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Research article

Cinnamic acid ameliorate gentamicin-induced liver dysfunctions and nephrotoxicity in rats through induction of antioxidant activities

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ABSTRACT

This study was the first to evaluate the possible protective effects of cinnamic acid (CA) against Gentamicin (GM) induced liver and kidney dysfunctions in rats. Adult male Wistar rats were randomly assigned to 4 equal groups (n = 8): Control group (saline, 0.5 ml/day), CA group (CA, 50 mg/kg/day), GM group (GM, 100 mg/kg/day), and GM + CA group (100 & 50 mg/kg/day). Following 12 days of treatments, blood and 24 h urine samples were collected and kidneys were taken out for biochemical, histopathological, and molecular studies. Following CA treatment, renal function markers and transaminases activities including serum urea (59.92%) and creatinine (50.41%), protein excretion rate (43.67%), and serum activities of aspartate aminotransferase (AST) (54.34%) and alanine aminotransferase (ALT) (47.26%) significantly reduced in the treated group as compared with the GM group ($P < 0.05$). Also, CA could significantly ameliorate the levels of triglyceride (29.70%), cholesterol (13.02%), very low-density lipoprotein (29.69%) and high-density lipoprotein-cholesterol (7.28%). CA could also attenuate oxidative stress through a decrease of serum malondialdehyde (MDA) (50.86%) and nitric oxide (NO) (0.85%) and an increase of renal catalase (CAT) (196.14%) and glutathione peroxidase (GPX) activities (45.88%) as well as GPX mRNA expression (44.42-fold) as compared with the GM group ($P < 0.05$). Moreover, histopathological evaluations revealed attenuated tubular damages and reduced inflammatory cellular infiltration in CA treated animals. Overall, CA alleviates GM-induced nephrotoxicity and alterations in transaminases activities in rats through its antioxidant activities.

1. Introduction

Among aminoglycoside antibiotics, gentamicin (GM) is well known to be clinically effective against infections induced by Gram-negative bacteria [1]. Despite its clinical advantages, the most important complication of aminoglycosides, including GM, is nephrotoxicity, which limits their clinical utilization [2]. Recently, it has been reported that one dose of GM can initiate acute kidney injury (AKI) [3, 4]. Nephrotoxicity signs are observed in about 30% of the patients following seven days of GM treatment [5]. The precise underlying mechanisms of GM-induced nephropathy have not been fully known. However, it has been demonstrated that the GM nephrotoxicity is associated with the accumulation of GM in the tubular epithelial cells, predominantly in the proximal tubules and also in the distal and collecting ducts [6, 7, 8]. In tubular cells, GM triggers different pathological events such as oxidative stress, inflammation, apoptosis, necrosis, phospholipidosis, endoplasmic reticulum (ER) stress, mitochondrial dysfunction, etc. These events can lead to cell death, tubular dysfunction, and a decrease in glomerular filtration rate (GFR) [2]. Oxidative stress seems to be a principal molecular mechanism in the development of GM nephrotoxicity [9]. GM directly enhances the generation of mitochondrial reactive oxygen species (ROS) [2]. These free radicals cause deleterious effects on biomolecules such as proteins, lipids, and nucleic acids [2]. The main implications of GM induced ROS overproduction is the promotion of inflammation and suppression of the endogenous antioxidant system [2, 10]. Therefore, numerous preclinical and clinical studies have focused on antioxidants or drugs with promising anti-oxidative, anti-inflammatory, and renoprotective effects in the

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past decade [4, 10, 11, 12]. The liver is constantly exposed to drugs and toxins and plays a key role in detoxification of them [13]. There are different studies about the adverse effects of GM on the liver in animal models [14, 15, 16]. These studies revealed that GM can directly induce hepatotoxicity in rats through oxidative stress and apoptosis and finally lead to the elevation of the serum activities of transaminases (i.e., ALT and AST) and ALP [14, 15, 16]. Furthermore, some researchers believe that AKI due to GM can cause liver dysfunction, named "remote organ injury" [15]. Cinnamic acid (CA) (Figure 1) is a major active phenolic component of Cinnamon (*Cinnamomum cassia*) [17]. Previous studies have discovered different pharmacological properties of CA. This phenolic compound has been reported to possess anti-oxidative, anti--inflammatory, anti-tumoral, anti-microbial, and anti-fungal characteristics [18, 19, 20, 21, 22]. CA exerts anti-diabetic and hypoglycemic activities through modulation of glycogenesis and gluconeogenesis and amelioration of glucose tolerance and insulin secretion [17, 23, 24]. Additionally, the protective outcomes of CA treatment on ischemia/reperfusion damages have been determined previously [25]. Other valuable characteristics such as splenoprotective [26], hepatoprotective and cardioprotective [27, 28], functions have been attributed to CA. Ameliorative effects of CA on neuroinflammation [29], memory deficits [23], and neurodegenerative disorders such as Alzheimer's disease [30] and Parkinson's disease [31] have been investigated in several studies. Furthermore, there is evidence about the vasodilator activity of CA through the nitric oxide (NO)–cGMP–PKG pathway [32]. Recently, El-Sayed et al. showed the renoprotective effects of CA against renal injuries induced by cisplatin [33]. CA exhibited high antioxidant activity due to the presence of vinyl fragments in its structure. This characteristic attracts our interest to study this natural compound as a promising target for the management of pathologic conditions related to oxidative stress [23, 34]. Therefore, this study was designed for the first time to determine the possible protective effects of CA against GM induced renal and liver dysfunctions in rats.

