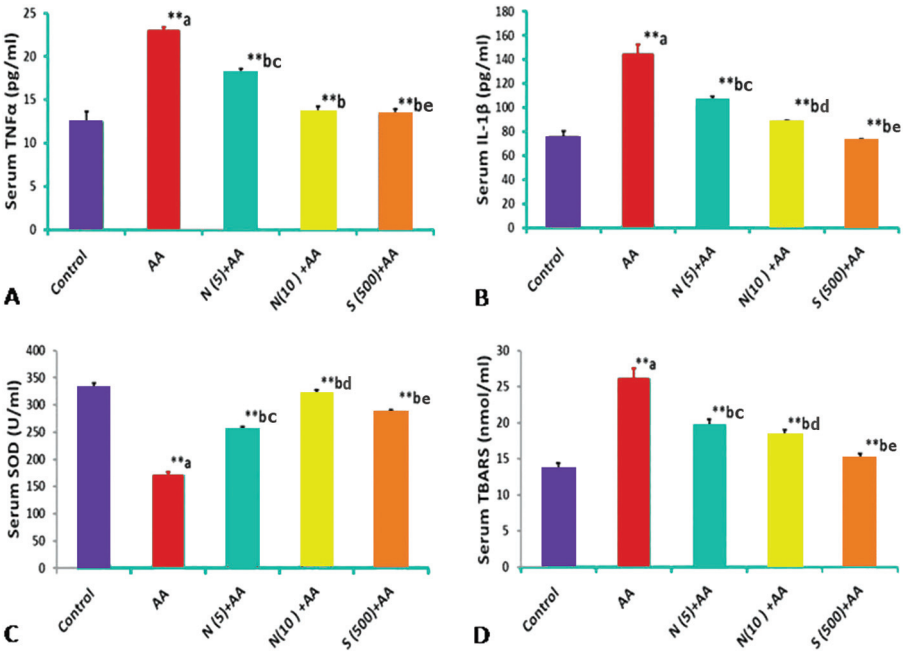
Effects on serum thiobarbituric acid reactive substances

Serum TBARS levels in the AA group was significantly increased compared with the control group (P=0.004). This increase was significantly prevented by pretreatment with either nebivolol (5 and 10 mg/kg) or sulfasalazine, compared with the AA group (P=0.004). However, the effect of sulfasalazine was highly significant compared with nebivolol 5 and 10 mg/kg/day (P=0.004). There was a significant difference between results of the nebivolol 5 mg/kg/day group and the nebivolol 10 mg/kg/day group (P=0.045) (Fig. 5d).

Discussion

The experimental model of AA-induced colitis in rats resembles human UC [36], where oxidative stress plays a major role [37]. In our study, AA caused significant elevation in serum levels and colonic contents of TBARS and decreased colonic contents of GSH. This was previously confirmed by Cetinkaya *et al.* [4] in the same animal model. Our results showed that nebivolol (5 and 10 mg/kg/day) increased GSH and decreased colonic TBARS levels. This is in agreement with the findings of Morsy and Heeba [24] who proved that nebivolol significantly reduced renal levels of TBARS and increased renal levels of reduced GSH compared with untreated rats. Our results were similar to Toblli *et al.* [38] who

Figure 5



Mean±SD of serum TNF-α (a), IL-1 (b), SOD (c), and TBARS (d) between the study groups (n=6) [$^{*}P<0.05$ (significant), $^{**}P<0.01$ (highly significant)] (a) compared with the control group, (b) compared with the AA group, (c) compared with N(10), (d) compared with S, and (e) compared with N5. AA, acetic acid; IL-1, interleukin-1; N, nebivolol; S, sulfasalazine; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substance; TNF-α, tumor necrosis factor-α.

reported that nebivolol increased renal levels of reduced GSH and normalized renal TBARS levels in rats.

