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Inhibition of Ethanol-Induced Gastric Mucosal Damage by Carvedilol in Male Wistar Albino Rats: Possible Biochemical Changes

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Abstract: The effect of acute carvedilol (a third-generation nonselective β-blocker) pretreatment on gastric mucosal injury induced by 80% ethanol was investigated in male Wistar albino rats. The effects caused by pylorous ligation, accumulated gastric acid secretions and ethanol-induced changes in gastric mucus secretions, levels of proteins, nucleic acid, malondialdehyde (MDA) and non-protein sulfhydryl groups (NP-SH) in the stomach wall were investigated. The gastric ulcers were induced by administration of 1 mL of 80% ethanol, as a necrotizing agent into the stomach. Carvedilol pretreatment at two oral doses of 30 and 60 mg kg^{-1} body weight were found to protect against the ulcerogenic effects of ethanol. Same dose regimen of carvedilol offered significant protection against ethanol-induced damage on the parameters evaluated for histopathology. Furthermore, the pretreatment afforded a significant inhibition of pylorous ligated accumulation of gastric acid secretions and ethanol-induced depletion of stomach wall mucus, nucleic acids, proteins and NP-SH contents. Only higher dose of carvedilol provided inhibition of ethanol-induced increase in MDA concentration. The protective effects of carvedilol against gastric secretion or damage to the gastric-wall mucosa may be mediated through its effects on mucus production and NP-SH concentrations, possible free-radical scavenging ability and/or cytoprotective properties.

Key words: Carvedilol, β-blocker, gastric secretions, nucleic-acids, sulfhydryls, lipid-peroxides, ulceration, glutathione

INTRODUCTION

Carvedilol is a third-generation nonselective β-adrenoceptor antagonist used for the treatment of hypertension (Weber *et al*., 2006). It has been shown to have multiple functions such as neuroprotection (Savitz *et al*., 2000) and myocardial (Kopecky, 2006), as well as endothelial protection (Vyssoulis *et al*., 2004). Carvedilol was shown to scavenge oxygen free radicals and inhibit lipid peroxidation in biological systems (Kumar *et al*., 2000; Huang *et al*., 2007). Several reports suggest the cardioprotective effect of carvedilol may be through its antioxidant activity, which is not shared by all β-adrenergic receptor antagonists (Dulin and Abraham, 2004). Moreover, the antioxidant protection of carvedilol occurs through a chain-breaking mechanism in postischemic hearts in rat (Kramer and Weglicki, 1996). General pharmacological screening of carvedilol in rats showed a decrease in gastric secretion (Hirohashi *et al*., 1990). On the basis of current literature review it can be inferred that carvedilol may contribute to the gastric mucosal defense.

Gastric ulcers are one of the most widespread diseases worldwide and are believed to be due to an imbalance between aggressive factors such as acid and pepsin and the maintenance of mucosal integrity through endogenous defense mechanisms (Wallace and Granger, 1996). Ethanol consumption is one of the many factors that increase the risk of gastric ulcer (Ray *et al*., 1990). Experimentally, many ethanol lesion models are widely used to induce gastric ulcers in animals (Sheeba and Asha, 2006). It was reported that the pathogenesis of acute experimental gastric lesion may involve generation of oxygen-derived free radicals, primarily superoxide anions, hydroxyl radicals and lipid peroxides (Al-Shabanah *et al*., 2000). Disturbed gastric motility and reduced gastric mucosal blood flow were also important pathogenic elements in gastric ulcer malignancy (Brozozwski *et al*., 2000). Furthermore, the imbalance between gastrotoxic agents and protective mechanisms may results in an acute inflammation accompanied by neutrophils infiltration of gastric mucosa (Konturek *et al*., 2000).

Complications from portal hypertension are responsible for disabling morbidity and mortality in patients with cirrhosis (Weber *et al*., 2006). To lower the risk of life-threatening gastrointestinal hemorrhage from esophageal varices, a number of controlled studies support the use of β-blockers for both primary and secondary prophylaxis. Despite the availability of guidelines addressing these issues, the actual

effectiveness of β-blocker therapy in clinical practice remains unknown. Therefore, the present study is designed to determine the effect of carvedilol on gastric acid secretion and chemically-induced ulcers in Wistar albino rats.

MATERIALS AND METHODS

The present study was designed and studied in Quality Control and Research Laboratory, College of Pharmacy, King Saud University, Riyadh during 2007-2008 academic year.