2. Materials and methods

2.1. Chemicals

Tris-Ethylenediaminetetraacetic acid (Tris-EDTA), EDTA, and Tris-Hcl were taken from Merck Company (Germany). Commercial kits for the evaluation of lipid profile, renal functional parameters, transaminases, and alkaline phosphatase (ALP) were provided from Pars Azmoon Company (Tehran, Iran). Trizol reagent and CA (purity: ≥99%) were acquired from Sigma Aldrich Company (St. Louis, MO, USA). SYBER Green qPCR Master Mix 2x and cDNA synthesis kit were obtained from Yekta Tajhiz Azma Company (Iran). Finally, GM was provided by Alborz Darou Company (80 mg/2mL, AMP).

2.2. Animals and experimental design

Thirty-two adult male Wistar rats weighing from 180 to 200 g were provided from the laboratory animal unit of the Razi Herbal Medicines Research Center in Lorestan, Iran. The rats were stored in a room under controlled temperature (25 ± 1 °C), optimum humidity (50 ± 10%), and 12-h light/dark cycle. The enrolled animals were randomly assigned to 4 equal groups (n = 8) as follows:

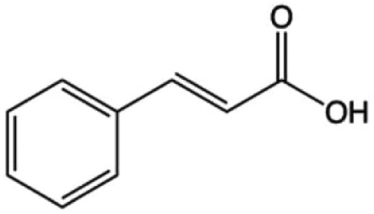

Figure 1. Chemical structure of CA (C9H8O2).

1. Control group: Saline (0.5 ml/day) was intraperitoneally administrated for 12 days.
2. CA group: This group was intraperitoneally treated with CA (50 mg/kg/day) [28, 40] for 12 days.
3. GM group: GM (100 mg/kg/day) was given intraperitoneally for 12 days [35, 36].
4. Treated group (GM + CA): GM (100 mg/kg/day) and CA (50 mg/kg/day) were intraperitoneally administrated for 12 days. Saline was used as a vehicle for CA [24]. CA was given to the rats 1 h after GM injection.
5. This study was reviewed and approved by the Lorestan University of Medical Science's Ethics Committee (code: LUMS.REC.1397.160). Also, this research was also following the guidelines of the National Health and Medical Research Council.

2.3. Sample collection

On day 12, the animals were maintained in separate metabolic cages for taking 24-h urine samples. The samples were centrifuged (1400 rpm, 5 min) and the supernatant was isolated for the evaluation of the protein excretion rate. After 24-h urine sampling, ketamine (75 mg/kg intraperitoneally) and xylazine (13 mg/kg intraperitoneally) were used to anesthetize the animals. Under anesthesia, cardiac blood samples were collected from the rats, and then centrifuged (3000 rpm, 4 °C, 15 min) for serum isolation. Collected serum samples were saved at -20 °C for future biochemical analysis. The right kidney was dissociated carefully and fixed subsequently in 10% neutral formaldehyde for the histopathological assessments. The left kidney was selected for biochemical and molecular analysis. One-half of the left kidney was homogenized in chilly phosphate-buffered saline (PBS, pH 7.4). The prepared homogenate was centrifuged (18,000×g, 4 °C) for 30 min and the supernatant was removed for renal biochemical analysis. The other part was put in the Trizol reagent (Sigma-Aldrich Company, USA) and saved at -70 °C for the assessment of mRNA expression levels of antioxidant enzymes.

2.4. Kidney histopathological assessment

Fixed tissue samples with 10% formaldehyde were dehydrated in a series of ascending graded ethanol, cleared with xylene, and finally embedded in paraffin. After that, paraffin sections of kidneys were cut at 5μm thickness using a microtome and stained with periodic acid Schiff (PAS) staining method. PAS stained sections were studied under a light microscope (Olympus CX-31, Philippines).

