



Cinnamic acid Attenuates Cisplatin-Induced Hepatotoxicity and Nephrotoxicity

Amany A. Tohamy¹, Ahmed M. Aref², Ahmed E. Abdel Moneim^{1,*} and Romissaa H. Sayed¹

¹Department of Zoology & Entomology, Faculty of Science, Helwan University, Cairo, Egypt.

²Department of Biological Science, Faculty of Dentistry, Modern Sciences and Arts University (MSA), Cairo, Egypt.

Address correspondence to:

Ahmed Esmat Abdel Moneim

Department of Zoology and Entomology

Faculty of Science

Helwan University

11795 Helwan, Cairo, Egypt

Tel. number: (+20) 1153322079.

e-mail address: aest1977@hotmail.com

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Abstract

We investigated the effects of cinnamic acid (CA, 20 mg/kg body weight) on cisplatin (CP)-induced hepato and nephrotoxicity in mice. CP (5 mg/kg bwt) was injected intraperitoneally and CA was given by gastric gavage for 5 days pre- and post-CP injection. After 5 days of CP injection, CP-induced injuries of the hepatic and renal tissues which were evidenced (i) histopathological damage of the hepatic and renal tissues, (ii) as increases in liver and kidney function parameters, (iii) as increases in lipid peroxidation and nitric oxide, and (iv) as decrease in glutathione content. In contrast, the oral administration of CA concurrently to CP intoxicated mice brought back lipid peroxidation, nitric oxide, glutathione levels to near normalcy. Moreover, the histological observations evidenced that CA effectively rescues the liver and kidney from CP mediated oxidative damage. Therefore, cinnamic acid can be considered a potential candidate for protection of hepato-and nephrotoxicity induced by cisplatin.

Introduction

cisplatin (CP; cis-diamminedichloroplatinum, *cis*-[Pt(II)(NH₃)₂Cl₂] or [PtCl₂(NH₃)₂]) is one of the most potent chemotherapy drugs widely used for cancer treatment [1]. Cisplatin and its derivatives are effective agents in cancer therapy. They have been widely used to treat a variety of tumor types including testicular, ovarian, head, and cervical carcinoma and other different types of cancer including: sarcoma cancers, cancers of soft tissue, bones, muscles, and blood vessels [1-2].

CP exerts its action through the following mechanism. After entering the target cells, CP binds to the cellular DNA to form a covalent complex. The drug causes reversible alkylation of guanine and adenine, and forms intra- and interstrand cross-links in the DNA, thereby inhibiting elongation of DNA by DNA polymerase (inhibition of DNA transcription and replication). In addition, the formation of intrastrand cross-links results in changes in the conformation of the cells. These changes induce apoptosis and necrosis of the cancer cells, and underlie the antitumor effect of the drug [3].

CP has clinical benefit for several types of solid tumors; however, the efficiency of CP is often accompanied by toxic side effects and tumor resistance, which in turn leads to secondary malignancies [1]. CP causes generation of reactive oxygen species (ROS), such as superoxide anion and hydroxyl radical which deplete glutathione levels and inhibit the

activity of antioxidant enzymes in renal tissue. ROS may produce cellular injury and necrosis via several mechanisms including peroxidation of membrane lipids, protein denaturation and DNA damage [4]. Some reports show that CP induces ROS formation *in vivo*, which is responsible for the severe side effects of CP therapy, including nephrotoxicity and hepatotoxicity, which in turn, are reduced by the addition of antioxidants.

In rats, CP increases lipid peroxidation and alters the thiol status of the tissue with concomitant alterations in the enzymatic antioxidants. Glutathione and glutathione reductase levels are significantly decreased after CP therapy, whereas glutathione peroxidase, gamma-glutamyl transpeptidase and catalase show a significant increase [1,5].

Many antioxidant agents were investigated for their preventive abilities against cisplatin-induced nephrotoxicity. Some researches advised the use of enriched diets with natural antioxidants like vitamin E, ascorbic acid, and methionine [4,6]. Other studies reported that the use of sulfhydryl-containing drugs, such as captopril, diethyldithiocarbamate, sodium thiosulfate, *N*-acetylcysteine, and lipoic acid, could also exert antioxidant activity [7,8]. Cinnamon, scientifically named *Cinnamomum spp*, is a plant with many uses as a herbal medicine, containing mucilage, tannin, sugar, resin, and essential oil, among which cinnamic acid (CA), a major active phenolic ingredient in cinnamon displays many pharmacological properties, such as antioxidant and antimicrobial activity. CA is reported to possess antioxidant, antibacterial and anti-inflammatory effects [9,10]. The majority of naturally occurring phenolics retain antioxidative and anti-inflammatory properties which appear to contribute to their chemopreventive or chemoprotective activity [11]. Another researches demonstrated that the cinnamon-derived food factor CA is a potent activator of the Nrf2-orchestrated antioxidant response in cultured human epithelial colon cells [12]. Hence, this study aimed to assess the possible protective effect of cinnamic acid (CA) against cisplatin-induced hepatotoxicity and nephrotoxicity.

