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Toxicology

Cadmium-induced hepatocellular injury: Modulatory effects of γ-glutamyl cysteine on the biomarkers of inflammation, DNA damage, and apoptotic cell death

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ABSTRACT

Cadmium is an extremely toxic pollutant that reaches human body through intake of the industrially polluted food and water as well as through cigarette smoking and exposure to polluted air. Cadmium accumulates in different body organs especially the liver. It induces tissue injury largely through inflammation and oxidative stress-based mechanisms. The aim of the current study was to investigate the ability of γ glutamyl cysteine (γGC) to protect against cadmium-induced hepatocellular injury employing Wistar rats as a mammalian model. The results of the current work indicated that γGC upregulated the level of the anti-inflammatory cytokine IL-10 and downregulated the levels of the pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β) in the cadmium-exposed rats. In addition, γGC reduced the liver tissues cadmium content in the cadmium-treated rats, suppressed the cadmium-induced hepatocellular apoptosis and oxidative modifications of cellular DNA, lipids, and proteins. Additionally, γGC enhanced the antioxidant potential of the liver tissues in the cadmium-treated rats as evidenced by a remarkable increase in the activity of the antioxidant enzymes superoxide dismutase and glutathione peroxidase and significant increase in the levels of the total antioxidant capacity and reduced glutathione as well as a significant reduction in oxidized to reduced glutathione (GSSG/GSH) ratio. Moreover, it effectively improved liver cell integrity in the cadmium-treated rats as demonstrated by a significant reduction in the serum activity of the liver enzymes (ALT and AST) and amelioration of the cadmium-evoked histopathological alterations. Together, these findings underscore, for the first time, the alleviating effects of γGC against cadmium-induced hepatocellular injury that is potentially mediated through reduction of liver tissue cadmium content along with modulation of both hepatocellular redox status and inflammatory cytokines.

1. Introduction

Cadmium is a highly toxic environmental pollutant that accumulates in different body organs especially the liver [1,2]. Cadmium toxicity has been associated with various organs dysfunction including the liver [3–5]. Cadmium, thus, has recently been ranked the top seventh toxic substance [6]. Industrially-contaminated food and water, cigarette smoking, and polluted air represent major exposure sources of general population to cadmium [7].

Although cadmium is not a redox reactive metal, its toxicity is predominantly based on induction of oxidative stress [8,9]. Cadmium displaces divalent cations from the active sites of different enzymes including the antioxidant enzymes, leading to disruption of the cellular redox homeostasis [10,11]. In the same context, cadmium increases the release of iron from biological membranes which subsequently participate in the generation of the reactive oxygen species, ROS [12]. Cumulative data have indicated the high affinity of cadmium to the thiol-containing compounds including the critically important endogenous

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antioxidant reduced glutathione, GSH [13,14]. Depletion of GSH, thus, largely contributes to the cadmium-induced tissue injury [15]. Cadmium toxicity has also been associated with inflammatory responses that include modulation of the pro-inflammatory and anti-inflammatory cytokines [16,17]. Several studies have reported substantial roles of oxidative stress in initiation of the inflammatory cascades [18–20]. Cadmium-triggered inflammatory response is, thus, potentially mediated through cadmium-induced oxidative stress. Additionally, cadmium toxicity has been associated with oxidative modifications of the cellular DNA, lipids, and proteins [9,21]. Tandon, et al., demonstrated that the antioxidant N-acetyl L-cysteine (NAC) and mannitol decreased the cadmium-induced hepatocellular injury and oxidative stress [22]. The authors also showed that combining the antioxidants with a metal chelator was more effective in protection against cadmium-induced oxidative stress. In has also been shown that vitamin E protected against cadmium-induced hepatotoxicity via antioxidant mechanisms [23]. Improving liver tissue redox status and/or cadmium chelation may, thus, protect against cadmium-induced liver tissue injury.

Gamma glutamyl cysteine (γGC) is a dipeptide that has displayed a forceful antioxidant properties in different experimental models [24–26]. It is a cofactor for the antioxidant enzyme glutathione peroxidase 1 [24]. Metal chelating properties has also been reported for γGC [25,27]. The aim of the current study was, thus, to explore the possible protective effect of γGC against cadmium chloride-induced hepatocellular injury using rats as an experimental model. Liver was chosen in our experiment because cadmium chlorides accumulates largely in the liver compared to other forms of cadmium [28]. In addition, liver is the organ which initially receives, via the portal circulation, most of the orally administered cadmium, reviewed in [29].

2. Material and methods

2.1. Animals

Male Wistar rats weighing 210–230 g (50 days old on average) were supplied by King Fahd medical research center, King Abdulaziz University, Jeddah, Saudi Arabia. Rats were housed in polypropylene cages (four animals per cage), at temperature of 23 ± 2 °C, humidity of 60 ± 10%, and a 12 h/12 h light/dark cycle). Standard commercially available rodent chow and double distilled water were freely allowed. Rats were acclimatized to Taif University animal facility for a ten-day period prior to experimentation. All measures were made to reduce animal suffering during the laboratory work. Animals care, treatment, and sampling were executed in accordance with the guide of Taif University Research Ethical Committee and in agree with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

2.2. Chemicals and kits

Cadmium chloride (99.99% purity), thiobarbituric acid (TBA, ≤ 98% purity), 2,4-dinitrophenyl hydrazine (DNPH, 97% purity), and 5,5′- dithiobis 2-nitrobenzoic acid (DTNB, ≤ 98% purity) were obtained from Sigma-Aldrich (St Louis, MO, USA). γ-glutamyl cysteine was obtained from Biospecialties International (Mayfield, NSW, Australia). All other chemicals were of highest purity (American chemical society reagent grade). Total antioxidant capacity, superoxide dismutase, and glutathione peroxidase assay kits were purchased from Cayman Chemical Company (Ann Arbor, MI, USA). IL-10, TNF-α, IL-6, and IL-1β assay kits were obtained from Ray Biotech (Norcross, GA, USA). Caspase-3 kit was purchased from R & D systems (Minneapolis, MN, USA). 8-oxo-2`-deoxyguanosine assay kit was purchased from Trevigen (HT 8-oxo-dG, Trevigen, Inc., Gaithersburg, MD, USA). DNA extraction kit was purchased from Qiagen (DNeasy Blood & Tissue Kit, Qiagen, Hilden, Germany). Liver enzymes alanine transaminase (ALT) and aspartate transaminase (AST) assay kits were purchased from Human (Max-Planck-Ring, Wiesbaden Germany). Oxidized/reduced glutathione detection kit was purchased from Enzo Life Science Inc. (Enzo Diagnostics, NY, USA).