Animals: Male Wistar albino rats (home bred) all roughly the same age (eight weeks old), weighing 180-200 g, were used in the present study. All the animals were maintained under controlled conditions of temperature (22±1°C), humidity (55%) and light (12 h dark and 12 h light). They were provided free access to Purina rat chow (Manufactured by Grain Silos and Flour Mills Organization, Riyadh, Saudi Arabia) and water. All procedures including euthanasia procedure were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research (1996) as well as the Ethical Guidelines of the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Experimental design: For each study, the rats were randomly divided in different groups (6 rats in each group) and were fasted 36 h with water *ad libitum*. Carvedilol tablets (Roche, Mannheim, Germany) were powdered and suspended in 0.25% carboxymethyl cellulose (CMC) solution. The suspension was administered orally by calculating the doses of 30 and 60 mg kg^{-1} to fasted rats. The selection of the higher dose (60 mg kg^{-1} body weight) used in the present study was based from earlier study reports performed by De *et al*. (2002) and Boesen *et al*. (2004). Cimetidine (50 mg kg^{-1} body weight) was used as reference drug (positive control). The number of animals used per group is usually the minimum necessary to test reliability for statistical significance for these types of experiments. Among the various parameters that may be studied in the impairment of gastric mucosal integrity and protection, we focused the attention on the alteration in endogenous levels of proteins, nucleic acids (DNA and RNA), malondialdehyde (MDA) and non-protein sulfhydryl groups (NP-SH). Histopathological observation was then used to confirm the carvedilol and ethanol interaction in the gastric mucosa. The tissue samples whether for biochemical analysis or histopathological screening were coded and kept them as blind.

Estimation of gastric secretion in pylorus ligated (Shay) rats: The method of Shay rat ulcer (Shay *et al*., 1945) was adopted in the current investigation. Rats were fasted for 36 h with access to water *ad libitum* before the pylorus was ligated under anesthesia. The abdomen was opened by a small midline incision below the xiphoid process. Then, pylorus portion of stomach was slightly lifted out and ligated. Precaution was taken to avoid traction to the pylorus or damage to its blood supply. The stomach was placed carefully in the abdomen and the wound was sutured by interrupted sutures. Carvedilol and cimetidine suspensions were administered intraduodenally immediately after pylorus ligation. The animals were sacrificed 6 h following pylorus ligation. The stomach was then removed, content collected, volume measured, centrifuged and subjected to analysis for titratable acidity against 0.01 N NaOH to pH 7 using a digital pH meter. The total acid output was calculated (meq L^{-1}).

Gastric lesions induced by ethanol: Each animal in the test groups was given 1 mL of 80% ethanol by gavage, as a necrotizing agent, which was known to produce gastric lesions (Al-Shabanah *et al*., 2000; Al-Bekairi *et al*., 1992). Based on the gastric emptying time in fasted rats, carvedilol (30 and 60 mg kg^{-1}) and cimetidine were given 60 min before the administration of the necrotizing agent (80% ethanol). The animals were killed by decapitation 1 h after ethanol treatment. The stomach was excised and opened along the greater curvature. After washing with normal saline, the gastric lesions were quantified using a binocular magnifier. The ulcers were scored according to the method of Valcavi *et al*. (1982) and assessed on the basis of their dimensions: Deep circular ulcers more than 8 mm = 10; 7-8 mm = 7; 5-6 mm = 6; 4-5 mm = 5; 3-4 mm = 4; 2-3 mm = 3; 1-2 mm = 2; 0-1 mm = 1. Furthermore, the deep linear ulcer more than 10 mm in length = 6 and linear ulcer less than 10 mm in length = 3. The score for each single lesion were then summed up for the determination of ulcer index.

Gastric wall mucus determination: The modified procedure of Corne *et al*. (1974) was used to determine gastric-mucus. The glandular segments from the stomach were removed and weighed then transferred immediately to 1% Alcian blue solution (in sucrose solution buffered with sodium acetate, pH 5). The excess dye was removed by rinsing with sucrose solution. The dye complexed with the gastric wall mucus was extracted with 10 mL of 0.5 M magnesium chloride solution. A 4 mL aliquot of blue extract was then shaken with an equal volume of diethyl ether. The resulting emulsion was centrifuged and the absorbance of the aqueous layer

was recorded at 580 nm. The quantity of Alcian blue extracted (net) per grams of wet glandular tissue was then calculated.

Estimation of protein and nucleic acids in stomach: The levels of proteins and nucleic acids in the stomach were determined according to the following procedures: the stomachs were rapidly dissected from the animals, frozen in liquid nitrogen and stored at -70°C until they were analyzed for total proteins and nucleic acids (DNA and RNA). Total protein was determined by the method of Lowry *et al*. (1951). The method described by Bregman (1983) was used to determine levels of nucleic acids. Each stomach tissues were homogenized in ice-cold distilled water. The homogenates were extracted in different concentrations of cold and hot trichloroacetic acid (TCA) and 95% ethanol. DNA was determined by treating the nucleic acid extract with diphenylamine reagent and measuring the intensity of the blue colour at 600 nm. For quantification of RNA, the nucleic acid extract was treated with orcinol reagent and the green color was recorded at 660 nm.

Estimation of NP-SH in stomach: Gastric mucosal NP-SH was measured according to the method of Sedlak and Lindsay (1968). The glandular stomach (400 mg) was removed and homogenized in 8.0 mL of ice-cold 0.02 M ethylenediaminetetraacetic acid (EDTA). The homogenate (5.0 mL) was mixed with distilled water (4 mL) and 1 mL (50% w/v) aqueous TCA and centrifuged. The supernatants (2 mL aliquots) were then mixed with 4 mL of Tris buffer, (pH 8.9), 0.1 mL of 0.4% 5,5´-dithiobis (2-nitrobezoic acid) (DTNB) was added and the sample was shaken. The absorbance was read within 5 min of addition of DTNB, at 412 nm, against a reagent blank with no homogenate.

