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Research Article
Protective Effect of Carvedilol on Paracetamol-induced Hepatotoxicity; Role of Modulation Inflammation and Lipid Peroxidation

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Abstract

Background and Objective: Hepatotoxicity induced by hepatotoxins has been regarded as one of the most serious health problems. The present study aimed to investigate the possible protective effects of carvedilol against paracetamol-induced hepatotoxicity. **Methodology:** Thirty two male rats were randomly divided into four groups as follows: vehicle control, hepatotoxicity control, N-acetyl cysteine (300 mg kg^{-1}; p.o.) and carvedilol (30 mg kg^{-1}; p.o.). Seven days after initiation of treatments, hepatotoxicity was induced by a single oral administration of paracetamol (1 g kg^{-1}). At the end of the experimental period, blood samples were collected for estimation of serumalanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and gamma-glutamyltransferase (GGT) activities as well as serum Total Protein (TP) level as markers of hepatic dysfunction. In addition, hepatic malondialdehyde (MDA), glutathione (GSH) and Nitric Oxide (NO) contents were assessed as oxidative and nitrosative stress markers. Serum tumor necrosis factor-alpha (TNF-α) and interleukins-1beta (IL-1β) levels were also determined as inflammatory markers. Moreover, histopathological and immunohistochemical studies were performed. **Results:** Paracetamol administration resulted in a significant elevation of ALT, AST, LDH and GGT activities, MDA and NO contents, as well as TNF-α and IL-1β levels coupled by significant reduction of TP level and GSH content. Pretreatment with carvedilol mitigated paracetamol-induced biochemical, histological and immuno-histochemical changes. **Conclusion:** It has been concluded that carvedilol could alleviate hepatotoxicity induced by paracetamol, most probably through its antioxidant and anti-inflammatory properties. It may be of therapeutic value in treatment of paracetamol-induced hepatotoxicity.

Key words: Carvedilol, hepatotoxicity, interleukins-1beta, N-acetyl cysteine, tumor necrosis factor-alpha

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Paracetamol is a regularly used antipyretic and analgesic agent. It is safe and well tolerated at therapeutic doses; however, an overdose could lead to severe hepatotoxicity[1]. At therapeutic level, it is mainly metabolized by glucuronide and sulfate conjugation and then excreted, meanwhile a small amount is metabolized by cytochrome P450, mainly CYP 2E1, to N-acetyl-p-benzoquinoneimine (NAPQI), a highly reactive metabolite, which undergoes glutathione conjugation and eliminated via the kidney. However, at overdose the conjugation pathway becomes saturated leading to increased generation of NAPQI. Moreover, glutathione depletion occurs and NAPQI irreversibly binds to cellular and mitochondrial proteins leading to liver damage and necrosis[2]. Extreme generation of NAPQI elicits consequent stimulation of the pro-inflammatory cytokines, tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β), which in turn reinforces tissue necrosis[3].

N-acetyl cysteine (NAC) is a cysteine prodrug which increases the intracellular glutathione (GSH) concentration, hence it has proven efficacy against paracetamol-induced hepatotoxicity[4]. Furthermore, it has anti-inflammatory features through reducing nuclear factor-kappa B (NF-κB) activation which in turn decreases the overproduction of TNF-α and IL-1β and the expression of inflammatory mediators, inducible nitric oxide synthase (iNOS) and cycloxegenase (COX-2)[5]. In addition, it could be used as chemopreventive agent due to its anti-mutagenic and anti-carcinogenic properties[6].

Carvedilol, a non-selective β-blocker with α_1 blocking ability is a vasodilating agent used in treatment of hypertension and congestive heart failure[7]. It has been demonstrated to provide greater benefit than traditional β-blockers by virtue of its antioxidant, anti-inflammatory and anti-fibrotic propertiesin cardiac and hepatic studies[8,9]. It has proven efficacy against anthracycline-induced cardiotoxicity, nephrotoxicity and ischemia-reperfusion injury[10-12].

The present study was designed to explore the possible protecting actions of carvedilol against paracetamol-induced hepatotoxicity and to compare these results with the effects of NAC.

MATERIALS AND METHODS

Animals: Adult male Wistar albino rats (130-150 g) obtained from the National Research Centre (Cairo, Egypt) were used in this study. Animals were kept in plastic well ventilated cages under suitable environmental conditions (temperature 22±2°C, humidity 60±4%) with 12 h light/dark cycle, allowed free access to food and water *ad libitum* and allowed to acclimatize for one week. All animal experiments were carried out according to the guidelines of the Ethics Committee of Faculty of Pharmacy, Beni-Suef University, Egypt.

Drugs and chemicals: The N-acetyl cysteine and carvedilol were purchased from Sigma-Aldrich (USA) and Fluka (USA), respectively. They were dissolved in isotonic saline (0.9%) solution and orally administered in doses[13,14] of 300 mg kg^{-1} and 30 mg kg^{-1}, respectively. Other chemicals used in the present study were of the highest grade commercially available.