2.3. Experimental design and treatment protocol

Thirty-two male Wistar rats that were matched for age and body weight were randomly allocated into four groups of eight-animal each. Normal control group (N Ctrl): Rats were ip injected with saline (vehicle) daily for a twelve-week experimental period. γ-glutamyl cysteine control group (GC Ctrl): animals were injected with γ-glutamyl cysteine (100 mg/kg, ip daily for the twelve-week experimental period). Animals in N Ctrl and GC Ctrl groups were allowed to drink double distilled water ad libitum. The dose of γ-glutamyl cysteine is consistent with previous work [25]. Cadmium only-treated group (CD): rats in this group were allowed to drink double distilled water that contains 50 mg/L cadmium chloride over the twelve-week experimental period [30]. Cadmium and γ-glutamyl cysteine-treated group (CD + GC): Rats in this group were handled as the CD group except that they were also treated with γ-glutamyl cysteine (100 mg/kg, ip daily) over the twelve-week experimental period.

2.4. Sample preparation

One day after the last dose of γGC, animals in all of the experimental groups were euthanized under anesthesia (pentobarbital sodium: 65 mg/kg body weight, ip injection) [31] and blood as well as liver tissue samples were collected. Blood was obtained from the heart via cardiac puncture and centrifuged (10,000 × g, 4 °C for 15 min) to separate serum for evaluation of serum activity of alanine transaminase (ALT) and aspartate transaminase (AST). Liver tissues were quickly bathed in cold saline and divided into four parts for cadmium determination, homogenization, DNA extraction, and histopathological investigation. For cadmium determination, liver tissue sample was handled as described in measured parameters (section 2.5.4.). For homogenization, liver tissue samples were weighed, homogenized (10% w/v in phosphate-buffered saline), and centrifuged (10,000 × g, 4 °C for 15 min) to separate supernatants for determination of all of the proposed parameters. DNA was extracted from liver tissues using the commercially available Qiagen kit (DNeasy Blood & Tissue Kit, Qiagen, Hilden, Germany) according to the manufacturer's instructions. Liver tissue samples designated for histopathological investigation were handled as described in the Histopathological Examination section (2.5.10.).

2.5. Measured parameters

2.5.1. Evaluation of the inflammatory cytokines

The levels of the anti-inflammatory cytokine IL-10 and the pro-inflammatory cytokines TNF-α, IL-6, and IL-1β in the liver tissue homogenates were evaluated using ELISA kits (Ray Biotech, GA, USA) in accordance with the manufacturer's guide. The protocol employs biotinylated antibodies and streptavidin–HRP conjugate and the detection was done using TMB (3, 3′, 5, 5′-tetramethylbenzidine) solution. The concentrations of all cytokines were calculated using standard curves.

2.5.2. Evaluation of DNA oxidation marker 8-hydroxy-2′–deoxyguanosine

The biomarker of oxidative DNA damage, 8 − OH-dG, in liver tissues of different experimental groups was evaluated using the commercially available HT 8 − OH-dG ELISA II kit (R&D Systems) as guided by the manufacturer's instructions. The kit uses a standard 8 − OH-dG precoated to a plate wells. The 8 − OH-dG monoclonal antibody in the assay reagent binds competitively to the pre-coated 8 − OH-dG as well as to that in the sample solution. Antibody bound to 8 − OH-dG in the sample is washed away during a washing step while antibody bound to

the 8 − OH-dG pre-coated to the well is retained. Detection was performed with HRP-conjugate and colorimetric substrate. The intensity of the colored produced is inversely proportional to amount of 8 − OH-dG present in sample.

2.5.3. Determination of caspase 3 activity

Caspase-3 activity, the apoptotic biomarker, was measured using the commercially available kit (R &D systems) according to the manufacturer's instructions. Briefly, an aliquot of the liver tissue homogenate supernatant was incubated with the labeled substrate DEVD-pNA (acetyl-Asp-Glu-Val-Asp p-nitroaniline). The cleavage of the peptide by caspase 3 releases the chromophore pNA. Spectrophotometric determination of pNA at 405 nm was used to evaluate caspase 3 activity. Absorbance were recorded after 90 min incubation of the liver tissue homogenates with the enzyme substrate, DEVD-pNA.

2.5.4. Measurement of lipid peroxidation and cadmium level in the liver tissues

Lipid peroxidation level in the liver tissue homogenates was measured by evaluating thiobarbituric acid reactive substances (TBARS) according to the method described previously [32]. Extinction coefficient of 155 mM^{-1} cm^{-1} was used to calculate TBARS concentrations. Cadmium content in the liver tissues was determined after samples digestion in a mixture of 70% perchloric acid and 50% nitric acid (1:1) using atomic absorption spectrophotometry (PerkinElmer, Model 2380) as described previously [33].

2.5.5. Assessment of protein carbonyl content

Protein carbonyl content, an index of protein oxidative modification, in liver tissue homogenates was assessed using DNPH according to the method described previously [34]. Extinction coefficient (at 370 nm) of 22,000 M^{-1} cm^{-1} was used to calculate the concentration of protein carbonyl content in different samples.

2.5.6. Evaluation of the antioxidant enzymes activity

Activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in the liver tissue samples were evaluated using the commercially available kits (Cayman chemicals) in accordance with the manufacturer's instructions. SOD assay is based on the formation of a colored formazan dye by the action of superoxide anions (generated by hypoxanthine-xanthine oxidase system) on a tetrazolium salt. Dismutaion of the generated superoxide anion by SOD in the test sample decreases the formazan dye formation. Monitoring the optical density of formazan at 450 nm was used to evaluate SOD activity in the test samples. GPx assay employs GPx in the test sample to reduce hydrogen peroxide using GSH. Glutathione reductase and NADPH are then used to regenerate GSH. Monitoring the decline in the optical density of NADPH at 340 was used to evaluate GPx activity in the test samples.