Estimation of MDA concentrations in stomach: The method described by Ohkawa *et al*. (1979) was followed with little modifications. The stomach tissues (200 mg) were homogenized in aqueous 0.15 M KCl to give 10% homogenate. One milliliter of homogenate was mixed with 1 mL of 10% TCA and centrifuged at 3,000 rpm for 15 min. Then, 1 mL supernatant was mixed with 1 mL of 0.67% 2-thiobarbutaric acid. The test tubes were closed by glass stoppers and placed in a boiling water bath for 15 min. Tubes were allowed to cool down at room temperature. Optical density of the clear pink supernatants was recorded at 532 nm. Malondialdehyde bis (dimethyl acetal) was used as standard.

Histopathological assessment: The gastric tissue samples were preserved in 10% natural buffered formalin and processed for routine paraffin block preparation. Using an American optical rotary microtome, sections of thickness about 3 µm were cut and stained with hematoxyline and eosin (Culling, 1974). The slides were then examined under a microscope for pathomorphological changes as congestion, haemorrhage, oedema and erosions using an arbitrary scale for the assessment of severity of these changes. The tissues were kept blind with the histopathologist.

Histological grading for possible gastric injury: Microscopic reactive, reparative or degenerative changes seen in the mucosal layer were scored as follows: mucosal congestion = 1; mucosal hemorrhage = 2; Glandular disarray/degeneration = 3; mucosal erosions = 4; Frank ulcer formation= 5. Polymorphonuclear cell infiltrates in addition to the monocytic leukocytes were estimated in gastric tissues as: rare = 0; occasional = 1; several = 2; numerous = 3. Irregularity in the thickness of the submucosa, which could signify edema, accompanied with increased vascular congestion was scored as follows: focal =1 and diffuse = 2. Degenerative changes exhibited by the smooth muscles, including atrophy, nuclear pyknosis, cytoplasmic vacuolations and necroses were scored as: focal =1 and diffuse = 2. The final injury was estimated: score of 0 – 2 = within normal; score of 3-9 = mild injury; score of 10 – 17 = moderate injury; score of >17 = severe injury.

Statistical analysis: The data were expressed as Mean±Standard Error of Means (SEM). The difference between treatment groups was compared statistically via., analysis of variance (One-way ANOVA) using SPSS software and Post hoc Tukey-Kramer multiple comparison tests were used to analyze the different studies undertaken. p values of less than 0.05 were considered statistically significant. The statistical software named SSPS was used for analyzing the data.

RESULTS

Effects on the gastric secretions in 6 h pylorus-ligated (Shay) rats: In control rats, pylorus ligation for 6 h resulted in accumulation of gastric secretions (13.58±0.98 mL) and titrable acidity (70.87±4.67 meq L^{-1}). Treatment with both doses of carvedilol (30 and 60 mg kg^{-1} body weight) showed a significant decrease in the volume and gastric contents (10.17±0.55 mL; $p<0.05$) and (6.98±0.47 mL; $p<0.01$), respectively, compared to control rats. Gastric content in cimetidine treated group was significantly (6.17±0.19 mL; $p<0.001$) less than control group (Fig. 1a). A significant decrease in titrable acidity was also observed in the rats treated with 60 mg kg^{-1} (45.78±4.92; $p<0.05$) of carvedilol but not with 30 mg kg^{-1} (60.89±3.54). However, in cimetidine pretreated group the

titrable acidity was (37.48±2.97 meq L^{-1}) which is significantly ($p<0.01$) less than control group (Fig. 1b).

Effects on the gastric lesions induced by 80% ethanol: One hour after the treatments with 80% ethanol to rats, an extensive gastric lesion (28.45±3.67) in the glandular mucosa of stomach was observed (Fig. 2). Pretreatment with carvedilol (30 and 60 mg kg^{-1}) and cimetidine (50 mg kg^{-1}) resulted in a significant protection against ulceration induced by 80% ethanol ($p<0.05$, $p<0.001$ and $p<0.001$, respectively) (Fig. 2).

Effects on ethanol-induced changes in gastric wall mucus: The treatment with 80% ethanol significantly decreased the Alcian blue binding capacity of gastric wall mucus (302.81±12.58 µg g^{-1}; $p<0.05$) as compared to control rats (415.66±9.01 µg g^{-1}). Pretreatment with carvedilol in the doses of 30 mg kg^{-1} (441.7±27.6; $p<0.05$) and 60 mg kg^{-1} (432.3±31.6; $p<0.01$) enhanced Alcian blue binding capacity of gastric mucosa. Cimetidine (50 mg kg^{-1}) pretreatment also significantly (386.8±17.3; $p<0.01$) increased the Alcian blue binding capacity of gastric mucosa when compared to ethanol ingested group (Fig. 3).