2.5. Biochemical assessment

2.5.1. Determination of renal functional markers

Serum levels of creatinine and urea, as the markers of renal function, were determined by an auto-analyzer (Olympus AU-600, Tokyo, Japan) according to the protocols of commercial kits (Pars Azmoon Company, Tehran, Iran). Protein excretion rate was evaluated by turbidimetric determination of total protein in 24-h urine samples according to the method of Shahangian and colleagues [37].

2.5.2. Evaluation of serum activities of transaminases and ALP

The serum activities of ALP and transaminases including aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed on an auto-analyzer (Olympus AU-600, Tokyo, Japan) applying commercial kits (Pars Azmoon Company, Tehran, Iran) similar to our previous study [38].

2.5.3. Determination of lipid profile

The levels of triglyceride (TG), cholesterol (Chol), and high-density lipoprotein-cholesterol (HDL-C) in collected serum samples were evaluated by a biochemical analyzer (Olympus AU-600, Tokyo, Japan) and commercial kits (Pars Azmoon, Tehran, Iran). The levels of low-density

lipoprotein (LDL) and very-low-density lipoprotein (VLDL) were determined in this study based on the equation of Friedewald and colleagues [39].

2.6. Measurement of oxidative stress biomarkers

2.6.1. Malondialdehyde (MDA)

The serum and kidney levels of MDA, as the marker of lipid peroxidation, were assessed according to thiobarbituric acid (TBA) test [40]. The absorbance was spectrophotometrically determined at 532 nm.

2.6.2. Glutathione peroxidase (GPX)

The serum and kidney activities of glutathione peroxidase (GPX) were established according to the method of Rotruck and colleagues [41]. The absorbance was determined at 420 nm using an ELISA reader and GPX activity is shown as U/mg protein.

2.6.3. Catalase (CAT)

The assay of CAT activities in serum and kidney was carried out based on the Sinha method [42]. For initiation of the reaction, a sample (20 μL of serum or supernatant) was added in 2 ml of 30 mM hydrogen peroxide (H_2O_2) in a 50 mM potassium phosphate buffer with pH 7.0. Enzyme units were considered as mM of used H_2O_2 per min g or mL.

2.6.4. Nitric oxide (NO)

Serum NO levels were determined through the assessment of the nitrite, as the end product of NO, which was conducted according to the method of Giustarini and colleagues [43].

2.7. RNA isolation and quantitative real-time RT-PCR

The mRNA expressions of the antioxidant enzymes (CAT and GPX) were evaluated using quantitative real-time reverse-transcription polymerase chain reaction (RT-PCR) technique. Total RNA was isolated from the collected kidney samples using Trizol reagent (Sigma Aldrich Company, USA), as fully described in our previous study [44]. Following RNA isolation, its integrity and purity were established by a NanoDrop spectrophotometer (Biochrom WPA Biowave II, UK) and agarose gel (2%) electrophoresis, respectively. The synthetization of cDNA was conducted from RNA samples (2μg) using cDNA synthesis kit (YT4500, Yekta Tajhiz Azma, Iran) according to its manufacturer's guides. The expressions of target genes (GPX and CAT) and reference gene (β-actin) were determined by quantitative RT-PCR applying SYBER Green qPCR Master Mix 2x (YT2551, Yekta Tajhiz Azma, Iran). Reactions were conducted in triplicate on Rotor-Gene 6000 (Corbett Research) and samples were amplified with the following thermal cycling condition including a pre-cycling heat activation at 95 °C for 3 min, followed by 40 cycles of heat denaturation at 95 °C for 5 s and annealing and extension at 60 °C for 30 s. The $2^{-\Delta\Delta CT}$ standard method was applied to analyze the results of quantitative real-time RT-PCR [45]. The sequence of primers used for each gene is available in Table 1.

3. Statistical analysis

All of the values were represented as the mean ± standard error (SE). Multiple comparisons between groups were implemented using a one-way ANOVA test followed by LSD test for biochemical parameters and Dunnett T3 test for real-time RT-PCR results. Statistical analyses were conducted by SPSS statistics software (SPSS; version 20). The statistically significant difference between groups was set at P-value < 0.05. Graphs were constructed using GraphPad Prism version 8.0.2 for Windows.

Table 1. The sequence of used primers for quantitative real-time RT-PCR.