2.5.7. Determination of reduced glutathione and oxidized to reduced glutathione ratio

Level of the reduced glutathione (GSH) in the liver tissue homogenates was measured spectrophotometrically using DTNB [35]. Briefly, liver tissue homogenates were deprotonated with trichloroacetic acid solution (10% w/v). After centrifugation (10,000 × g for 10 min at 4 °C), the supernatant was reacted with 10 mM DTNB and the optical density was measured at 412 nm. Oxidized to reduced glutathione ratio was determined using the commercially available Glutathione (GSSG/GSH) detection kit (Enzo Diagnostics, NY, USA) according to the manufacturer's instruction.

2.5.8. Assessment of total antioxidant capacity

The total antioxidant capacity (TAC) was determined in the liver tissue homogenates using the commercially available kit (Cayman total antioxidant assay) according to the manufacturer's instructions. The assay based on the ability of the antioxidants in the liver tissue samples to suppress the oxidation of 2, 2-azino-di-[3-ethylbenzthiazoline sulphonate (ABTS). Spectrophotometric monitoring of the oxidation product of ABTS at 405 nm was used to evaluate the TAC in the liver tissue homogenates.

2.5.9. Determination of serum activity of the liver enzymes

Serum activity of ALT and AST was determined using the commercially available kits; GOT (ASAT) IFCC mod liquiUV and GPT (ALAT) IFCC mod liquiUV (Human, Max-Planck-Ring, Wiesbaden Germany) according to the manufacturer's instructions. The principle of ALT and AST assay is based on the ability of these enzymes to catalyze the transamination reactions between 2-oxoglutarate and L- alanine (ALT) and between 2-oxoglutarate and L-aspartate (AST) to form pyruvate and oxaloacetate respectively which can be reduced in the presence of NADH + H^+ to L-lactate and L-malate respectively. During these reactions, NADH + H^+ is oxidized to NAD^+. The activity of these enzymes in the samples was evaluated via monitoring the rate of reduction in the NADH + H^+ optical density at 340 nm.

2.5.10. Histopathological examination

Autopsy samples that were taken from the liver of different experimental groups were fixed in 10% formalin in saline for twenty four hours. Samples were then handled, stained with hematoxylin & eosin (H &E), and examined as previously described [25].

2.6. Statistical analysis and calculations

Statistical comparisons among different experimental groups were executed using one way analysis of variance (ANOVA) followed by Tukey-Kramer test. Obtained data were presented as mean ± standard deviation (M ± SD). At $p > 0.05$, statistically difference was considered significant. SigmaPlot 12 statistics software (Systat Software, Inc., San Jose, CA) was employed to execute statistics and create graphs. Estimated daily elemental cadmium intake was calculated in different experimental groups based on their average daily water consumption (Table 2). The mean body weight and the percentage of body weight gain for rats in all groups were calculated for the first 6 weeks as well as for the last 6 weeks of the experiment (Table 1). Liver weight to

Table 1
Body weight, weight gain, and liver weight to body weight percentage.

Group	Mean body weight (gm) (Mean ± SD)			Body weight gain (% of initial weight)		Liver/ body weight % (Mean ± SD)
	Initial weight	After 6 weeks	After 12 weeks	After 6 weeks	After 12 weeks	
N Ctrl	218.1 ± 7.2	280.3 ± 9.3	305.7 ± 10.2	28.5 %	40.2%	3.59 ± 0.24
GC Ctrl	216.9 ± 6.2	283.2 ± 8.3	309.1 ± 11.1	30.6%	42.5%	3.55 ± 0.29
CD	218.5 ± 8.1	245.4 ± 7.1*	257.5 ± 7.9*	12.3%	17.8%	3.53 ± 0.31
CD + GC	219.9 ± 9.9	267.2 ± 7.9 #	292.4 ± 9.3#	21.5%	33%	3.48 ± 0.32

(*) Significant difference from N Ctrl group, $p < 0.05$ (n = 8).
(#) Significant difference from CD group, $p < 0.05$ (n = 8).

Table 2
Water, cadmium, and food consumption.

Group	Mean water consumption (ml/rat/day)		Estimated elemental cadmium intake (mg/rat/day)		Food consumption (gm/rat/day)	
	During the first 6 weeks	During the last 6 weeks	During the first 6 weeks	During the last 6 weeks	During the first 6 weeks	During the last 6 weeks
N Ctrl	25	29	–	–	13	15
GC Ctrl	25	30	–	–	12	15
CD	23	25	0.705	0.766	12	13
CD + GC	24	26	0.736	0.797	13	14

body weight perentage was also calculated at the end of the experiment (Table 1).

3. Results

3.1. Gamma glutamyl cysteine modulates the levels of the inflammatory cytokines in the cadmium-treated rats

Treating rats with cadmium significantly reduced the level of the anti-inflammatory cytokine IL-10 to 38% of its value in the normal control group (Fig. 1a). In contrast, it increased the levels of the pro-inflammatory cytokine TNF-α to 340%, IL-6 to 430%, and IL-1β to 337% of their values in the normal control group (Fig. 1b–d). γGC effectively modulated the observed cadmium-induced changes in the inflammatory cytokines levels. It significantly upregulated IL-10 to 170% of its level in the CD group and downregulated TNF-α to 46%, IL-6 to 50%, and IL-1β to 53% of their values in the CD group (Fig. 1), denoting the attenuating effect of γGC against cadmium-induced inflammatory response.

3.2. Gamma glutamyl cysteine attenuates cadmium-evoked oxidative modifications of cellular macromolecules and suppresses the apoptotic cell death

Because oxidative stress plays important roles in the induction of the inflammatory response [18–20,36], oxidative damage to the critical biomolecules in liver tissues were evaluated to explore the possible underlining mechanism of the observed cadmium-induced inflammatory response. The results indicated that exposure to cadmium resulted in 2.1-fold increase in the level of the oxidative DNA damage biomarker 8-Oxo-dG in the CD group compared to the normal control group (Fig. 2b). Besides, it induced 1.7-fold increase in the lipid peroxidation marker malondialdehyde (MDA), and 2.2-fold increase in the level of the protein oxidation marker protein carbonyl content (PCC) in the CD group compared to the normal control group (Fig. 3). Co-treatment with γGC effectively reduced oxidation of DNA (reduced to 60% of its level in the CD group), lipid peroxidation (reduced to 67% of its level in the CD group), and protein oxidative modifications (reduced to 48% of its level in the CD group, Fig. 2 and 3). In addition, Cadmium induced 2.9-fold increase