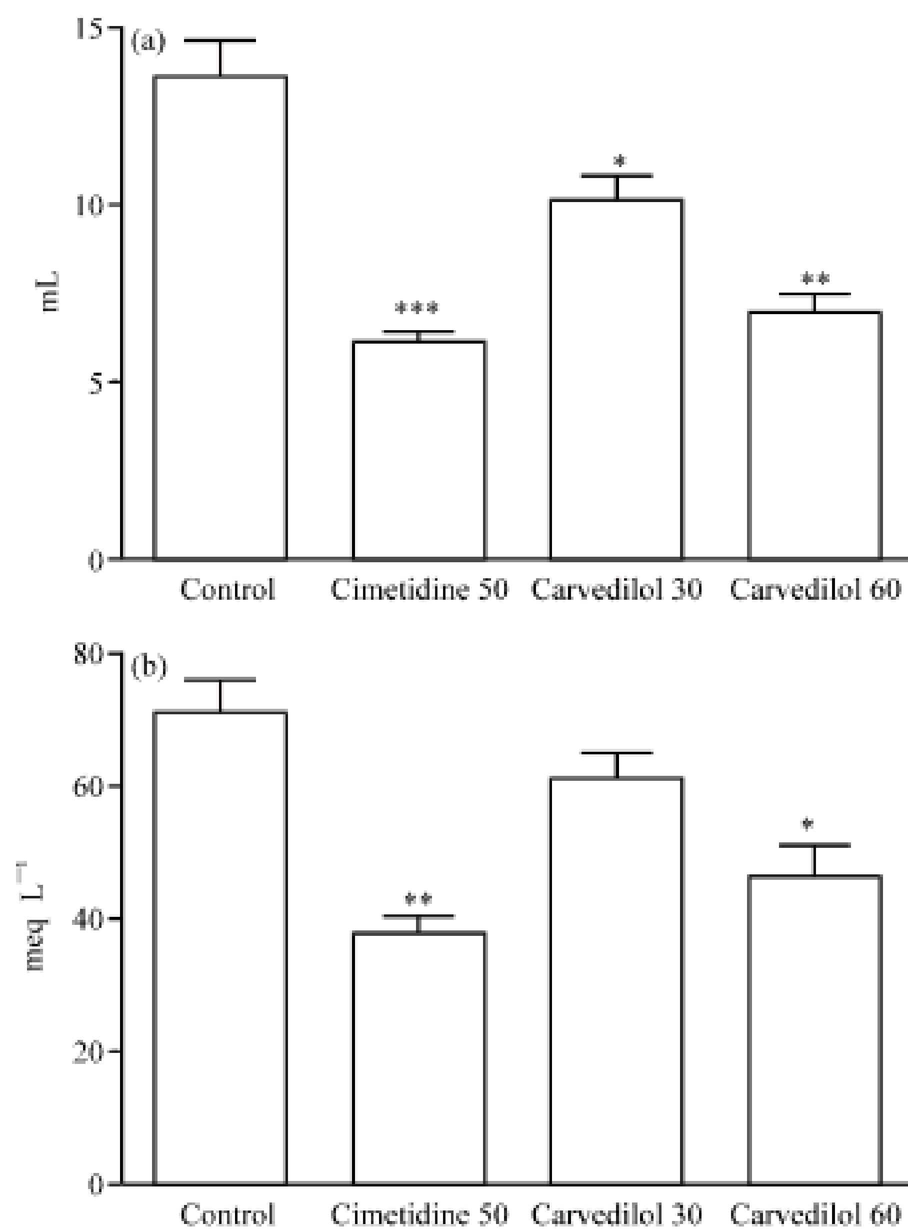

Fig. 1: (a) Effect of carvedilol (30 and 60 mg kg^{-1} body weight) on the gastric secretions and (b) acidity in Pylorus ligated rats. Cimetidine and carvedilol treated groups were statistically compared to control treated group. *$p<0.05$, **$p<0.01$ and ***$p<0.001$. Data were expressed as Mean±SEM and analyzed using one-way ANOVA and Post hoc Tukey-Kramer multiple comparison tests. Six rats were used in each group