Experimental design: Following the acclimatization week, animals were allocated into four groups (n = 8) as follows: Group I (vehicle control) and group II (hepatotoxicity control) received isotonic saline (0.9%, p.o.) for 7 successive days. Groups III and IV received NAC and carvedilol, respectively for 7 successive days. After an overnight fasting, animals in groups II, III and IV received paracetamol (1 g kg^{-1}; p.o.)[15] for the induction of hepatotoxicity. Twenty four hours later, blood samples were collected from the retro-orbital venous plexus under light anesthesia, allowed to clot and then centrifuged at 3000 rpm for 20 min for serum separation. The separated sera were stored at -80°C until further biochemical analysis. Serum was used for the estimation of ALT, AST, LDH and GGT activities as well as TP, TNF-α and IL-1β levels. Instantly after collection of blood, rats were euthanized and livers were removed, washed with ice-cold physiological saline (0.9%), blotted dry on a filter paper and weighed. Each liver was divided into two parts, the first part was fixed in 10% formol saline for histopathological examination and immunohistochemical determination of iNOS and COX-2 activities; meantime the second remaining part was homogenized in ice-cold physiological saline. The homogenate was centrifuged at 6000 rpm for 15 min at 4°C using a cooling centrifuge (Sigma, 3-30 K, Germany) and the obtained supernatant was used for the biochemical estimation of hepatic MDA, GSH and NO contents.

Biochemical estimations

Assessment of hepatotoxicity biomarkers: Serum ALT and AST activities were determined using commercially available kits (Randox, UK) and were expressed as U L^{-1}. Serum GGT and LDH activities were assessed using diagnostic kits (Analyticon, Germany) and (Human, Germany), respectively and were expressed as U L^{-1}. Serum TP concentration was measured

using biochemical kit (Diamond Diagnostic, Egypt) and was expressed as g dL^{-1}.

Assessment of oxidative and nitrosative stress biomarkers: Hepatic lipid peroxides content was estimated according to the method described by Uchiyama and Mihara[16] and expressed as nmol g^{-1} tissue.

Hepatic GSH content was determined as described in the method of Beutler *et al.*[17] and was expressed as mg g^{-1} tissue. Hepatic NO content was estimated as total nitrate/nitrite (NOx) using Griess reagent[18] and was expressed as µmol g^{-1} tissue.

Assessment of inflammatory biomarkers: Serum TNF-α and IL-1β levels were determined using ELISA kits (Koma Biotech, Korea) and (Abcam, USA), respectively and both were expressed as pg mL^{-1}.

Histopathological examination of liver tissues: Liver tissue specimens were fixed in 10% formol saline, then trimmed off, washed and dehydrated in ascending grades of alcohol. The dehydrated specimens were then cleared in xylene, embedded in paraffin blocks and sectioned at 4-6 µm thick. The obtained tissue sections were deparaffinized using xylol and stained using hematoxylin and eosin (H and E) for histopathological examination through the electric light microscope[19]. The frequency and severity of lesions in the livers were assessed semi-quantitatively as previously reported[20] using a scale where, grade 0: No apparent injury, grade I: Swelling of hepatocytes, grade II: Ballooning of hepatocytes, grade III: Lipid droplets in hepatocytes and grade IV: Necrosis of hepatocytes. In addition, a scoring system was used to establish the severity of hepatic inflammation[21] where, grade 0: None, grade 1: Scattered neutrophils, occasional mononuclear cells, 1 or 2 foci per 20x objective, grade 2: Neutrophils associated with ballooned hepatocytes, mild chronic inflammation, 3 or 4 foci per 20x objective and grade 3: Acute and chronic inflammation, neutrophils may concentrate in zone III, over 4 foci per 20x objective.