Gene	Primer position	Primers sequences (5′→3′)
GPX	Forward	GGTGTTCCAGTGCGCAGAT
	Reverse	TCGAACCCGATATAGAAGCCCT
CAT	Forward	ATTGCCGTCCGATTCTCC
	Reverse	CCAGTTACCATCTTCAGTGTAG
β-actin	Forward	TATCGGCAATGAGCGGTTCC
	Reverse	AGCACTGTGTTGGCATAGAGG

4. Results

4.1. Histopathological study

Histopathological evaluation of PAS-stained kidney sections from the animals in the control (Figure 2A) and CA (Figure 2B) groups showed normal histological appearances. The kidney sections of these groups indicated tubules with minimal interstitial spaces, intact brush borders, and unbroken basement membrane (Figure 2A and B). By contrast, GM treated rats indicated tubules with loss of brush border (Figure 2C, arrowheads) and discontinuous basement membrane (Figure 2C, arrows). Furthermore, high levels of tubular necrosis (Figure 2C and D, stars), massive tubular casts (Figure 2C and D, stars), and desquamation of tubular epithelial cells (Figure 2C and D, stars) were also other findings observed in kidney sections from the GM group. As represented in Figure 2E, a marked increase of inflammatory cellular infiltration (arrows) in renal interstitial space was also observed in GM treated animals. By contrast, the treatment of nephrotoxic animals with CA could notably improve all of the above histopathological findings in comparison with the GM group. As shown in Figure 2F, nephrotoxic rats treated with CA exhibited tubules with a well-preserved brush border (arrowheads) and unbroken basement membrane (arrows). CA treatment could also considerably decrease tubular necrosis, cast formation, and tubulorrhexis compared with the GM group (Figure 2F, stars). Besides, CA reduced inflammatory cellular infiltration (orange arrows) in kidney interstitium (Figure 2F).

4.2. Biochemical analysis

4.2.1. Lipid profile

The effects of CA on lipid profile and atherogenic indices are illustrated in Table 2. 12 days of GM administration resulted in dyslipidemia manifested by high levels of TG, Chol, LDL, and VLDL (1.91-fold, 1.37-fold, 1.91-fold, and 3.40-fold, respectively) and low level of HDL-C (1.13-fold) compared with the control group ($P < 0.001$). Treatments with 50 mg/kg CA caused a decrease in the levels of TG, Chol, and VLDL (29.70%, 13.02%, and 29.69%, respectively) and an increase in HDL-C levels (7.28%) in comparison with the GM group ($P < 0.01$).

4.2.2. Renal functional markers

GM administration for 12 days led to acute kidney injury (AKI), which was reflected by the augmentation of serum creatinine (4.03-fold) and urea (3.54-fold) and protein excretion rate (2.84-fold) compared to the control group (Figure 3A, B, and C) ($P < 0.001$). By contrast, the levels of renal functional markers (50.41%, 59.92%, and 43.67%, respectively) significantly reduced in the treated group following the CA treatment when compared with the GM group (Figure 3A, B, and C) ($P < 0.001$). However, CA was unable to restore renal functional parameters to normal levels observed in the control group.

4.2.3. The activities of ALP and transaminases

The serum activities of AST, ALT, and ALP in GM-treated rats were significantly higher (2.01-fold, 1.67-fold, and 1.62-fold, respectively) when compared to those in healthy rats (Figure 4A, B, and C) ($P < 0.001$). Treatment with CA could decrease serum activities of transaminases, AST (54.34%) and ALT (47.26%) in the treated group in comparison with the GM group (Figure 4A and 4B). However, there was no significant variation in the activity of ALP ($P = 0.79$). CA could regenerate serum activities of AST and ALT to normal activities observed in the control group.