Our findings were supported by Morsy and Heeba [39], who demonstrated that nebivolol effectively protected against cold stress-induced gastric ulcers, probably by its antioxidant activity as evidenced by significant decreases in gastric mucosal TBARS concentration. Similarly Khan *et al.* [40] showed that nebivolol protected against lipid peroxidation. Mohammad [41] studied the effect of nebivolol on methotrexate-induced liver toxicity. He reported that nebivolol reduced TBARS and increased GSH levels. Similarly, Akgullu *et al.* [42] found that nebivolol pretreatment of rats showing hyperhomocysteinemia-induced oxidative stress significantly decreased the high TBARS levels and increased the GSH levels in the heart, liver, brain, and kidney tissues. The inhibitory effect of nebivolol on lipid peroxidation could be a consequence of its antioxidant activity and/or its suppressing effect on iNOS expression. Fatani *et al.* [36] studied the effect of carvedilol pretreatment on a AA-colitis model and reported similar findings. Carvedilol was the first third-generation β-blocker with antioxidant and free radical scavenger activity [43]. Its antioxidant property resides in its carbazole moiety, partly shared by nebivolol [44].

In the AA group, the colonic SOD activity was severely reduced because of oxidative stress. Similar findings were noticed by Aleisa *et al.* [45] who worked on a similar animal model. In our study, nebivolol pretreatment at both doses prevented the AA-induced reduction in colonic SOD. On the contrary, Ilhan *et al.* [46] found that nebivolol treatment prevented the increase in SOD activities produced by ischemia/reperfusion (I/R) in spinal cord tissue. Gideroglu *et al.* [47] proved the protective effect of nebivolol on skin flap survival in tissue injury, where SOD enzyme activity in the nebivolol group was found to be significantly higher than in the control group. The protective effect of nebivolol is mostly due to direct ROS-scavenging action [48,49]. Nebivolol exhibited antioxidant effects by reducing superoxide production by inhibiting NADPH oxidase and preventing eNOS uncoupling. Another possible mechanism for the drug's efficacy is acting primarily within the mitochondria at the site of ROS production or as a ROS scavenger, inhibiting superoxide anion or hydrogen peroxide.

In the AA group, there was significant elevation in serum TNF-α and IL-1β when compared with the control group. This was previously reported by Tahan *et al.* [50]. In our study, pretreatment of the animals with nebivolol (5 and 10 mg/kg/day) significantly decreased TNF- α and IL-1β. Similarly, Morsy and Heeba [24] showed that nebivolol significantly reduced renal levels of TNF-α, compared with untreated rats. The effect of nebivolol on TNF-α level is consistent with the results of Garbin *et al.* [51] who found that nebivolol significantly reduced oxidative stress-induced TNF-α gene expression in human umbilical vein. Furthermore, nebivolol downregulated TNF-α gene expression in human coronary sites as reported by Wolf *et al.* [20]. The observed anti-inflammatory effect of nebivolol in the present study, by decreasing MPO, was also reported by Münzel and Gori [19]. In addition, Ilhan *et al.* [46] found that nebivolol treatment prevented the increase of MPO in spinal cord tissue produced by I/R.

In the AA group, caspase 3 protein expression was upregulated. This can be explained by the results of Kaushal *et al.* [52] who reported that binding of TNF-α to its receptors activates receptor-dependent pathways of apoptosis through activation of caspases including caspase 3. Our results showed that nebivolol pretreatment by either dose decreased caspase 3 protein expression. Similarly, Morsy and Heeba [24] showed that nebivolol significantly reduced renal levels of caspase 3 compared with untreated rats. In addition, Uzar *et al.* [53] reported that nebivolol had antiapoptotic effects by decreasing caspase 3 immunoreactivity in cerebral I/R in rats. Nebivolol treatment prevented the total oxidant status, malondialdehyde levels, from increasing in brains of rats exposed to I/R. In conclusion, nebivolol protected rats from ischemia-induced brain injury. This may be due to the indirect prevention of oxidative stress and apoptosis. Similarly, nebivolol was found to reduce the expressions of proadhesion and inflammatory molecules on the endothelial wall and reduce apoptosis and measures of oxidative stress [20,54]. These findings are consistent with the results Gandhi *et al.* [55] who showed that nebivolol reduced inflammation and apoptosis after renal I/R injury and that nebivolol had potent antiapoptotic and anti-inflammatory properties due to its NO-releasing property. Some β-blockers were also shown to prevent myocardial apoptosis [56,57]. Although the mechanism is not fully clear, in-vitro studies have shown that noradrenaline (via β1 receptors) activates caspases that are basic apoptotic enzymes [58].