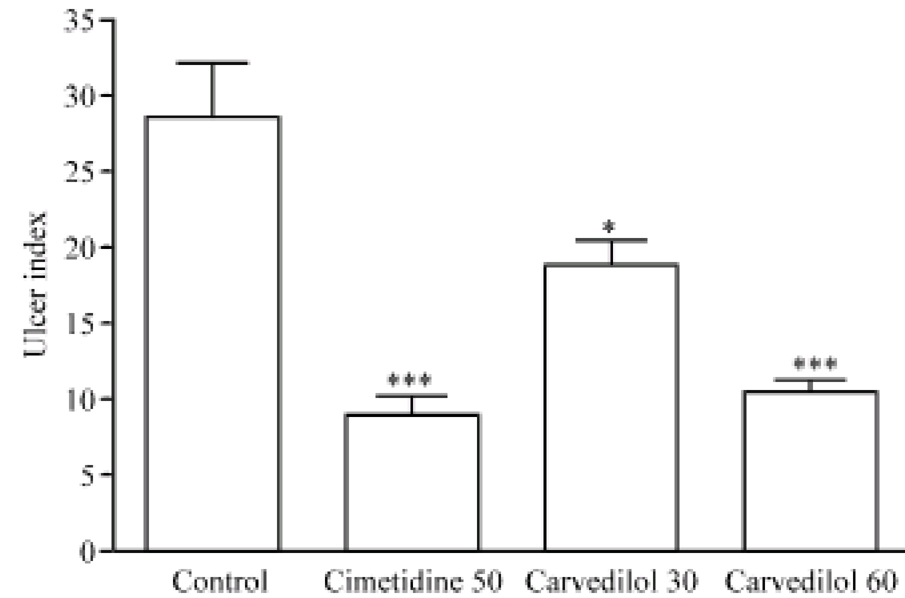

Fig. 2: Effect of carvedilol (30 and 60 mg kg^{-1} body weight) on the induction of gastric ulcers by 80% ethanol in male Wistar albino rats. Cimetidine and carvedilol treated groups were statistically compared to control treated group. *$p<0.05$, **$p<0.01$ and ***$p<0.001$. Data were expressed as Mean±SEM and analyzed using one-way ANOVA and Post hoc Tukey-Kramer multiple comparison tests. Six rats were used in each group

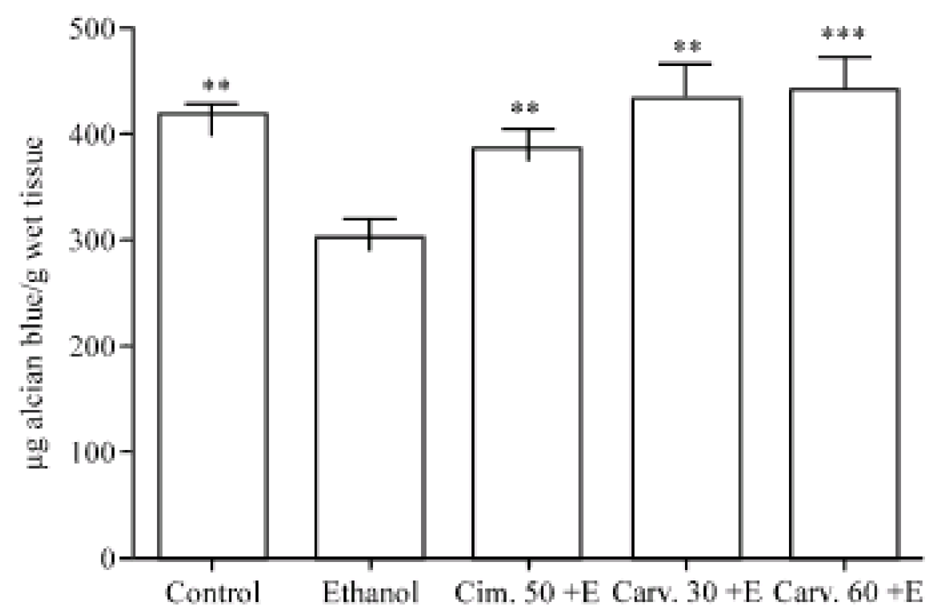

Fig. 3: Effect of carvedilol (30 and 60 mg kg^{-1} body weight) on the induction of changes in gastric wall mucus by 80% ethanol in male Wistar albino rats. Control group was statistically compared to ethanol (E) group. Cimetidine (Cim.) and carvedilol (Carv.) treated groups were statistically compared to ethanol treated group. *$p<0.05$, **$p<0.01$ and ***$p<0.001$. Data were expressed as Mean±SEM and analyzed one-way ANOVA and Post hoc Tukey-Kramer multiple comparison tests. Six rats were used in each group

Effects on protein and nucleic acid concentrations: Carvedilol (30 and 60 mg kg^{-1}) treatment to fasted rats showed no significant changes in stomach nucleic acids and total protein concentrations as compared to control group of rats (Table 1). Treatment with 80% ethanol to fasted rats caused significant reduction in the nucleic acid ($p<0.01$) and in total protein ($p<0.05$) concentrations of gastric mucosa as compared to control group (Table 1). The decrease in nucleic acid levels (DNA and RNA) at the stomach wall by 80% ethanol was significantly inhibited following pretreatment with the lower dose ($p<0.05$ for DNA and $p<0.01$ for RNA) as well as with the higher dose ($p<0.001$) of carvedilol. Cimetidine pretreatment to the ethanol ingested rats significantly ($p<0.001$) protected the decrease in nucleic acids. Furthermore, the decrease in total protein by 80% ethanol was significantly inhibited following carvedilol pretreatment at both doses ($p<0.05$; 30 and 60 mg kg^{-1} body weight) and cimetidine (50 mg kg^{-1}), respectively (Table 1).