Immuno-histochemical estimation of inducible nitric oxide synthase and cycloxegenase-2 activities: Hepatic iNOS and COX-2 activities were determined according to the following immuno-histochemical protocol[22]. Liver tissue blocks from each group were initially checked by hematoxylin-eosin (H and E)-stained sections to select the representative block for immuno-histochemical staining. Four micron sections from each formalin-fixed paraffin blocks were immuno-stained using the primary antibodies against iNOS (1:50, monoclonal, neomarkers, Fremont, CA, USA) and COX-2 (1:100, monoclonal, Neomarkers, Fremont, CA, USA). Immuno-histochemistry was performed by the labeled streptavidin-biotin method, using the ultra vision large volume detection system (Lab Vision, Fremont, CA, USA) kit. The existence of immuno-histochemical staining against iNOS and COX-2 display early phase of inflammation. Sections were de-paraffinized and rehydrated in graded ethanol. After being rinsed in distilled water, sections were micro-waved for 5 min at 600 W in 0.01 mol L^{-1} sodium citrate buffer (pH 6.0); this step was repeated 3 times. The slides were immersed in 3% H_2O_2 in distilled water for 5 min and then in blocking solution for 30 min in order to block endogenous peroxidase activity as well as unspecific binding sites. Sections were then rinsed in Phosphate Buffered Saline (PBS) and incubated at room temperature with the primary antibody for 60 min followed by rinsing in PBS. Negative controls were performed by omitting the primary antibody. The degree of staining was assorted according to the extent and intensity of the staining. Two independent observers screened all sections as a semi-quantitative evaluation of iNOS and COX-2 immuno-staining. The intensity of staining was scored on a scale of 0-3 where, 0 = negative staining, 1 = weakly positive staining, 2 = moderately positive staining and 3 = strongly positive staining. The extent of positivity was estimated on a scale of 0-4, in which 0 = negative, 1 = positive staining in 1-25% of cells, 2 = positive staining in 26-50%, 3 = positive staining in 51-75% and 4 = positive staining in 76-100%. The combined staining score (extension+intensity) $\geq$3 was considered as positive staining.

Statistical analysis: All data were expressed as Means±Standard Error of Mean (SEM). Statistical analysis was performed using GraphPad Prism (GraphPad software, version 5, Inc., San Diego, USA). Comparison of means was done using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test. The level of significance was set at $p<0.05$.

RESULTS

Effect on hepatotoxicity biomarkers: Paracetamol significantly increased serum ALT and AST activities by 220.4 and 177.37%, respectively compared to the vehicle control group. Pretreatment with NAC and carvedilol significantly decreased ALT activity by 60.65 and 44.98%, respectively as compared to hepatotoxicity control group. Similarly, NAC and carvedilol significantly reduced AST activity by 70.72 and

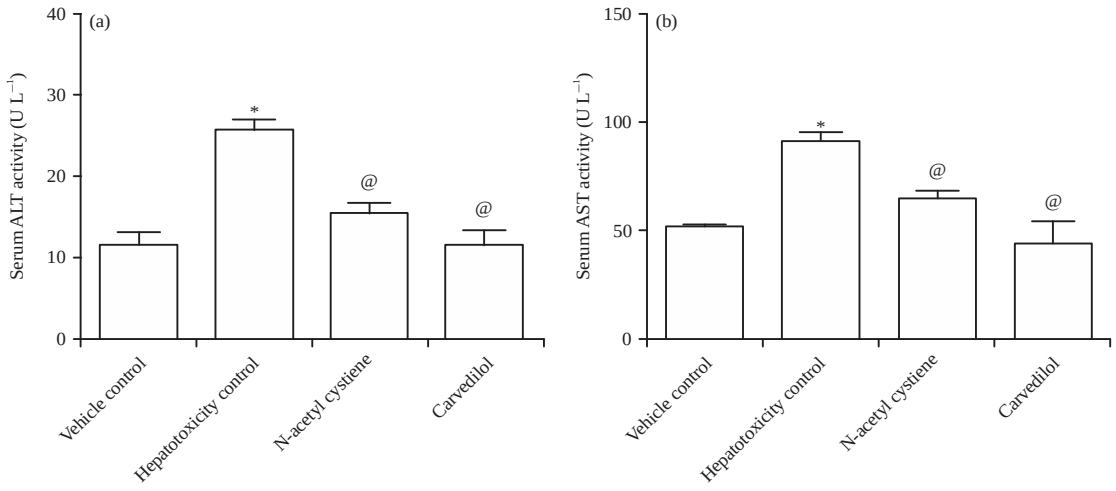

Fig.1(a-b): Effect of N-acetyl cysteine (300 mg kg^{-1}; p.o.) and carvedilol (30 mg kg^{-1}; p.o.) on (a) Serum alanine aminotransferase (ALT) and (b) Aspartate aminotransferase (AST) in paracetamol-induced hepatotoxicity rat model, each bar represents the Mean±SEM (n = 8), hepatotoxicity was induced by administration of a single dose of paracetamol (1 g kg^{-1}; p.o.), statistical analysis was carried out by ANOVA followed by Tukey-Kramer multiple comparisons test, *Significantly different from vehicle control value at $p<0.05$, @Significantly different from hepatotoxic control value at $p<0.05$

47.63%, respectively as compared with hepatotoxicity control group (Fig. 1a, b). There was no significant difference between NAC and carvedilol regarding their effects on serum ALT and AST activities.

Paracetamol administration resulted in significant elevation in serum LDH and GGT activities by 624.4 and 297.32%, respectively when compared to the vehicle control group. Pretreatment of rats with NAC significantly reduced LDH and GGT activities by 54.36 and 64.26%, respectively as compared with paracetamol control group (Fig. 2a, b).