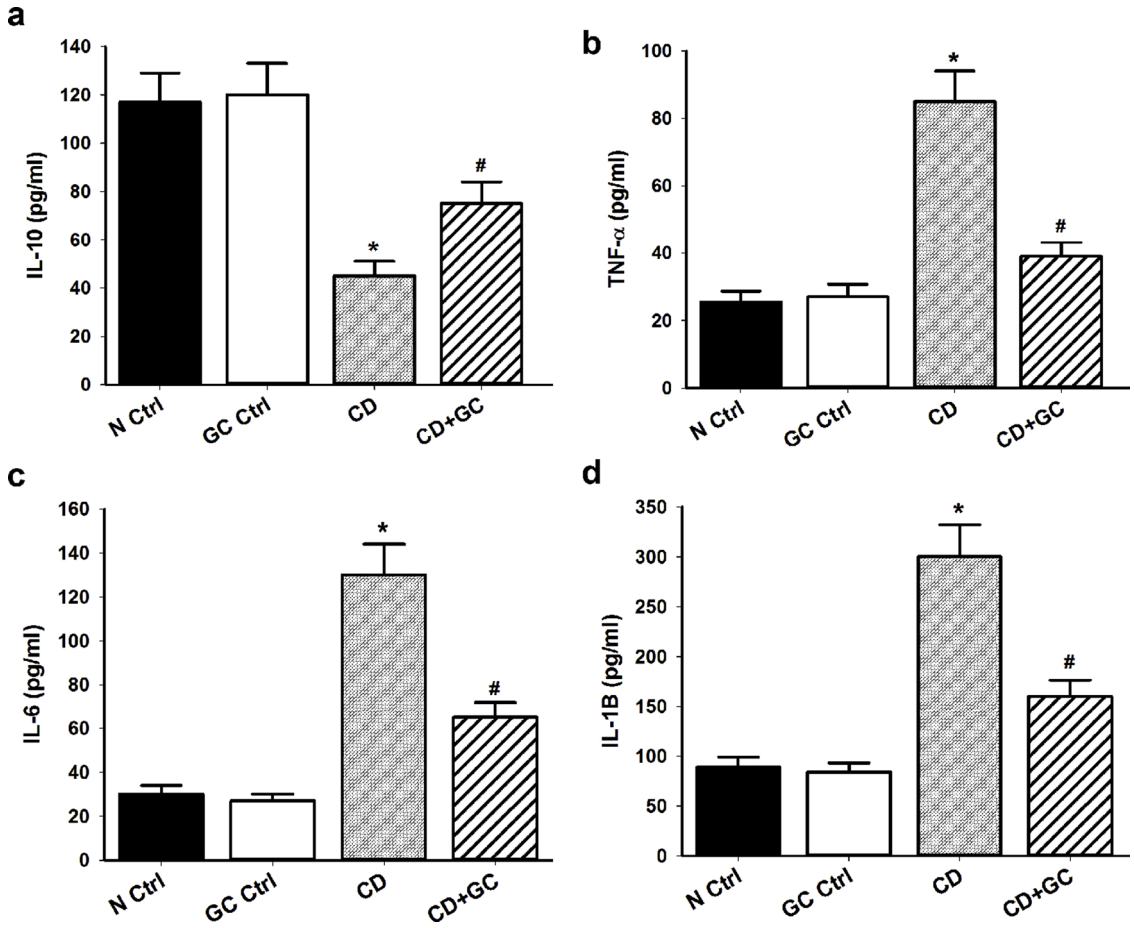

Fig. 1. Effect of γ GC on the levels of the inflammatory cytokines in the liver tissues of the cadmium-treated rats. Levels of the anti-inflammatory cytokines interleukin 10, IL-10 (a); the pro-inflammatory cytokine tumor necrosis factor alpha, TNF-α (b); interleukin 6, IL-6 (c); and interleukin 1 beta, IL-1β (d) were determined in the liver tissue homogenates of normal control group (N Ctrl), γ glutamyl cysteine control group (GC Ctrl), Cadmium-treated group (CD), and cadmium and γ glutamyl cysteine-treated group (CD + GC). Data are presented as mean ± standard deviation. (*) significant difference from N Ctrl, (#) significant difference from CD, $p < 0.05$ (n = 8).