Histopathological examination confirmed the previous results. The AA group showed a significant increase in

animals' colon weights. In AA-induced colitis, the wet weight of the inflamed colon was considered an indicator of the grade of inflammation [59]. This was confirmed by histopathological findings as severe tissue ulceration and dense inflammatory infiltrate. This was in agreement with El-Abhar *et al*. [60]. Our results showed that the pretreatment with either dose of nebivolol significantly reduced colonic weight, indicating decreased colon inflammation. The protective effect of nebivolol may be attributed to its vasodilator activity and endothelium protection power [19,61].

Conclusion

Our study showed a dose-dependent protective activity of nebivolol against experimentally induced UC by AA in Wistar rats. This could be attributed to its anti-inflammatory, antioxidant, and antiapoptotic properties. Nebivolol can be promising in the treatment of UC, ameliorating its severity. Additional studies with larger numbers of animals are required to support these findings and assess other parameters, as well as compare nebivolol with drugs in the same class with clarification of its antiapoptotic and anti-inflammatory mechanisms of action with clinical evaluation of its use in UC.

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Conflicts of interest

There are no conflicts of interest.

References

1 Baumgart DC, Sandborn WJ. Inflammatory bowel disease: clinical aspects and established and evolving therapies. Lancet 2007; 369:1641–1657.

2 Olden KW. Diagnosis of irritable bowel syndrome. Gastroenterology 2002; 122:1701–1714.

3 Dröge W. Free radicals in the physiological control of cell function. Physiol Rev 2002; 82:47–95.

4 Cetinkaya A, Bulbuloglu E, Kurutas EB, Ciralik H, Kantarceken B, Buyukbese MA. Beneficial effects of N-acetylcysteine on acetic acid-induced colitis in rats. Tohoku J Exp Med 2005; 206:131–139.

5 Cetinkaya A, Bulbuloglu E, Kantarceken B, Ciralik H, Kurutas EB, Buyukbese MA, Gumusalan Y. Effects of L-carnitine on oxidant/antioxidant status in acetic acid-induced colitis. Dig Dis Sci 2006; 51:488–494.

6 Reiff C, Kelly D. Inflammatory bowel disease, gut bacteria and probiotic therapy. Int J Med Microbiol 2010; 300:25–33.

7 Hagiwara C, Tanaka M, Kudo H. Increase in colorectal epithelial apoptotic cells in patients with ulcerative colitis ultimately requiring surgery. J Gastroenterol Hepatol 2002; 17:758–764.

8 Qiu W, Wu B, Wang X, Buchanan ME, Regueiro MD, Hartman DJ, *et al.* PUMA-mediated intestinal epithelial apoptosis contributes to ulcerative colitis in humans and mice. J Clin Invest 2011; 121:1722–1732.

9 Dieckgraefe BK, Crimmins DL, Landt V, Houchen C, Anant S, Porche-Sorbet R, Ladenson JH. Expression of the regenerating gene family in inflammatory bowel disease mucosa: Reg Ialpha upregulation, processing, and antiapoptotic activity. J Investig Med 2002; 50:421–434

10 Sträter J, Wellisch I, Riedl S, Walczak H, Koretz K, Tandara A, *et al.* CD95 (APO-1/Fas)-mediated apoptosis in colon epithelial cells: a possible role in ulcerative colitis. Gastroenterology 1997; 113:160–167.