Moreover, paracetamol-induced hepatotoxicity significantly decreased serum TP level to 59.23% as compared to the vehicle control group. Prophylactic treatment of hepatotoxic rats withcarvedilol significantly elevated TP level by 235.42% as compared with hepatotoxicity control group (Fig. 2c).

Effect on oxidative and nitrosative stress biomarkers: Induction of hepatotoxicity by paracetamol resulted in significant elevation of hepatic MDA content by 181.66% and significant decrease in hepatic GSH content by 39.28% compared to the vehicle control group. Pretreatment with NAC and carvedilol significantly reduced paracetamol-induced elevation of MDA by 70.75 and 48.5%, respectively compared to hepatotoxicity control group without any significant difference with respect to NAC (Table 1).

Paracetamol administration resulted in significant elevation in hepatic NOx content by 149.53% compared with the vehicle control group. Likewise, prophylactic administration of NAC significantly increased hepatic NOx content by 132.6% as compared to hepatotoxicity control group, meanwhile carvedilol pretreatment nearly normalized hepatic NOx content (Table 1).

Effect on inflammatory biomarkers: Induction of hepatotoxicity by paracetamol significantly increased serum levels of TNF-α and IL-1β by 268.72 and 490.13%, respectively compared to the vehicle control group. Pretreatment with NAC and carvedilol significantly decreased the elevated TNF-α level by 68.85 and 28%, respectively compared with hepatotoxicity control group. Carvedilol showed better effect on serum TNF-α level than NAC. Similarly, prophylactic administration of NAC and carvedilol significantly reduced the elevated IL-1β level by 61.34 and 60%, respectively when compared to hepatotoxicity control animals (Table 2). There was no significant difference between NAC and carvedilol regarding their effects on serum IL-1β level.

Effect on liver histopathology: Histopathological examination of hepatic tissues of vehicle control group revealed organization of hepatic cords and normal histological structure of hepatic lobules (grade 0), without any markers of vascular or inflammatory changes (grade 0) (Fig. 3a, b). On the other hand, hepatic sections of paracetamol-induced hepatotoxic rats revealed moderate vascular congestion of central veins and hepatic sinusoids in