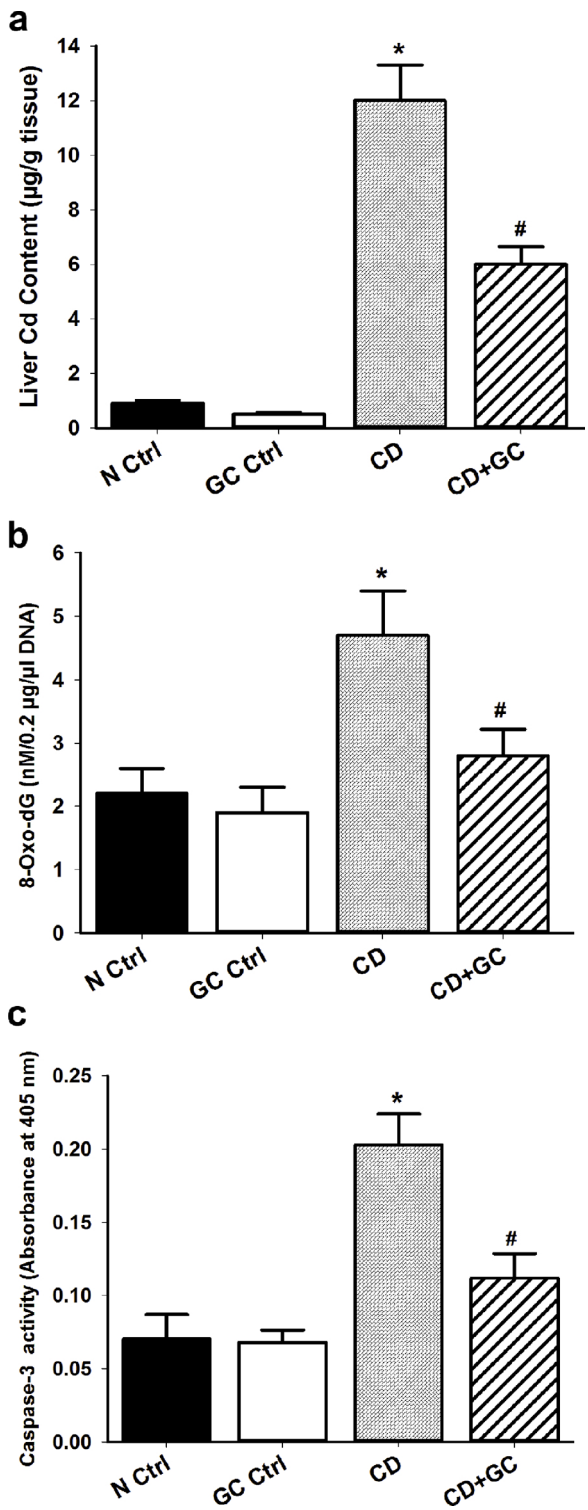

Fig. 2. Effect of γGC on the levels of cadmium, of 8-oxo-2dG, and the activity of caspase 3 in the liver tissues of the cadmium-treated rats. Cadmium content (a), level of the DNA oxidation marker 8-oxo-dG (b), and the activity of the apoptotic cell death maker caspase 3 (c) were determined in the liver tissue homogenates of normal control group (N Ctrl), γ glutamyl cysteine control group (GC Ctrl), Cadmium-treated group (CD), and cadmium and γ glutamyl cysteine-treated group (CD + GC). Data are presented as mean ± standard deviation. (*) significant difference from N Ctrl, (#) significant difference from CD, $p < 0.05$ (n = 8).

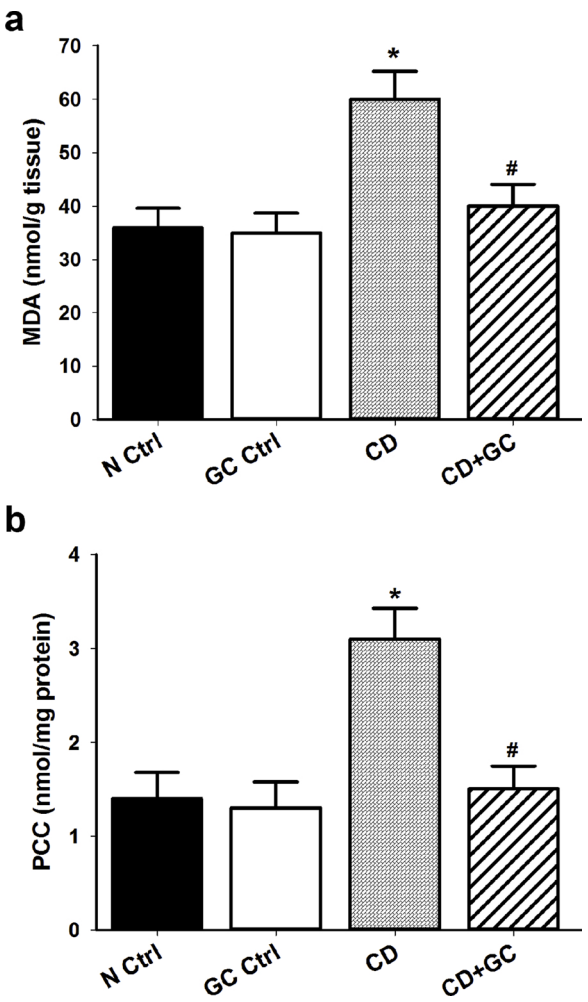

Fig. 3. Effect of γ GC on the levels of lipids and proteins oxidation biomarkers in the liver tissues of the cadmium-treated rats. Lipid peroxidation marker MDA (a), and protein oxidation marker PCC (b) were determined in liver tissues of normal control group (N Ctrl), γ glutamyl cysteine control group (GC Ctrl), Cadmium-treated group (CD), and cadmium and γ glutamyl cysteine-treated group (CD + GC). Data are presented as mean ± standard deviation. (*) significant difference from N Ctrl, (#) significant difference from CD, $p < 0.05$ (n = 8).

in the activity of the apoptotic biomarker caspase 3 (Fig. 2c). Treating rats with γGC markedly reduced caspase 3 activity (reduced to 55% of its activity in the CD group), supporting the ameliorating effect of γGC against cadmium-induced hepatocellular injury.

3.3. Gamma glutamyl cysteine decreases liver tissue content of cadmium and enhances liver antioxidant defense in the cadmium-treated rats

Cadmium administration significantly increased liver tissue cadmium content in CD group. Co-administration of γGC, however, significantly reduced the cadmium level in the liver tissues, denoting potential cadmium chelating ability of γGC (Fig. 2a). Additionally, administration of γGC significantly counteracted the cadmium-induced decline in the antioxidant status of liver tissues. Cadmium decreased the activity of the antioxidant enzymes SOD and GPx to 38% and 46% of their activities in the normal control group respectively (Fig. 4 a&b). Similarly, it decreased the levels of the reduced glutathione (GSH) and the total antioxidant capacity (TAC) of the liver tissues to 45% and 58% of their levels in the normal control group respectively (Fig. 4 c&d). In addition, it effectively increased the ratio of oxidized to reduced glutathione (GSSG/GSH) in CD group as compared to that of the normal