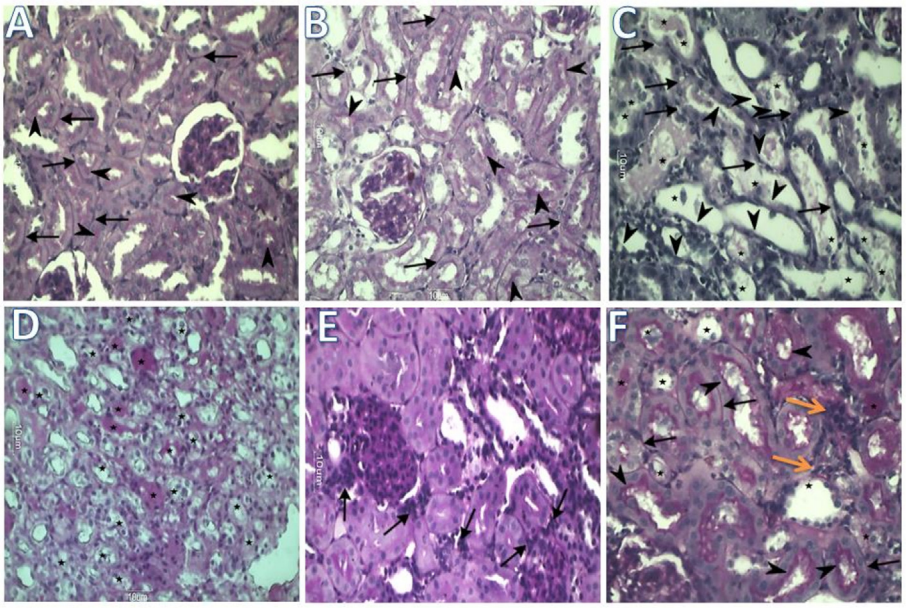

Figure 2. The effects of CA (50 mg/kg) on GM-induced histopathological changes observed in the photomicrographs of renal sections (×400) stained with periodic acid Schiff (PAS). A) A kidney section from a rat in the control group represents a normal architecture of the kidney (arrowheads indicate the intact brush border and arrows show the basement membrane). B) Kidney micrograph of an animal in the CA group (treated with CA only) indicates normal kidney architecture similar to that of the control group. C) Kidney histopathological section from an animal in the GM group illustrates the loss of brush border (arrowheads) and basement membrane (arrows). Furthermore, high levels of tubular necrosis (stars), cast formation (stars), and desquamation of tubular epithelial cells (stars) are also other findings in this micrograph. D) Another kidney micrograph from the GM group shows high levels of tubular necrosis (stars) and cast formation (stars). E) Kidney photomicrograph from the GM group illustrates increased cellular infiltration (arrows). F) A histopathological section from a rat kidney in the treated group (GM + CA) implies the improvement of renal tubulopathy. This section represents a decrease of tubular necrosis (stars), tubular cast (stars), and leukocyte infiltration (orange arrows). Additionally, continuous basement membrane (arrows) and tubules with brush border at the apical surface of their epithelial cells (arrowheads) are also observed in this micrograph.

Table 2. The effects of CA on the serum levels of lipid profile and NO in GM nephrotoxicity in rats.

Parameters	Control	CA	GM	GM + CA
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
TG (mg/dL)	75.83 ± 4.47	64.33 ± 10.61#	145.33 ± 13.56*	102.16 ± 13.55#
Chol (mg/dL)	69.83 ± 1.83	69.83 ± 1.47#	96.00 ± 5.16*	83.50 ± 2.60*#
VLDL (mg/dL)	15.16 ± 0.89	14.70 ± 0.91#	29.06 ± 2,71*	20.43 ± 2.71#
LDL (mg/dL)	7.43 ± 1.34	8.56 ± 1.40#	25.30 ± 6.58*	18.41 ± 1.89*
HDL-C (mg/dL)	47.23 ± 0.47	46.57 ± 0.44#	41.62 ± 0.45*	44.65 ± 0.62*#
NO (nmol/dL)	1.275 ± 0.00092	1.274 ± 0.00157#	1.291 ± 0.00288*	1.280 ± 0.00125*#

Note: All of the values are represented as mean ± standard error (SE). One-way ANOVA followed by a post hoc LSD test was used for comparison between different groups. *Significant change in comparison with the control group at $P < 0.05$. #Significant change in comparison with GM group at $P < 0.05$.
CA: Cinnamic acid; TG: Triglyceride; Cho: Cholesterol; VLDL: Very low-density lipoprotein; LDL: Low-density lipoprotein; HDL-C: High-density lipoprotein-cholesterol; NO: Nitric oxide.

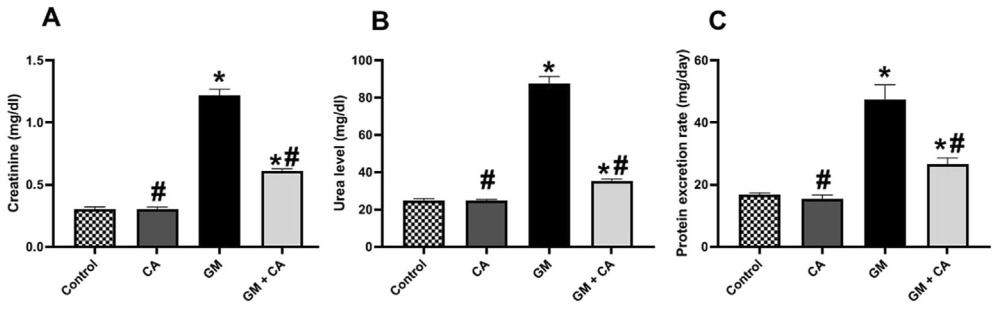

Figure 3. The effects of CA administration on renal functional parameters including serum creatinine (A) and urea (B) and protein excretion rate (C) in GM nephrotoxicity. Data are represented as Mean ± SE. One-way ANOVA followed by a post hoc LSD test was used for comparison between different groups. *Significant change in comparison with the control group at $P < 0.05$; #Significant change in comparison with the GM group at $P < 0.05$.