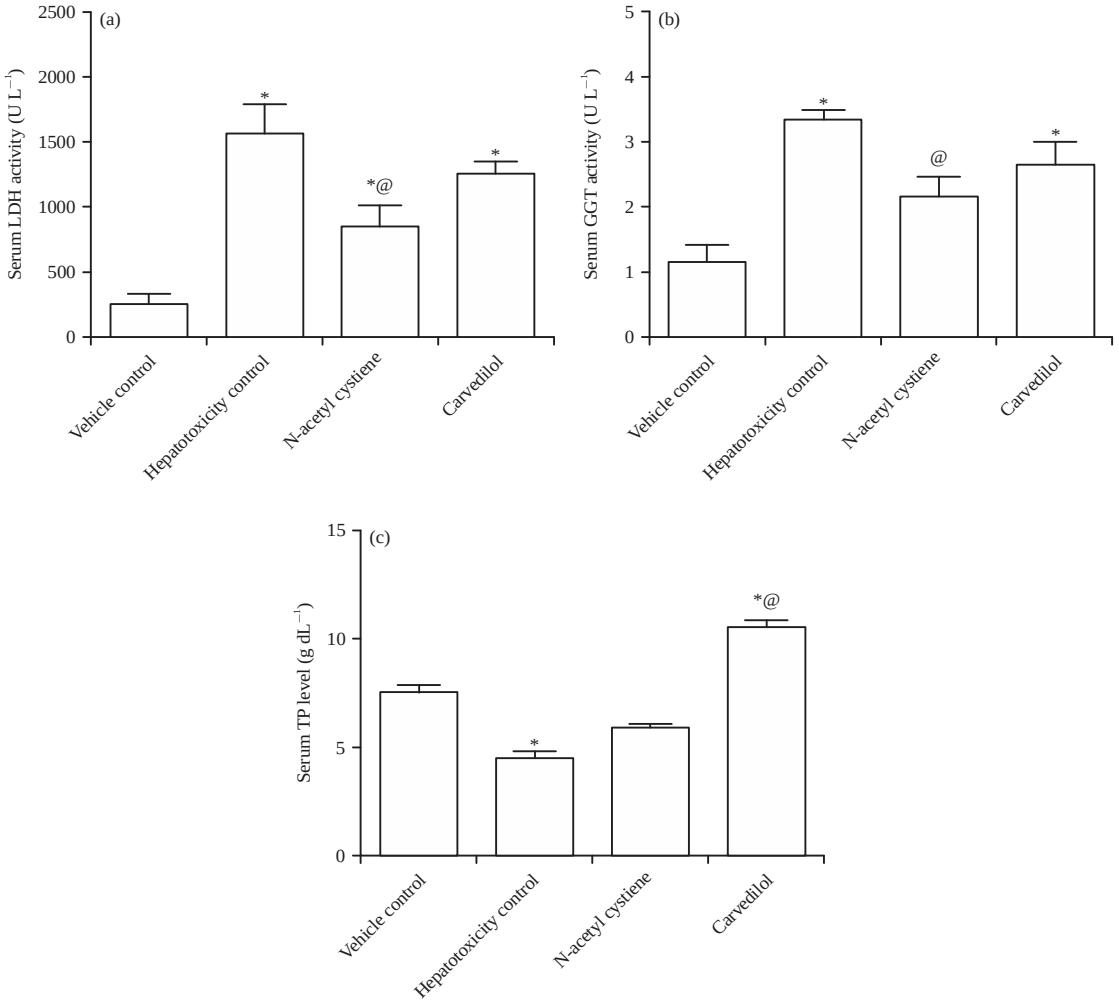

Fig. 2(a-c): Effect of N-acetyl cysteine (300 mg kg^{-1}, p.o.) and carvedilol (30 mg kg^{-1}, p.o.) on (a) Serum lactate dehydrogenase (LDH), (b) Gamma-glutamyltransferase (GGT) and (c) Total protein (TP) in paracetamol-induced hepatotoxicity rat model, each bar represents the Mean±SEM (n = 8), hepatotoxicity was induced by administration of a single dose of paracetamol (1 g kg^{-1}, p.o.), statistical analysis was carried out by ANOVA followed by Tukey-Kramer multiple comparisons test, *Significantly different from vehicle control value at p<0.05, @Significantly different from hepatotoxic control value at p<0.05

Table 1: Effect of N-acetyl cysteine (300 mg kg^{-1}, p.o.) and carvedilol (30 mg kg^{-1}, p.o.) on oxidative and nitrosative stress biomarkers in paracetamol-induced hepatotoxicity rat model

		Paracetamol treated rats		
Parameters	Vehicle control	Hepatotoxicity control	NAC	Carvedilol
MDA (nmol g^{-1} tissue)	75.8±7.28	137.7±8.52*	97.43±6.93$^{@}$	66.78±4.83$^{@}$
GSH (mg g^{-1} tissue)	1.40±0.18	0.550±0.04*	2.010±0.16$^{@}$	0.655±0.146^{b}
NOx (µmol g^{-1} tissue)	858±76.92	1283±66.4*	1700±103.2*$^{@}$	971.4±52.3^{b}

Values are expressed as Means±SEM (n = 8), statistical analysis was carried out by ANOVA followed by Tukey-kramer multiple comparisons test, *Significantly different from vehicle control value at p<0.05, $^{@}$Significantly different from hepatotoxicity control value at p<0.05, ^{b}Significantly different from NAC value at p<0.05

addition to moderate inflammatory changes (grade 2). Red blood cells pooling in the sinusoids with inflammatory cells mainly lymphocytes and macrophages infiltrating the necrotic foci of hepatocytes. Fatty change of hepatocytes which characterized by signet ring cells appeared as cluster scatter all over of hepatic lobules. Centrilobular necrosis and hyperplasia of kupffer cells were also seen (grade IV) (Fig. 3c, d).