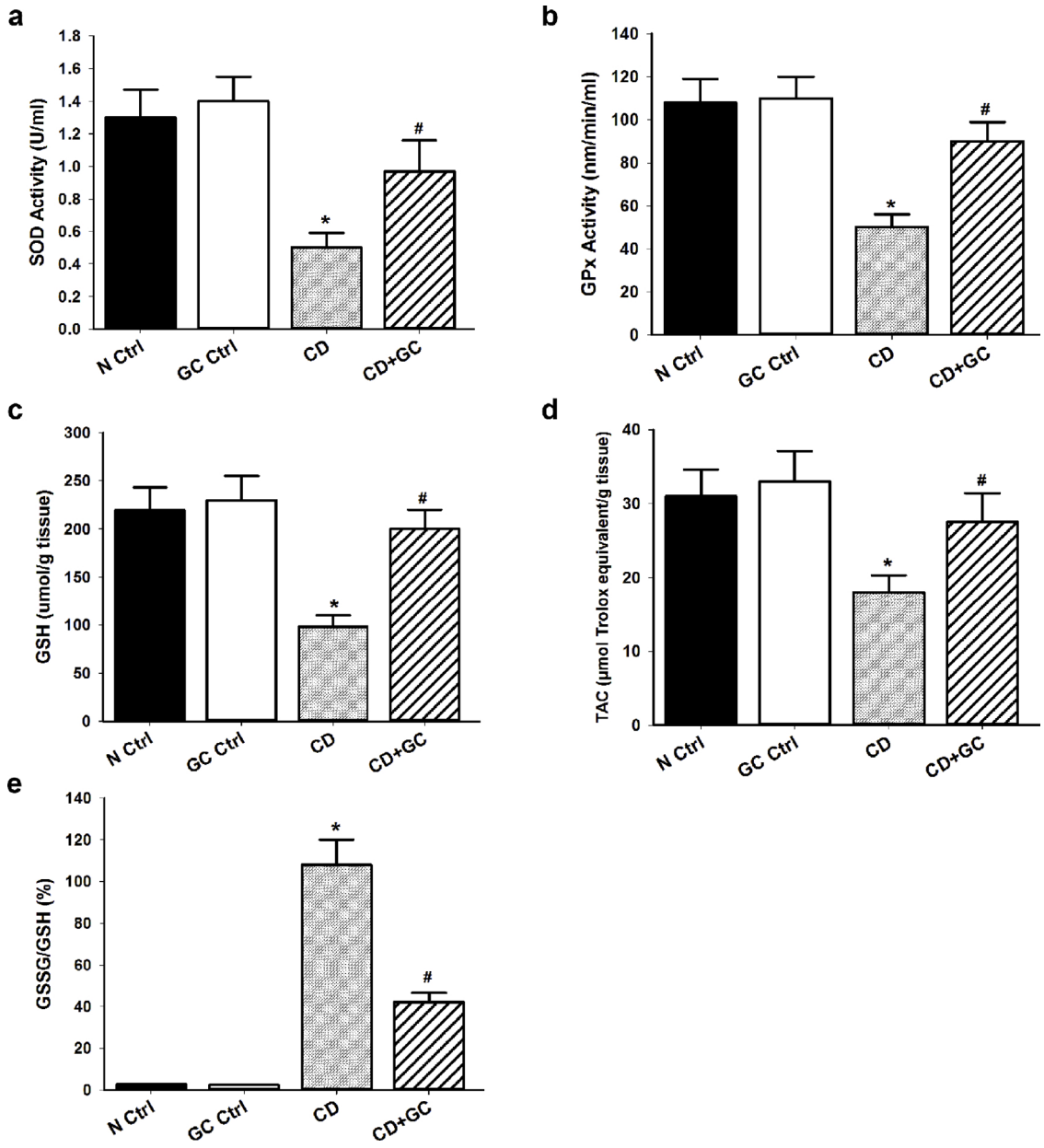

Fig. 4. Effect of γ GC on the antioxidant status of the liver tissues of the cadmium-treated rats. Activity of superoxide dismutase SOD (a), glutathione peroxidase GPx (b), reduced glutathione GSH (c), the total antioxidant capacity TAC, (d), and oxidized to reduced glutathione ratio (e) were evaluated in the liver tissue homogenates of normal control group (N Ctrl), γ glutamyl cysteine control group (GC Ctrl), Cadmium-treated group (CD), and cadmium and γ glutamyl cysteine-treated group (CD + GC). Data are presented as mean ± standard deviation. (*) significant difference from N Ctrl, (#) significant difference from CD, $p < 0.05$ (n = 8).

control group (Fig. 4e). Co-administration of γGC, however, boosted the enzymatic activity of SOD and GPx to 190% and 180% of their activities in the CD group respectively. Likewise, γGC effectively increased the levels of both GSH and TAC to 210% and 153% of their values in the CD group (Fig. 4) and significantly reduced the GSSG/GSH ratio (Fig. 4e), reinforcing the ameliorating effect of γGC against the cadmium-induced hepatocellular injury.

3.4. Gamma glutamyl cysteine improves hepatocellular integrity and ameliorates cadmium-induced liver histopathological changes in the cadmium-treated rats

Cadmium administration dramatically increased the serum activity of the intracellular liver enzymes ALT (289% of the N Ctrl group) and AST (173% of the N Ctrl group), indicating liver tissue injury. Co-administration of γGC, however, significantly reduced the serum activity of ALT (reduced to 46% of its level in the CD group, Fig. 5a) and AST (reduced to 74% of its level in the CD group, Fig. 5b), reflecting improved hepatocellular integrity. Histopathological examination of the liver tissues revealed that cadmium induced histopathological aberrations that include hepatocellular degeneration, portal vein dilatation as well as liver tissues fibrotic changes (Fig. 6 c) as compared to the normal control group that showed normal histological picture (Fig. 6a). Treating rats with γGC mitigated the observed histopathological alterations (Fig. 6d).

4. Discussion

With twenty-year half-life, continuous exposure to even little amounts of cadmium imposes substantial toxicity due to its accumulating