4.2.4. Oxidative stress biomarkers

In the serum and kidney homogenates of GM-treated animals, MDA concentrations (Figure 5A) significantly increased (2.25-fold and 1.68-fold, respectively) when compared to controls ($P < 0.001$). Treatment with CA could reduce serum and renal MDA concentrations (50.86% and 13.53%, respectively) in the treated group in comparison with the GM group. However, the decrease in renal MDA concentration was not statistically significant ($P = 0.13$). In the CA + GM group, serum MDA level

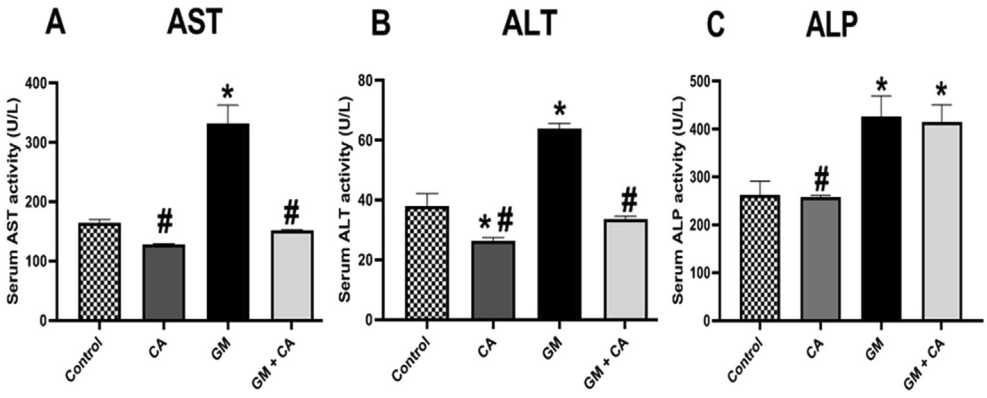

Figure 4. The effects of treatment with CA on serum activities of AST (A), ALT (B), and ALP (C) in rats with GM nephrotoxicity. Data are represented as Mean ± SE. One-way ANOVA followed by a post hoc LSD test was used for comparison between different groups. *Significant change in comparison with the control group at $P < 0.05$; #Significant change in comparison with GM group at $P < 0.05$. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase (ALT); ALP: alkaline phosphatase (ALP).

regenerated close to normal concentration in the control group. GPX activities in the serum and kidney are indicated in Figure 5B. There was no statistically significant difference in the serum activity of GPX among studied groups ($P > 0.05$). The induction of GM nephrotoxicity was accompanied by a significant decrease (1.52-fold) in renal GPX activity in comparison with the control group ($P < 0.001$). The biochemical results implied that renal GPX activity significantly promoted (45.88%) in the treated group when compared to the GM group ($P < 0.001$). 12 days of CA treatment returned renal GPX activity close to control levels. GM nephrotoxicity considerably reduced the serum and renal activities of CAT (1.72-fold and 2.76-fold, respectively) in comparison to the control group (Figure 5C) ($P < 0.01$). Biochemical analysis revealed that kidney CAT activity was significantly higher (2.96-fold) in the treated group than that of in the GM group (Figure 5C) ($P < 0.01$). CA treatment for 12 consecutive days considerably recovered renal CAT activity to normal levels observed in controls. Following the treatment of nephrotoxic animals with CA, serum CAT activity showed no statistical difference compared to the GM group (Figure 5C) ($P > 0.05$). The concentration of NO in serum was found to be augmented in the GM group than that of the control group (Table 2) ($P < 0.001$). Whereas, CA treatment could reduce NO concentration in the treated group compared to the GM group ($P < 0.001$) (Table 2).

4.3. Gene expressions of renal antioxidant enzymes

As shown in Figure 6, markedly decreased levels of renal mRNA expression of antioxidant enzymes CAT and GPX (34.72-fold and 62.5-fold, respectively) were observed in untreated nephrotoxic rats following 12 days of GM nephrotoxicity than in controls ($P < 0.0001$). Expression levels of CAT and GPX were additionally investigated in the treated group, with their renal expressions being 7.13-fold and 44.42-fold, respectively, greater in treated nephrotoxic animals than in untreated nephrotoxic rats. However, the increment of mRNA expression levels of CAT was not statistically ($P = 0.87$).