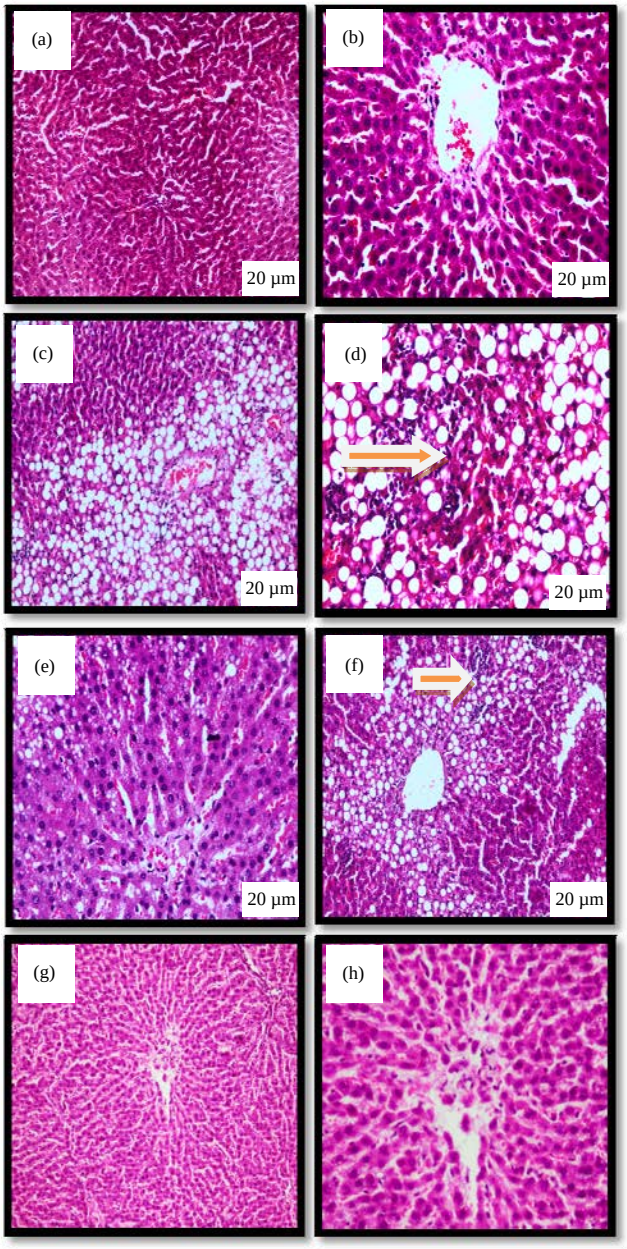

Fig. 3(a-h): Histopathological changes in liver tissues of (a, b) Vehicle control, (c, d) Hepatotoxicity control, (e, f) N-acetyl cysteine treated and (g, h) Carvedilol treated rats. (a) Photomicrograph of liver section of vehicle control rats showing normal histological structure of hepatic lobules and organization of hepatic cords (X100), (b) Showing no signs of vascular or inflammatory changes (X200), (c) Photomicrograph of liver section of hepatotoxic control rats showing vascular congestion of the central veins and damaged hepatocytes with areas of fatty change (X200), (d) Showing red blood cells pooling in the sinusoids with inflammatory cells infiltrating the necrotic foci of hepatocytes (arrow) (X400), (e) Photomicrograph of liver section of N-acetyl cysteine-treated rats showing vascular congestion of central veins and hepatic sinusoids as well as fatty degeneration of hepatocytes (X200), (f) Showing centrilobular necrobiotic changes with focal aggregation of inflammatory cells (arrow) (X200), (g) Photomicrograph of liver section of carvedilol-treated rats showing normal lobular structure with mild dilatation of hepatic sinusoids (X100) and (h) Showing mild swelling of hepatocytes (X200) (H and E)

Table 2: Effect of N-acetyl cysteine (300 mg kg^{-1}, p.o.) and carvedilol (30 mg kg^{-1}, p.o.) on inflammatory biomarkers in paracetamol-induced hepatotoxicity rat model

		Paracetamol treated rats		
Parameters	Vehicle control	Hepatotoxicity control	NAC	Carvedilol
TNF-α (pg mL^{-1})	40.86±3.9	8109.8±6.16*	75.6±1.88*@	30.76±0.81@b
IL-1β (pg mL^{-1})	26.85±1.9	131.6±6.4*	80.73±3.76*@	79.01±2.97*@

Values are expressed as Means±SEM (n = 8), statistical analysis was carried out by ANOVA followed by Tukey-kramer multiple comparisons test, *Significantly different from vehicle control value at p<0.05, @Significantly different from hepatotoxicity control value at p<0.05, bSignificantly different from NAC value at p<0.05

Hepatic tissues of rats pretreated with NAC showed mild vascular and inflammatory changes in form of dilatation of central veins and sinusoids (grade 2) coupled by centrilobular degenerative changes appeared as fatty degeneration and apoptosis of hepatocytes (grade III) (Fig. 3e, f).

Normal histological structure of hepatic lobules with mild dilatation hepatic sinusoids, without any markers of vascular or inflammatory changes (grade 0) were observed inthe hepatic sections of rats pretreated with carvedilol. In addition, the hepatocytes revealed micro-vacuolation of hepatocytes by few numbers of droplets (grade I) (Fig. 3g, h).

Effect on immuno-histochemistry of iNOS and COX-2: The normal control ratsshowed no detectable iNOS or COX-2 stain in both cytoplasm and nuclei (0 = negative staining) (Fig. 4a, b), meanwhile paracetamol-intoxicated rats revealed uniform distribution of very strong immunostain by iNOS (4 = very strongly positive staining) and COX-2 appeared as brown granules in both cytoplasm and nuclei of degenerated hepatocytes (3 = strongly positive staining) (Fig. 4c, d).

Rats pretreated with NAC revealed positive iNOS stain in numerous numbers of hepatocytes nuclei (3 = strongly positive staining) coupled with positive COX-2 stain in few numbers of hepatocytes nuclei (1 = weakly positive staining) (Fig. 4e, f).

Carvedilol pretreated ratsshowed no detectable iNOS or COX-2 stain in both cytoplasm and nuclei (0 = negative staining) (Fig. 4g, h).