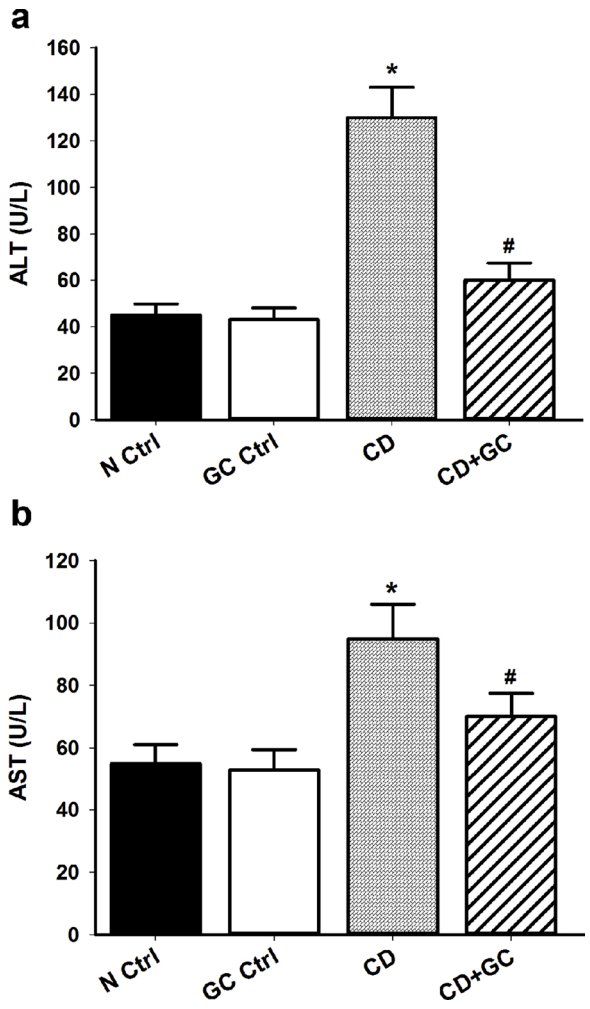

Fig. 5. Effect of γ GC on the activity of alanine transaminase and aspartate transaminase in the serum of the cadmium-treated rats. Activity of the liver enzymes alanine transaminase ALT (a) and aspartate transaminase AST (b) in serum of normal control group (N Ctrl), γ glutamyl cysteine control group (GC Ctrl), Cadmium-treated group (CD), and cadmium and γ glutamyl cysteine-treated group (CD + GC). Data are presented as mean ± standard deviation. (*) significant difference from N Ctrl group, (#) significant difference from CD group, $p < 0.05$ (n = 8).

properties [37]. Because cadmium accumulates largely in the liver tissues, hepatocytes represent a primary target for cadmium-induced tissue injury [2]. Cadmium toxicity has been associated with inflammatory responses. Cadmium decreases the level of the anti-inflammatory cytokine IL-10 and increases the levels of the pro-inflammatory cytokines including TNF-α [16,17]. In line with these data, the current work showed that cadmium significantly reduced the level of the anti-inflammatory cytokine IL-10 and increased the level of the pro-inflammatory cytokines TNF-α, IL-6, and IL-1β (Fig. 1). Co-administration of γGC, however, effectively increased the level of IL-10 and reduced the level of TNF-α, IL-6, and IL-1β (Fig. 1), highlighting the ameliorating effects of γGC against cadmium-induced hepatocellular inflammation.

It has been reported that ROS upregulate the expression of the proinflammatory cytokines including TNF-α [18,36]. Cellular redox status has also been shown to play a substantial role in production of TNF-α by the hepatic macrophages, Kupffer cells [19]. The link between the oxidative stress and the inflammatory response is thought to be mediated through ROS-induced modulation of gene expression of the inflammatory cytokines. ROS activates a variety of transcription factors including nuclear factor kappa B and AP-1 protein which, in turn, modulate gene expression of a multitude of pro-inflammatory and anti-inflammatory cytokines [20].

Cadmium disrupts the cellular redox homeostasis with subsequent induction of oxidative stress and oxidative modifications of the critical cellular macromolecules [8,9,21]. Numerous studies have unveiled different mechanisms underlining cadmium-induced disruption of cellular redox homeostasis. As a divalent cation, cadmium competes with similar divalent cations for the active sites of different enzymes and proteins [10,11,38]. Non-toxic divalent cations have been, thus, used to counteract the cadmium toxicity [39]. Zinc and selenium are divalent cations that are required for the activity of the antioxidant enzymes SOD and GPx respectively. Cadmium may, thus, dramatically affects SOD and GPx antioxidant activities, leading to disruption of cellular redox homeostasis. Importantly, cadmium increases the availability of iron, the redox reactive metal, with subsequent generation of ROS and oxidative modification of cellular macromolecules [12]. Additionally, cadmium depletes the critical cellular antioxidant GSH [13,14]. In line with these data, the current study showed that cadmium administration disrupted the redox homeostasis of the liver tissues as evidenced by increased lipid peroxidation, protein oxidation (Fig. 3), DNA oxidative modification (Fig. 2b), decreased activities of the antioxidant enzymes GPx and SOD, decreased levels of GSH and TAC, and increased ratio of GSSG/GSH (Fig. 4).

Antioxidants have been shown to play roles against cadmium toxicity. Previous studies have demonstrated that NAC and mannitol decreased the cadmium-induced hepatocellular oxidative stress via antioxidant mechanisms as evidenced by decreasing lipid peroxidation and lowering GSSG/GSH ratio as well as increasing the enzymatic antioxidants activity [22,40]. Shaikh, et al., also demonstrated that NAC and vitamin E protected against cadmium-induced hepatotoxicity via antioxidant mechanisms [23]. In addition to its antioxidant activity, Sevgiler, et al., showed that NAC reduced liver tissue content of cadmium, indicating potential metal chelating properties for NAC due its cysteine residue [41]. The cysteine-rich protein, metallothionein, has been known to play major roles in modulating cadmium toxicity [42]. In comparison, the current study showed that co-administration of γGC and cadmium resulted in a significant decrease in the liver tissue cadmium content and a significant improvement in the liver tissue redox status. Improved liver tissue redox status was evidenced by significant decrease in lipid peroxidation, protein oxidation, DNA oxidative modification, GSSG/GSH ratio as well as significant increase in the activity of the antioxidant enzymes GPx and SOD, level of GSH and the total antioxidant capacity in the liver tissues of CD + GG rat group compared of CD group. Since γGC, at the used dose, was not able to significantly increase the level of GSH in the control group (Fig. 4c), its ameliorating effects against cadmium-induced oxidative stress cannot be explained simply via increasing liver tissues content of the antioxidant GSH. The mechanism of the ameliorating effects of γGC is, thus, more likely linked to its ability to reduce liver tissue cadmium content. The improvement in the liver tissue redox status is possibly secondary to decreased liver tissue cadmium content as cadmium decreases the activity of the antioxidant enzymes by displacing their essential divalent cation cofactors [10,11] and increases the production of ROS via releasing the redox reactive iron for the biological membranes [12].

γGC is a dipeptide composed of cysteine and glutamic acid. In addition to the metal chelating activity of cysteine residue that has been demonstrated in NAC [41], glutamic acid has been shown to exhibit metal chelating properties [43]. The presence of both cysteine and glutamic acid residues in γGC may, thus, responsible for its ability to reduce liver tissue content of cadmium potentially through metal chelating activity. Role of γGC as a cofactor for GPx, the important antioxidant enzyme, cannot also be excluded [24]. The observed immunemodulatory activity of γGC on cadmium-induced inflammatory response may, thus, be mediated, at least partially, via cadmium chelation and antioxidant mechanisms.