11 Banks C, Bateman A, Payne R, Johnson P, Sheron N. Chemokine expression in IBD. Mucosal chemokine expression is unselectively increased in both ulcerative colitis and Crohn's disease. J Pathol 2003; 199:28–35.

12 Sturm A, Leite AZ, Danese S, Krivacic KA, West GA, Mohr S, *et al.* Divergent cell cycle kinetics underlie the distinct functional capacity of mucosal T cells in Crohn's disease and ulcerative colitis. Gut 2004; 53:1624–1631.

13 Ueyama H, Kiyohara T, Sawada N, Isozaki K, Kitamura S, Kondo S, *et al.* High Fas ligand expression on lymphocytes in lesions of ulcerative colitis. Gut 1998; 43:48–55.

14 Wu HG, Gong X, Yao LQ, Zhang W, Shi Y, Liu HR, *et al.* Mechanisms of acupuncture and moxibustion in regulation of epithelial cell apoptosis in rat ulcerative colitis. World J Gastroenterol 2004; 10:682–688.

15 Scorrano L, Korsmeyer SJ. Mechanisms of cytochrome c release by proapoptotic *BCL-2* family members. Biochem Biophys Res Commun 2003; 304:437–444.

16 Iimura M, Nakamura T, Shinozaki S, Iizuka B, Inoue Y, Suzuki S, Hayashi N. Bax is downregulated in inflamed colonic mucosa of ulcerative colitis. Gut 2000; 47:228–235.

17 Lih-Brody L, Powell SR, Collier KP, Reddy GM, Cerchia R, Kahn E, *et al.* Increased oxidative stress and decreased antioxidant defenses in mucosa of inflammatory bowel disease. Dig Dis Sci 1996; 41:2078–2086.

18 Gupta S, Wright HM. Nebivolol: a highly selective beta1-adrenergic receptor blocker that causes vasodilation by increasing nitric oxide. Cardiovasc Ther 2008; 26:189–202.

19 Münzel T, Gori T. Nebivolol: the somewhat-different beta-adrenergic receptor blocker. J Am Coll Cardiol 2009; 54:1491–1499.

20 Wolf SC, Sauter G, Jobst J, Kempf VA, Risler T, Brehm BR. Major differences in gene expression in human coronary smooth muscle cells after nebivolol or metoprolol treatment. Int J Cardiol 2008; 125:4–10.

21 Mercanoglu G, Safran N, Gungor M, Pamukcu B, Uzun H, Sezgin C, *et al.* The effects of nebivolol on apoptosis in a rat infarct model. Circ J 2008; 72:660–670.

22 Mascolo N, Izzo AA, Autore G, Maiello FM, Di Carlo G, Capasso F. Acetic acid-induced colitis in normal and essential fatty acid deficient rats. J Pharmacol Exp Ther 1995; 272:469–475.

23 Heeba GH, El-Hanafy AA. Nebivolol regulates eNOS and iNOS expressions and alleviates oxidative stress in cerebral ischemia/reperfusion injury in rats. Life Sci 2012; 90:388–395.

24 Morsy MA, Heeba GH. Nebivolol ameliorates cisplatin-induced nephrotoxicity in rats. Basic Clin Pharmacol Toxicol 2016.

25 Deshmukh CD, Veeresh B, Pawar AT. Protective effect of *Emblica officinalis* fruit extract on acetic acid induced colitis in rats. J Herbal Med Toxicol 2010; 4:83–87.

26 Kannan N, Guruvayoorappan C. Protective effect of *Bauhinia tomentosa* on acetic acid induced ulcerative colitis by regulating antioxidant and inflammatory mediators. Int Immunopharmacol 2013; 16:57–66.

27 Millar AD, Rampton DS, Chander CL, Claxson AW, Blades S, Coumbe A, *et al.* Evaluating the antioxidant potential of new treatments for inflammatory bowel disease using a rat model of colitis. Gut 1996; 39: 407–415.

28 Zhou SY, Mei QB, Liu L, Guo X, Qiu BS, Zhao DH, Cho CH. Delivery of glucocorticoid conjugate in rat gastrointestinal tract and its treatment for ulcerative colitis. Acta Pharmacol Sin 2001; 22:761–764.