DISCUSSION

In the present study, paracetamol-induced hepatotoxicity resulted in hepatic dysfunctionas manifested by significant increase in serum ALT, AST, LDH and GGT activities coupled by significant decrease in serum TP level. Similar results were previously reported[22,23]. It has been reported that oxidative stress is one of the major contributing factors in the development of hepatic cellular injury[24]. Therefore, aggravated free radical generation could result in cell membrane attack and the leakage of the cytoplasmic enzymes, ALT, AST and LDH, as well as the membrane-bound enzyme, GGT into the blood circulation due to alteration of membrane permeability[25]. The liver is considered the major organ responsible for synthesis of plasma proteins[26]. The observed decrease in serum TP level is also consistent with hepatic damage and could be a consequence of decreased number of cells responsible for protein synthesis in the liver as a result of necrosis[27].

Data of the present study revealed that carvedilol administration counteracted paracetamol-induced hepatic dysfunction as manifested by significant decrease in serum ALT and AST activities coupled with significant increase in serum TP level. Such results are in accordance with those of previous studies[9] and could be attributed to the scavenging properties of carvedilol or its metabolites resulting in maintenance of membrane permeability and integrity[28].

In the current study, oxidative stress was apparentin hepatotoxic control rats as evidenced by significant elevation of hepatic TBARS content, an indicator of lipid peroxidation, parallel to significant reduction of hepatic GSH content. These findings are in accordance with those described previously[29]. Oxidative stressis considered as an important factor in the pathophysiology of paracetamol-induced hepatotoxicity. Consequently, the augmentation in free radical production, mainly superoxide anion and hydrogen peroxide, provoke peroxidation of membrane phospholipids with increased production of MDA as an end product of lipid peroxidation[30]. In addition, the produced superoxide anion binds to nitric oxide generating peroxynitrite radical which in turn causes further lipid peroxidation and protein oxidation[31].

It has been previously reported that the toxic0 metabolite of paracetamol (NAPQI) is detoxified by GSH conjugation and eliminated by the kidney, however, at over dose of paracetamol, over production of NAPQI can leadto depletion of cytosolic and mitochondrial GSH as well as tissue necrosis[2].

Findings of the present investigation revealed that carvedilol pretreatment resulted in significant reduction of hepatic TBARS content without significant effect on hepatic GSH content. The obtained results could be related to the free radicalscavenging and iron chelating properties of carvedilol with subsequent protection against oxidative stress[32]. The antioxidant activity of carvedilol is derived from the carbazole moiety in its structure[33].

The current results demonstrated significant increase in hepatic NO content in hepatotoxicity control rats which finds