Previous studies have demonstrated that cadmium toxicity is associated with hepatocellular damage that was manifested by increased

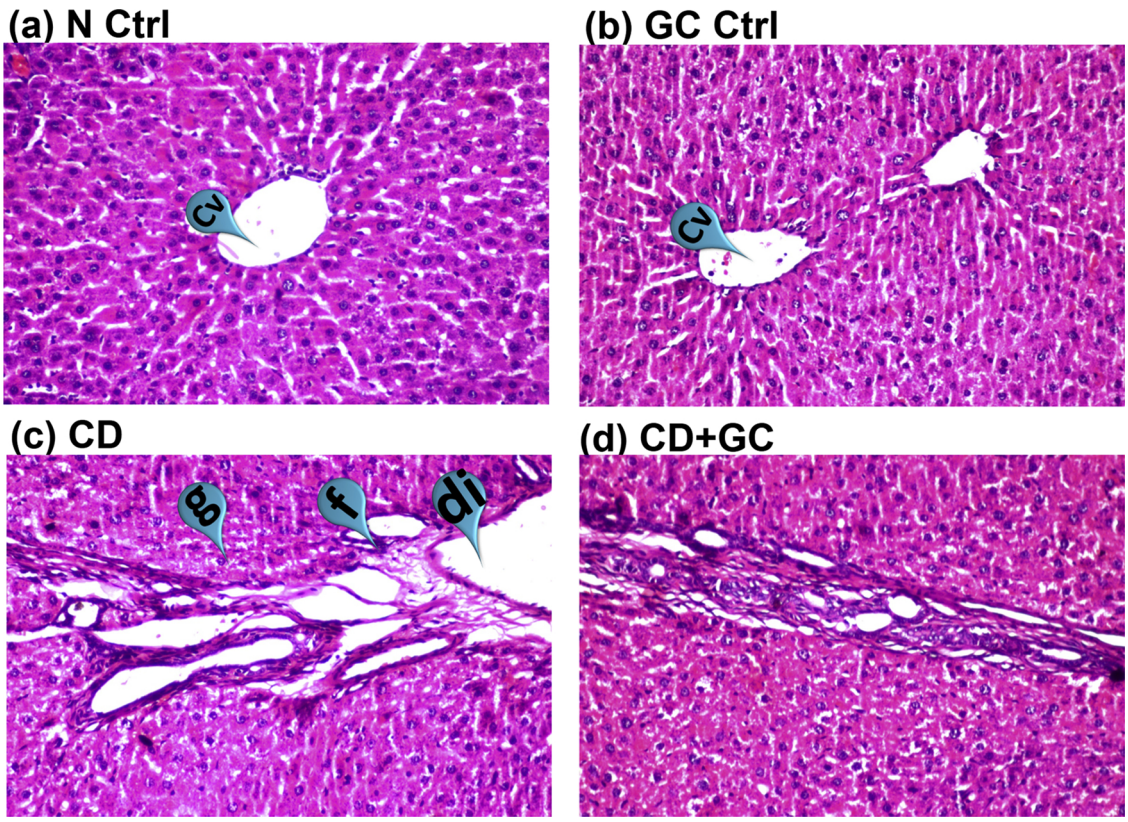

Fig. 6. Effect of γ GC on the histopathological changes to the liver tissues of the cadmium-treated rats. Photomicrographs from different experimental groups: (a) normal control group (N Ctrl), (b) γ glutamyl cysteine control group (GC Ctrl), (c) Cadmium-treated group (CD), and (d) cadmium and γ glutamyl cysteine-treated group (CD + GC). N Ctrl and GC Ctrl show normal histological structure of the liver tissues while CD group shows dilatation of the portal vein (di), hepatocellular degeneration (g) and fibrotic changes around the portal area (f). The photomicrographs were taken from liver tissues and stained with hematoxylin and eosin, 40X magnification, Cv = central vein.

serum activity of the liver enzymes ALT and AST [9,44] along with a variety of histopathological alterations to the liver tissues [45,46]. In line with these studies, the results of the current work revealed that cadmium administration upregulated the serum activity of both ALT and AST to 2.9 and 1.7 folds respectively as compared to the normal control group (Fig. 5), reflecting hepatocellular injury with subsequent leakage of the intracellular enzymes to the circulation. Likewise, cadmium evoked histopathological alterations in the hepatic tissues that include hepatocellular degeneration, portal vein dilatation as well as liver tissues fibrotic changes (Fig. 6). Lipid peroxidation in hepatocytes membranes may represent a plausible underlining cause of increased serum activity of the liver enzymes ALT and AST where lipid peroxidation may result in cell membrane disruption and leakage of the intracellular content including liver enzymes to the circulation. Damage to the cellular macromolecules may also contribute to the hepatocellular degeneration that was observed in the histopathological examination (Fig. 6c). Co-administration of γGC effectively reduced the serum activity of both ALT and AST (Fig. 5) and ameliorated the cadmium-induced histopathological alterations (Fig. 6 d), signifying the ameliorating activity of γGC against cadmium-induced liver tissue injury which potentially mediated through improving hepatocellular redox status secondary to decreased liver tissue cadmium content.

Regarding the hepatocellular apoptotic death, the current work demonstrated that cadmium administration increased the apoptotic cell death as indicated by 2.9-fold increase in caspase 3 activity (Fig. 2c). Oxidative stress and depletion of GSH have been implicated as major underlining causes for apoptotic cell death [47–49]. In oxidative stress-dependent mechanism, cadmium lowers the mitochondrial membrane potential in neuronal cells via blocking calcium channels with subsequent release of cytochrome c which eventually leads to caspase-dependent apoptotic cell death [50,51]. In addition, cadmium triggers ER stress, resulting in caspase-independent apoptotic cell death [52]. It is conceivable, thus, to correlate the observed cadmium-evoked hepatocellular apoptosis to the cadmium-induced oxidative stress and GSH depletion. γGC significantly reduced the apoptotic cell death as evidenced by the substantial decrease in the activity of the apoptotic cell death marker caspase 3 (Fig. 2c), reinforcing the attenuating activity of γGC against the cadmium-induced hepatocellular injury. It is reasonable to attribute the ability of γGC to decrease the hepatocellular apoptosis to its ability to decrease liver tissue cadmium content with subsequent alleviation of oxidative stress and rescuing GSH.

In conclusion, the findings of the current study point out the ameliorating activity of γGC against cadmium-induced hepatocellular injury in rats which is possibly mediated through decreasing liver tissue cadmium content, modulation of both hepatocellular redox status and inflammatory cytokines.

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Declarations of interest

None.

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