Effect of carvedilol on gastric mucosal NP-SH and MDA concentrations: After 1 h of carvedilol (30 and 60 mg kg^{-1}) treatment to fasted rats, no significant changes in stomach NP-SH and MDA concentrations compared to control group. Ethanol treatment significantly reduced the NP-SH concentrations in the gastric mucosa ($p<0.05$) as compared to the control (Table 2). Carvedilol pretreatment significantly prevented the decline in NP-SH concentrations by ethanol at the low ($p<0.05$) and high ($p<0.001$) doses as compared with the values obtained with ethanol alone (Table 2). In contrast, ethanol treatment caused significant ($p<0.05$) increase the MDA concentrations in gastric mucosa as compared to the control group. Pretreatment with carvedilol was found to inhibit the rise in MDA levels by ethanol at only the higher dose ($p<0.01$; 60 mg kg^{-1}) (Table 2). Cimetidine pretreatment to the ethanol ingested rats also showed significant ($P<0.001$) protection against the changes-induced by ethanol in stomach NP-SH and MDA concentrations, respectively.

Effect of carvedilol on histopathological changes induced by ethanol on gastric lesions: Figure 4, shows the appearance through gastric mucosa of one of the control rats (Fig. 4; Table 3; total damage score: 1). Treatment with ethanol to fasted rats caused considerable damage in glandular segments of stomach tissues in the form of necrosis, erosions, congestion, interluminal bleeding and hemorrhagic mucosal lesions in the stomach walls (Fig. 4; Table 3; total damage score: 16). Pretreatment with carvedilol (30 mg kg^{-1}) showed protection to the damaging action of ethanol evaluated for the same parameters (Fig. 4; Table 3; total damage score: 7). The higher dose (60 mg kg^{-1}) of carvedilol showed little more positive effect against the ethanolic damage (Fig. 4; Table 3; total score: 6).

Table 1: Effect of carvedilol pretreatment on nucleic acids and total protein content in the stomach wall of male Wistar albino rats treated with 80% ethanol

Treatment and dose (mg kg^{-1} body weight) gavage	DNA (μg/100 mg wet tissue)	RNA (μg/100 mg wet tissue)	Total protein (mg/100 mg wet tissue)
Control (0.25% CMC; 1 mL rat^{-1})	394.4±16.5	547.7±22.1	15.1±0.6
Carvedilol (30)	386.5±23.4	562.7±31.5	14.9±0.5
Carvedilol (60)	407.3±21.3	574.4±33.7	15.1±0.4
80% ethanol (1 mL rat^{-1})	278.2±10.4[a***]	341.2±17.5[a**]	12.4±0.4[a*]
Cimetidine (50) + 80% ethanol (1 mL rat^{-1})	374.2±15.7[b***]	524.0±19.8[b***]	15.1±0.5[b**]
Carvedilol (30) + 80% ethanol (1 mL rat^{-1})	312.7±9.8[b*]	436.5±12.7[b**]	13.6±0.3[b*]
Carvedilol (60) + 80% ethanol (1 mL rat^{-1})	354.2±8.8[b***]	497.4±9.8[b***]	14.7±0.6[b*]

[a]Ethanol group was statistically compared to control group. [b]Cimetidine and carvedilol treated groups were statistically compared to ethanol treated group. *$p<0.05$, **$p<0.01$ and ***$p<0.001$. Data were expressed as Mean±SEM and analyzed using one-way ANOVA and Post hoc Tukey-Kramer multiple comparison tests. Six rats were used in each group

Table 2: Effect of carvedilol pretreatment on NP-SH and MDA content in glandular stomachs of male Wistar albino rats treated with 80% ethanol

Treatment and dose (mg kg^{-1} body weight) gavage	NP-SH concentration (μg g^{-1} wet tissue)	MDA concentration (nmol g^{-1} wet tissue)
Control (0.25% CMC; 1 mL rat^{-1})	114.5±8.5	214.3±12.7
Carvedilol (30)	124.1±5.3	197.3±13.5
Carvedilol (60)	119.4±7.7	210.1±14.6
80% ethanol (1 mL rat^{-1})	57.9±3.8[a*]	321.5±17.8a[a*]
Cimetidine (50) + 80% ethanol (1 mL rat^{-1})	98.7±6.2[b***]	221.7±10.1[b***]
Carvedilol (30) + 80% ethanol (1 mL rat^{-1})	87.7±6.8[b*]	287.1±14.4
Carvedilol (60) + 80% ethanol (1 mL rat^{-1})	106.2±5.7[b***]	237.3±9.9[b**]

[a]Ethanol group was statistically compared to control group. [b]Cimetidine and carvedilol treated groups were statistically compared to ethanol treated group. *$p<0.05$, **$p<0.01$ and ***$p<0.001$. Data were expressed as Mean±SEM and analyzed one-way ANOVA and Post hoc Tukey-Kramer multiple comparison tests. Six rats were used in each group

Table 3: Effect of carvedilol pretreatment (30 and 60 mg kg^{-1} body weight) on the histopathological examination in gastric mucosal sections in a sample of control and experimental groups

Parameters	Control (0.25% CMC)	80% ethanol	Carvedilol (30) + 80% ethanol	Carvedilol (60) + 80% ethanol
		(1 mL rat^{-1})		
Mucosal congestion	0	1	1	1
Mucosal hemorrhage	0	2	0	0
Glandular disarray/degeneration	0	3	3	3
Mucosal erosions	0	4	0	0
Ulcer formation	0	0	0	0
Active inflammation	1	2	2	2
Submucosal distention	0	2	1	0
Muscularis layer degeneration	0	2	0	0
Total scores	1	16	7	6