5. Discussion

The present study is most likely the first to reveal the protective effects of CA on GM induced acute nephrotoxicity in the rat. Our results demonstrated that the destructive effects of GM on the rat's kidney can be ameliorated by 12 days of CA treatment. Most of the different parameters evaluated in our experiment were quickly regenerated in GM intoxicated animals following 12 days of CA administration. In our study, the histopathological analysis revealed tubulopathy induced by GM. GM-treated animals exhibited extreme tubular necrosis, tubulorrhexis, tubular casts, destroyed brush border, and basement membrane interruption in their kidneys. Also, increased inflammatory cellular infiltration was observed in kidney interstitium. GM induced tubulopathy has similarly been reported in several previous studies [3, 4, 12]. Augmented free radical generation mediated by GM can be responsible for the destruction of cellular membranes and the induction of necrosis in the kidney [46]. CA treatment during 12 days could considerably improve above renal histopathological alterations in comparison with the GM group. According to that several natural antioxidants can prevent GM induced kidney histopathological changes [36, 46, 47, 48]. It is most likely that the protective effects of CA against GM renal injuries are due to its antioxidant activities.

There are several pieces of evidence about GM-induced secondary hyperlipidemia in animal models [47, 48, 49, 50]. Similar results were found in our study, in which TG, Chol, LDL, and VLDL were increased and HDL-C was decreased in GM exposed animals. Previous studies have ascribed secondary hyperlipidemia to nephropathy progression caused by GM [1, 48]. The secondary hyperlipidemia can result from enhanced Chol biosynthesis in the liver because of elevated bioavailability of Chol precursor mevalonate as a consequence of diminished mevalonate catabolism in the damaged kidney [51]. Additionally, hypercholesterolemia may be associated with cholestasis, reflected by elevated ALP activity, or protein losing nephropathy in GM-intoxicated rats. Hypertriglyceridemia can be associated with nephrosis, which mitigates the elimination of the circulating TG-rich lipoproteins as a result of reduced plasma lipoprotein lipase (LPL) activity [52]. In this study, CA could reduce the levels of TG, Chol, and VLDL and increase HDL-C in the treated group that confirmed hypolipidemic and anti-atherogenic activities of CA. Several recent studies similarly indicated that natural antioxidants exert hypolipidemic effects against GM induced secondary hyperlipidemia [47, 48, 49, 50]. Although the precise mechanisms to justify the hypolipidemic and anti-atherogenic effects of CA are not fully elucidated in our study, some mechanisms are available in other studies about hypolipidemic effects of CA and its derivates. 1. CA decreases the absorption of dietary TGs in the intestine through inhibition of pancreatic lipase [28, 53]. 2. CA derivates can upregulate lecithin-cholesterol acyltransferase (LCAT), which is important in the esterification of Chol and the maturation of lipoproteins [54]. 3. CA derivates can increase plasma LPL activity and decrease tissue LPL activity [55]. 4. CA derivates can downregulate lipogenic genes (SREBP1c/1a, acetyl-CoA carboxylase, and fatty acid synthase) and upregulate lipolytic genes [56].

Our results exhibited that GM injection to rats led to acute renal failure, which was reflected by the increase of serum urea and creatinine as well as the enhancement of protein excretion rate. These outcomes are consistent with previous studies, which implied that GM induces renal function impairment [4, 48, 50, 57]. Significant elevations of serum urea and creatinine in GM intoxicated animals represent a decrease of glomerular filtration rate (GFR) [58, 59]. Also, a high level of protein excretion rate can result from GM induced disruption of blood renal filtration barrier and impairment of protein uptake by proximal tubules [59, 60]. The treatment of nephrotoxic animals with CA for 12 days considerably ameliorated serum urea and creatinine and protein excretion rate in comparison with the GM group. Our results complied with the