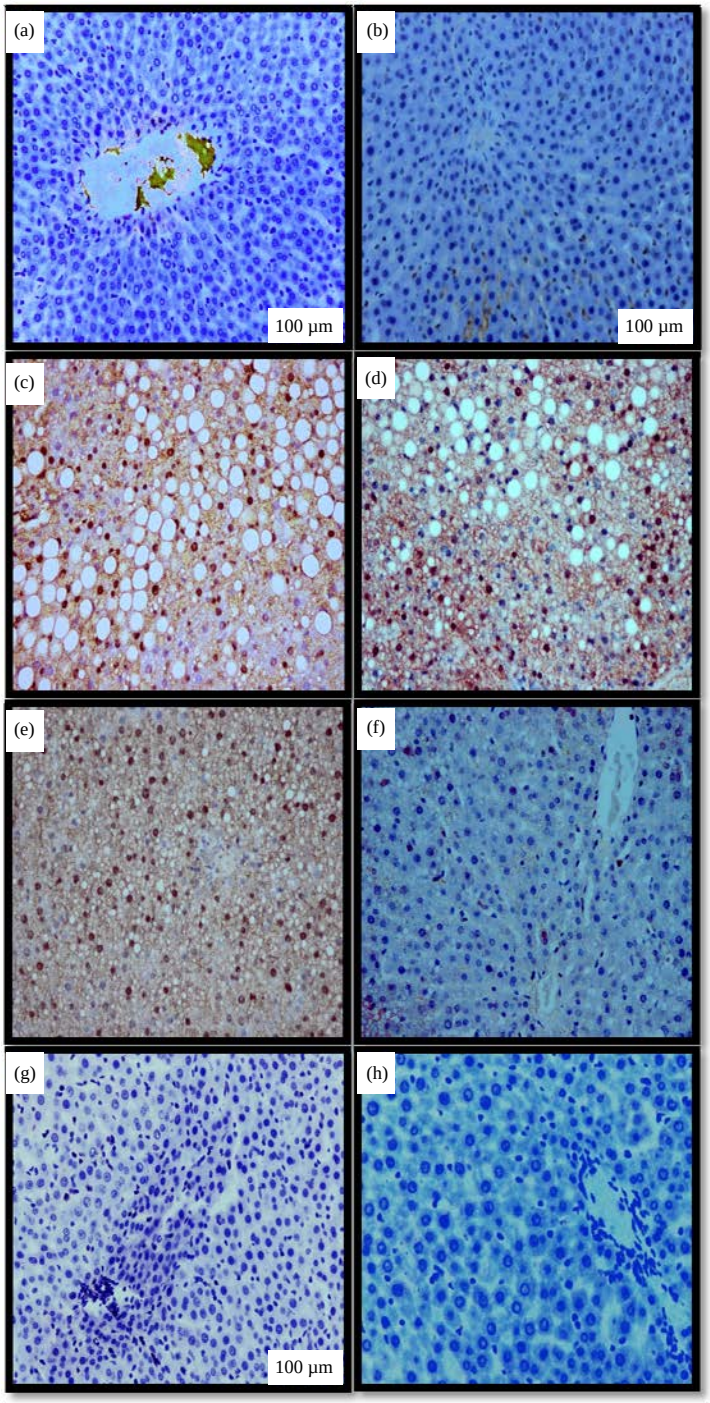

Fig. 4(a-h): Immuno-histochemical estimation of iNOS and COX-2 activitiesin liver tissues of (a, b) Vehicle control, (c, d) Hepatotoxicity control, (e, f) N-acetyl cysteine treated and (g, h) Carvedilol treated rats. In liver tissues of vehicle control rats, no detectable (a) iNOS or (b) COX-2 immuno-stained granules in both cytoplasm and nuclei was observed, (c) Meanwhile uniform distribution of very strong immuno-stain by iNOS in the hepatocytes in both cytoplasm and nuclei of degenerated cells and little number of hepatic nuclei positive COX-2, (d) Immuno-stain were observed in liver tissues of hepatotoxic control rats, (e) Positive iNOS stain, in numerous numbers of hepatocytes nuclei and few numbers of hepatocytes nuclei COX-2 positive stain, (f) Observed in liver tissues of N-acetyl cysteine-treated rats, (g) Meanwhile, in liver tissues of carvedilol-treated rats, no detectable (g) iNOS or (h) COX-2, immuno-stained granules in both cytoplasm and nuclei was observed (X200)

support in the findings of other investigators[22]. The observed effect could be attributed to the up-regulation of iNOS activity induced by TNF-α[34]. In addition, it can be suggested that elevated NO content could be a defense mechanism to compensate for continuous inactivation of NO by ROS including superoxide anion[35].

Paracetamol-induced hepatotoxicity resulted in significant increase in serum levels of the pro-inflammatory cytokines, including TNF-α and IL-1β. Similar results were previously reported[24]. The current observation could be explained through activation of kupffer cells by paracetamol overdose and oxidative stress leading to increased release of such pro-inflammatory cytokines[36,37].

According to the current results, prophylactic administration of carvedilol significantly decreased serum TNF-α and IL-1β levels which is in line withresults of previous study[38]. Such results could be attributed to the suppressing effect of carvedilol on mRNA expression and protein production of the aforementioned pro-inflammatory cytokines[39].

Induction of hepatotoxicity by paracetamol produced significant increase in iNOS and COX-2 activities. The current results coincide with those of previous studies[22,23]. The possible mechanism for the current results appearsto be related to the activation of NF-κB signaling pathway by paracetamol overdose and elevated TNF-α level[40,41].

Findings of the present study revealed that carvedilol supplementation significantly decreased iNOS and COX-2 activities which is in accordance with those of previous studies[38,42] and could be explained via the inhibitory effect of carvedilol on of NF-κB activation[43].

The histopathological examination of liver tissues of paracetamol treated rats revealed severe liver damage including moderate vascular congestion of central veins and hepatic sinusoids, moderate inflammatory changes, inflammatory cell infiltration, fatty degeneration of hepatocytes, centrilobular necrosis and hyperplasia of kupffer cells. The observed histopathological changes are in accordance with previous studies[15,22,23] which stated that paracetamol overdose caused cytoplasmic vacuolation of hepatocytes with sinusoidal congestion in addition to centrilobular necrosis and hepatocytes degeneration. These observations could be related to ROS generation, subsequent lipid peroxidation and inflammation induced by paracetamol.

The histopathological findings of the current study revealed that carvedilol administration greatly improved paracetamol-induced hepatic tissue damage by showing nearly normal histological structure of hepatic tissues without any markers of inflammatory changes. Such findings further support the biochemical and the immuno-histochemical findings and could be attributed to the antioxidant activity and iron-chelating ability of carvedilol reducing oxidative stress with subsequent reduction of histopathological alterations and restoration of normal physiological state of liver.

CONCLUSION

In conclusion, pretreatment of hepatotoxic rats with carvedilol attenuated most of the biochemical, histopathological and immuno-histochemical changes induced in rats by paracetamol. Such findings may be of considerable value in the treatment of hepatotoxicity in clinical practice.

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