29 Gaudio E, Taddei G, Vetuschi A, Sferra R, Frieri G, Ricciardi G, Caprilli R. Dextran sulfate sodium (DSS) colitis in rats: clinical, structural, and ultrastructural aspects. Dig Dis Sci 1999; 44:1458–1475.

30 Parlakpinar H, Ozer MK, Sahna E, Vardi N, Cigremis Y, Acet A. Amikacin-induced acute renal injury in rats: protective role of melatonin. J Pineal Res 2003; 35:85–90.

31 Sahna E, Parlakpinar H, Ozturk F, Cigremis Y, Acet A. The protective effects of physiological and pharmacological concentrations of melatonin on renal ischemia-reperfusion injury in rats. Urol Res 2003; 31:188–193.

32 Krawisz JE, Sharon P, Stenson WF. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Assessment of inflammation in rat and hamster models. Gastroenterology 1984; 87:1344–1350.

33 Kono Y. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Arch Biochem Biophys 1978; 186:189–195.

34 Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2– ΔΔCT method. Methods 2001; 25:402–408.

35 Dubovskiy IM, Martemyanov VV, Vorontsova YL, Rantala MJ, Gryzanova EV, Glupov VV. Effect of bacterial infection on antioxidant activity and lipid peroxidation in the midgut of *Galleria mellonella* L. larvae (Lepidoptera, Pyralidae). Comp Biochem Physiol C Toxicol Pharmacol 2008; 148:1–5.

36 Fatani AJ, Al-Hosaini KA, Ahmed MM, Abuohashish HM, Parmar MY, Al-Rejaie SS. Carvedilol attenuates inflammatory biomarkers and oxidative stress in a rat model of ulcerative colitis. Drug Dev Res 2015; 76:204–214.

37 Patel MA, Patel PK, Patel MB. Effects of ethanol extract of *Ficus bengalensis* (bark) on inflammatory bowel disease. Indian J Pharmacol 2010; 42:214–218.

38 Toblli JE, Cao G, Giani JF, Muñoz MC, Angerosa M, Dominici FP. Long-term treatment with nebivolol attenuates renal damage in Zucker diabetic fatty rats. J Hypertens 2011; 29:1613–1623.

39 Morsy MA, Heeba GH, Abdelwahab SA, Rofaeil RR. Protective effects of nebivolol against cold restraint stress-induced gastric ulcer in rats: role of NO, HO-1, and COX-1,2. Nitric Oxide 2012; 27:117–122.

40 Khan MU, Zhao W, Zhao T, Al Darazi F, Ahokas RA, Sun Y, *et al.* Nebivolol: a multifaceted antioxidant and cardioprotectant in hypertensive heart disease. J Cardiovasc Pharmacol 2013; 62:445–451.

41 Mohammad BI. Hepatoprotective potentials of nebivolol and aliskiren on methotrexate induced liver toxicity. Med J Babylon 2014; 7:16.

42 Akgullu C, Huyut MA, Boyacioglu M, Guleş O, Eryilmaz U, Hekim T, *et al.* Nebivolol to attenuate the effects of hyper-homocysteinaemia in rats. Atherosclerosis 2015; 240:33–39.

43 Yue TL, Cheng HY, Lysko PG, McKenna PJ, Feuerstein R, Gu JL, *et al.* Carvedilol, a new vasodilator and beta adrenoceptor antagonist, is an antioxidant and free radical scavenger. J Pharmacol Exp Ther 1992; 263:92–98.

44 Hess ML, Varma A. The third-generation beta-blocker: have we found the elusive, effective antioxidant?. J Cardiovasc Pharmacol 2013; 62:443–444.

45 Aleisa AM, Al-Rejaie SS, Abuohashish HM, Ola MS, Parmar MY, Ahmed MM. Pretreatment of Gymnema sylvestre revealed the protection against acetic acid-induced ulcerative colitis in rats. BMC Complement Altern Med 2014; 14:49.