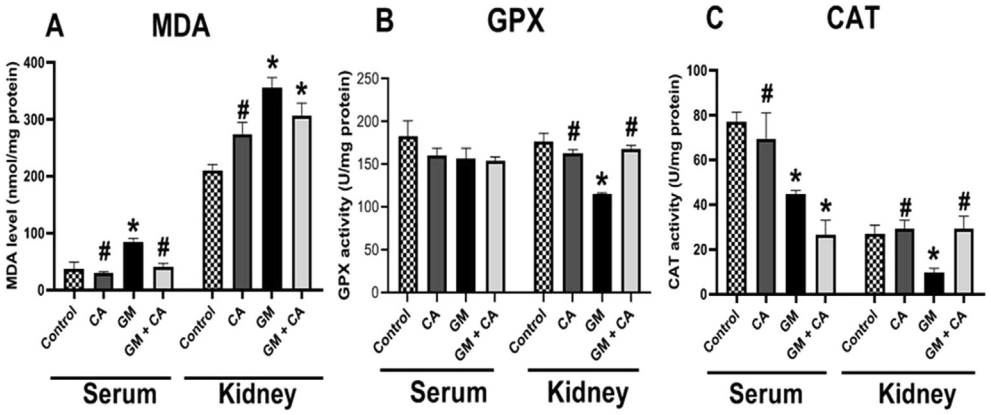

Figure 5. The effects of CA treatment on oxidative stress biomarkers including MDA (A), GPX (B), and CAT (C) in GM nephrotoxicity. Data are represented as Mean ± SE. One-way ANOVA followed by a post hoc LSD test was used for comparison between different groups. *Significant change in comparison with the control group at $P < 0.05$; #Significant change in comparison with GM group at $P < 0.05$. MDA: Malondialdehyde; GPX: Glutathione peroxidase; CAT: Catalase.

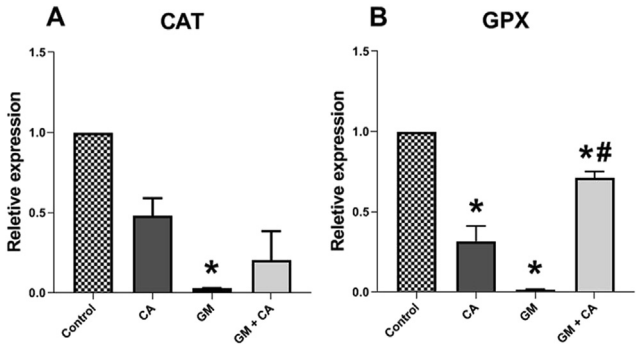

Figure 6. The effects of CA on the kidney mRNA expression levels of antioxidant enzymes including CAT (A) and GPX (B) in rats with GM nephrotoxicity. Data are represented as Mean ± SE. One-way ANOVA followed by a post hoc Dunnett T3 test was used for comparison between different groups. *Significant change in comparison with the control group at $P < 0.05$. #Significant change in comparison with the GM group at $P < 0.05$. CAT: Catalase; GPX: Glutathione peroxidase.

study of El-Sayed et al. representing the renoprotective effects of CA against renal injuries induced by cisplatin [33]. The decrease of serum urea and creatinine following CA treatment may be associated with improvement of GFR and blood renal filtration barrier function, as proposed for other antioxidants [59]. Reduced protein excretion rate in CA + GM treated rats may be related to the amelioration of glomerular barrier function and protein reabsorption by proximal tubules [59].

It is well known that GM can induce hepatotoxicity in rats through oxidative stress and apoptosis [14, 15, 16]. Similar to previous studies [14, 15], the serum activities of AST, ALT, and ALP were significantly higher in GM-treated rats that confirmed the liver injury. Following the CA treatment, the serum activities of AST and ALT significantly decreased in nephrotoxic animals. Amelioration of transaminases activities by CA in our study may be associated with its antioxidant activities. In this study, the assessment of transaminases activities was considered as a supplement or preliminary work. We suggest further studies to uncover the detailed mechanisms associated with hepatoprotective effects of CA against GM induced liver injuries.

Biochemical analysis showed that serum and renal MDA and serum NO concentrations significantly enhanced in GM-treated animals compared with controls. On the other hand, several studies determined that GM decreased the activities of antioxidant enzymes which are in accordance with our study. Thus, GM promotes the production of ROS and reactive nitrogen species (RNS) and suppresses the renal antioxidant system [4, 36, 47]. CA administration could reduce serum MDA and NO concentrations in the treated group. In addition, CA clearly recovered renal activities of CAT and GPX in CA + GM treated animals. Our study is in accordance with previous studies showing that CA can ameliorate oxidative stress in different pathological conditions [23, 26, 33]. Amelioration of oxidative stress by CA can be associated with its ROS scavenging activity [61] and/or its ability to upregulation of mRNA expression of antioxidant enzymes, as indicated in this study (Figure 6).

6. Conclusions

Our results indicated for the first time the effectiveness of CA administration in amelioration of renal dysfunction and altered transaminases activities in GM-intoxicated rats. In addition, CA showed hypolipidemic effects against GM-induced secondary hyperlipidemia. Antioxidant activities of CA might be responsible for its beneficial effects.

Declarations

Author contribution statement

Esmaeel Babaeenezhad: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Negar Nouryazdan, Maryam Nasri, Hassan Ahmadvand: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Mostafa Moradi Sarabi: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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