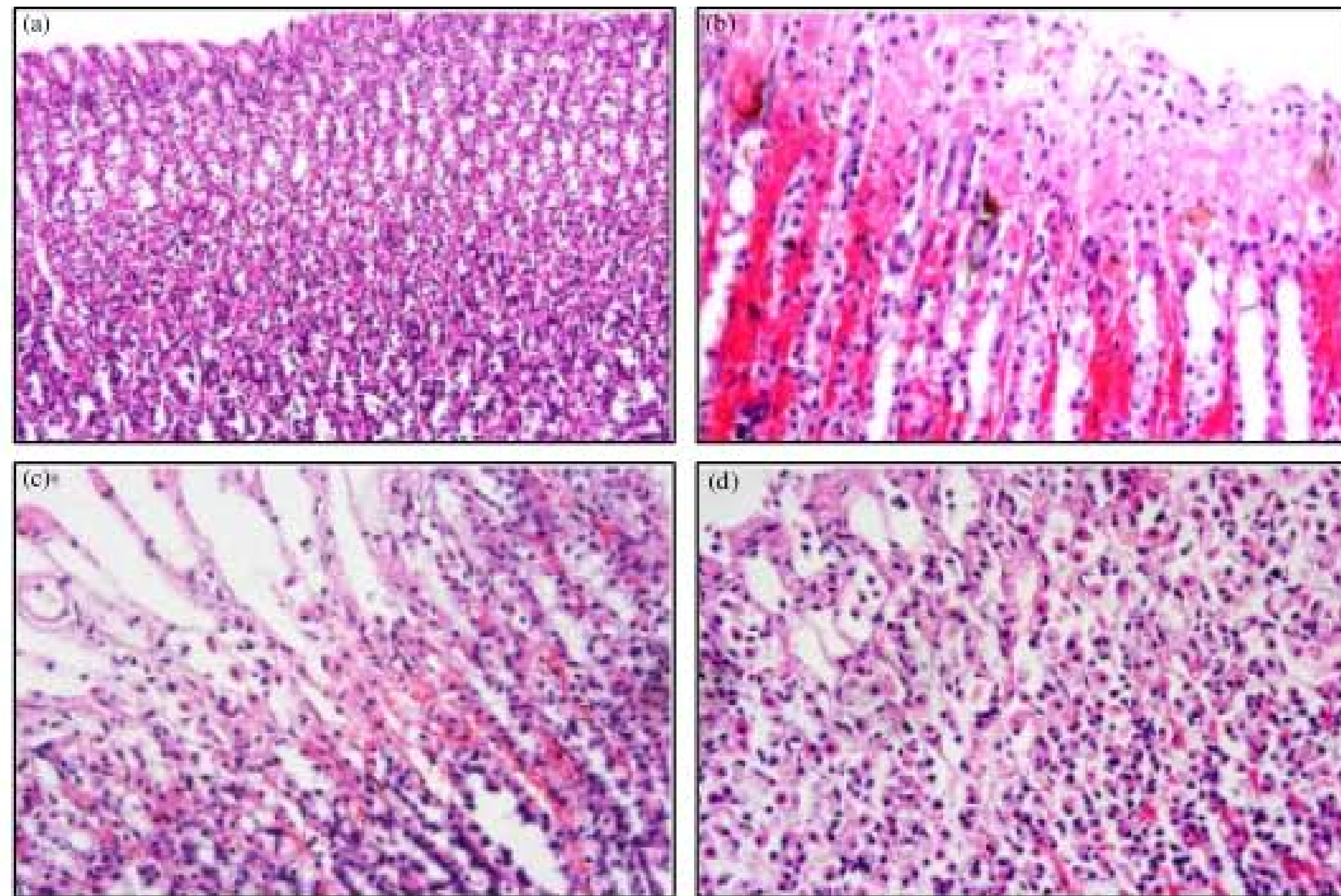

Fig. 4: Histological examination of gastric mucosal sections in control and experimental rats. (a) Section of gastric mucosa obtained from normal rats, (b) Section of gastric mucosa obtained from 80% ethanol treated rat showing fibrinoid necrosis of the foveolar surface epithelium, associated with destroyed mucosal glands, hemorrhage and a fibrinous exudates, (c) Section of gastric mucosa obtained from 80% ethanol treated rat after pretreatment of carvedilol at 30 mg kg^{-1} body weight showing superficial glandular distortion and degeneration only seen in the congested area and (d) Section of gastric mucosa obtained from 80% ethanol treated rat after pretreatment of carvedilol at 60 mg kg^{-1} body weight showing degenerated glands with effacement of superficial foveolar epithelium