46 Ilhan A, Yilmaz HR, Armutcu F, Gurel A, Akyol O. The protective effect of nebivolol on ischemia/reperfusion injury in rabbit spinal cord. Prog Neuropsychopharmacol Biol Psychiatry 2004; 28:1153–1160.

47 Gideroglu K, Alagoz S, Uygur F, Evinc R, Celikoz B, Bugdayci G. Effects of nebivolol on skin flap survival: a randomized experimental study in rats. Curr Ther Res Clin Exp 2008; 69:449–458.

48 De Groot AA, Mathy MJ, van Zwieten PA, Peters SL. Antioxidant activity of nebivolol in the rat aorta. J Cardiovasc Pharmacol 2004; 43:148–153.

49 Erdamar H, Sen N, Tavil Y, Yazici HU, Turfan M, Poyraz F, *et al.* The effect of nebivolol treatment on oxidative stress and antioxidant status in patients with cardiac syndrome-X. Coron Artery Dis 2009; 20:238–244.

50 Tahan G, Aytac E, Aytekin H, Gunduz F, Dogusoy G, Aydin S, *et al.* Vitamin E has a dual effect of anti-inflammatory and antioxidant activities in acetic acid-induced ulcerative colitis in rats. Can J Surg 2011; 54:333–338.

51 Garbin U, Fratta Pasini A, Stranieri C, Manfro S, Mozzini C, Boccioletti V, *et al.* Effects of nebivolol on endothelial gene expression during oxidative stress in human umbilical vein endothelial cells. Mediators Inflamm 2008; 2008:367590.

52 Kaushal GP, Kaushal V, Hong X, Shah SV. Role and regulation of activation of caspases in cisplatin-induced injury to renal tubular epithelial cells. Kidney Int 2001; 60:1726–1736.

53 Uzar E, Acar A, Evliyaoğlu O, Fırat U, Kamasak K, Göçmez C, *et al.* The anti-oxidant and anti-apoptotic effects of nebivolol and zofenopril in a model of cerebral ischemia/reperfusion in rats. Prog Neuropsychopharmacol Biol Psychiatry 2012; 36:22–28.

54 Celik T, Iyisoy A, Kardesoglu E, Fici F. The anti-inflammatory effects of nebivolol in human coronary smooth muscle cells: clinical implications. Int J Cardiol 2009; 133:415–416.

55 Gandhi C, Zalawadia R, Balaraman R. Nebivolol reduces experimentally induced warm renal ischemia reperfusion injury in rats. Ren Fail 2008; 30:921–930.

56 Sabbah HN, Sharov VG, Gupta RC, Todor A, Singh V, Goldstein S. Chronic therapy with metoprolol attenuates cardiomyocyte apoptosis in dogs with heart failure. J Am Coll Cardiol 2000; 36:1698–1705.

57 Su Q, Li L, Liu YC, Zhou Y, Lu YG, Wen WM. Effect of metoprolol on myocardial apoptosis and caspase-9 activation after coronary microembolization in rats. Exp Clin Cardiol 2013; 18:161–165.

58 Singh K, Xiao L, Remondino A, Sawyer DB, Colucci WS. Adrenergic regulation of cardiac myocyte apoptosis. J Cell Physiol 2001; 189:257–265.

59 Rachmilewitz D, Simon PL, Schwartz LW, Griswold DE, Fondacaro JD, Wasserman MA. Inflammatory mediators of experimental colitis in rats. Gastroenterology 1989; 97:326–337.

60 El-Abhar HS, Hammad LN, Gawad HS. Modulating effect of ginger extract on rats with ulcerative colitis. J Ethnopharmacol 2008; 118:367–372.

61 Feng MG, Prieto MC, Navar LG. Nebivolol-induced vasodilation of renal afferent arterioles involves β3-adrenergic receptor and nitric oxide synthase activation. Am J Physiol Renal Physiol 2012; 303:F775–F782.