DISCUSSION

Results of the present study demonstrated that two doses of carvedilol had the ability to decrease acid secretion and show protection against the ulceration caused by the necrotizing effect of ethanol in male Wistar albino rats. The results of histopathological assessment also revealed that pretreatment with carvedilol prevented congestion, hemorrhage, edema, necrosis, inflammatory and dysplastic changes and erosions caused by ethanol in the gastric tissue. The present results also showed significant decrease in the volume and acid output of gastric secretions in carvedilol administered Shay rats. Indeed, these results are in agreement with earlier study done for a general pharmacological screening of carvedilol in rats, which showed a decrease in gastric secretion (Hirohashi *et al*., 1990). Gastric blood circulation maintains the function of the stomach and thereby is closely linked to the pathogenesis and healing of gastrointestinal lesions. In the pylorus ligation model (Shay rats), it has been proposed that the interference in gastric blood circulation is responsible for the induction of ulcers (Blandizzi *et al*., 1999). Carvedilol is a nonselective β-blocker with α_1-adrenergic blocking activity and it has

been shown to decrease portal pressure in cirrhotic patients (Stanley *et al*., 1999; Talwalkar and Kamath, 2004). Recently, Lin *et al*. (2006) reported that carvedilol decreased portal pressure through a reduction of splanchnic blood flow in rats, which is linked to a decrease in hepatocollateral resistance and also decreased endothelial-related vasodilatory activities.

Increased mucus secretion by the gastric mucosal cells can prevent gastric ulceration by several mechanisms including lessening stomach wall friction during peristalsis as well as acting as effective barrier to back diffusion of hydrogen ions (Sevak *et al*., 2002). The gastric mucus coat is thought to be important in protecting gastric mucosa against ulcerogens and in facilitating the repair of the damaged gastric epithelium (Tariq *et al*., 2006). The mucus gel adhering to the gastric mucosal surface protects the underlying epithelium against acid (Giovanoni *et al*., 1999), pepsin (Jafri *et al*., 2001) and necrotizing agents such as absolute alcohol (Chen *et al*., 2005). In the present study, gastroprotective effect of acute carvedilol administration against the deleterious effects of 80% ethanol could be attributed to its anti-gastric acid secretory activity. Earlier studies demonstrated that natural prostaglandins and their synthetic analogues inhibit gastric acid secretions in animals and man (Pawlik *et al*., 2002) and prevent experimental ulcerations induced by a variety of destructive stimuli (Al-Shabanah *et al*., 2000). Kaan *et al*. (1996) reported that propranolol, a β-adrenoceptor, increases the prostaglandin E_2 levels in gastric mucosa which may explain its anti-ulcer effects. These reports as well as the current findings may have confirmed the cytoprotective nature of carvedilol (Tunez *et al*., 2006; Ronsein *et al*., 2005).

In addition, the results of the anti-ulcer and anti-secretary activity of the two doses of carvedilol were supported by the current data on the inhibitory effect on ethanol-induced depletion in nucleic acids, proteins and NP-SH and an increase in the levels of MDA in gastric mucosa. The exact mechanisms of the protection of ulcer and the anti-secretary effect of carvedilol are not known. However, it appears that the alteration in NP-SH induced by carvedilol may have a role in the present study. Indeed, depletors of NP-SH significantly potentiated gastric mucosal injury induced by some necrotizing agents (Hiraishi *et al*., 1994). Moreover, sulfhydryl compounds have the ability to bind free radicals produced in tissues following exposure to cytotoxic compounds (Al-Harbi *et al*., 1997). Lipid peroxidation of cell membranes may contribute to mucosal damage (Kusterer *et al*., 1987). Thus, the test doses of carvedilol pretreatment induced increase in NP-SH might have inhibited the impact of cytodestructive stimuli on nucleic acids, proteins and NP-SH possibly via inhibition of lipid peroxidation.

Further possible modes of action of protection by carvedilol may be due to its possible influence on reactive oxygen species. There is considerable evidence concerning the participation of reactive oxygen species in the etiology and pathophysiology of the gastric ulcer disease (Repetto and Llesuy, 2002; Khosla *et al*., 2004). It is well known that reactive oxygen species play a major role in the etiology and pathophysiology of human diseases in general and digestive system disorders in particular (Repetto and Llesuy, 2002). A link between reactive oxygen species and gastric ulceration was shown with pylorus ligation (Rastogi *et al*., 1998). It was also reported that there is a link between gastric ulceration and high concentration of ethanol treatments to rats (Al-Shabanah *et al*., 2000). Ethanol is known to be metabolized in the body to produce free radicals (Halliwell, 1991) while carvedilol is an established antioxidant, which is known to prevent lipid peroxidation (Kumar *et al*., 2000; Huang *et al*., 2007). Several reports suggest the cardioprotective effect of carvedilol may be through its antioxidant activity, which is not shared by all β-adrenergic receptor antagonists (Yuan *et al*., 2004). In additions carvedilol was shown to have a cytoprotective property in cultured cells (Tunez *et al*., 2006; Ronsein *et al*., 2005). Therefore, it is possible that the protective action, including antioxidant properties, of the two doses of carvedilol may be one way forward in minimizing gastric tissue injury induced by ethanol.

In summary, present results demonstrated that carvedilol has strong gastro-protective effect. Further investigations are still needed to confirm and evaluate its gastro-protective effect in experimental and clinical models.

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