Materials and methods

Chemicals

cis-Diammine platinum (II) dichloride, cinnamic acid and Tris-HCl were purchased from Sigma (St. Louis, MO, USA). Thiobarbituric acid and trichloroacetic acid were purchased from Merck. All other chemicals and reagents used in this study were of analytical grade. Double-distilled water was used as the solvent.

Animals and Experimental Design

Adult male albino mice weighing 23–25 g were obtained from The Holding Company for Biological Products and Vaccines (VACSERA, Cairo, Egypt). After an acclimatization period of one week, the animals were divided into four groups (7 mice per group) and housed in wire bottomed cages in a room under standard conditions of illumination with a 12-hours light-dark cycle at 25±1°C. They were provided with water and a balanced diet *ad libitum*. All animals received care in compliance with the Egyptian rules for animal protection.

First group (Con; Control Group) served as untreated control, the second group (CA Group) received a daily oral administration of 20 mg/kg bwt cinnamic acid dissolved in corn oil for 10 days, the third group (CP Group) received a single intraperitoneal injection of CP (5 mg/kg bwt) and left for 5 days and the forth group (CP-CA Group) received the same dose of cinnamic acid for 5 days before and after a single intraperitoneal injection of CP.

The level of the orally administered dosage of cinnamic acid (20 mg/kg bwt) was based on the previous work of Park [13] and the dose of CP selected on the basis of our previous studies [14-15].

The animals of all groups were sacrificed by fast decapitation, blood samples were collected, allowed to stand for half an hour and then centrifuged at 3000 rpm for 15 min at 4°C to separate serum which was stored at -20 °C for the different biochemical measurements. Part of the liver tissue and the right kidney was dissected out and fixed immediately in 10% neutral formaldehyde for histological study. Another part of the liver tissue and the left kidney was weighed and homogenized immediately to give 50% (w/v) homogenate in ice-cold medium containing 50 mM Tris-HCl, pH 7.4. The homogenate was centrifuged at 3000 rpm for 10 min at 4 °C. The supernatant was used for the various biochemical determinations.

Relative kidney weight

At the end of the experimental period, each mouse was weighed. The left kidney was then removed and weighed. Finally, the relative kidney weight was calculated by dividing left kidney weight by body weight and then multiplying it by 100.

Biochemical estimations

Liver and kidney functions tests

Colorimetric determination of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated by measuring the amount of pyruvate or oxaloacetate produced by forming 2, 4-dinitrophenylhydrazine according to the method of Reitman and Frankel [16]. Also, total bilirubin (TB) in serum was assayed according to the method of Schmidt and Eisenburg [17]. Additionally, serum creatinine, urea and uric acid were determined by commercially available diagnostic kits (Diamond Diagnostics-Egypt) according to the manufacturer's instructions.

Oxidative stress markers

Nitrite/nitrate (NO) and malondialdehyde (MDA) were assayed colorimetrically in kidney and liver homogenates according to the methods of Green et al. [18] and Ohkawa et al. [19], respectively, where MDA was determined by using 1 ml of trichloroacetic acid 10% and 1 ml of thiobarbituric acid 0.67% which were then heated in a boiling water bath for 30 min. Thiobarbituric acid reactive substances (TBARS) were determined by the absorbance at 535 nm and expressed as MDA formed. Nitric oxide was determined where in acid medium and in the presence of nitrite the formed nitrous acid diazotise sulphanilamide is coupled with N-(1-naphthyl) ethylenediamine. The resulting Azo dye had a bright reddish – purple color which can be measured at 540 nm.

In addition, The renal hepatic and glutathione (GSH) were determined by the methods of Ellman [20]. This method is based on the reduction of Elman's reagent (5,5` dithiobis (2-nitrobenzoic acid) "DTNB") with GSH to produce a yellow compound. The reduced chromogen was directly proportional to GSH concentration and its absorbance can be measured at 405 nm.

Histopathological examination

Tissue samples were fixed in 10% neutral formalin for 24 h and paraffin blocks were obtained and routinely processed for light microscopy. Slices of 4-5 µm were obtained from the prepared blocks and stained with hematoxylin-eosin. The preparations obtained were visualized under a microscope (Eclipse E200-LED, Nikon, Tokyo, Japan).

Statistical analysis

Differences between obtained values (mean ± SEM) were carried out by one way analysis of variance (ANOVA) followed by the Duncan test. A p-value of 0.05 or less was taken as a criterion for a statistically significant difference.

Results

Comparing the final body weights of mice in different groups, a significant weight loss ($p < 0.05$) was detected in CP-treated mice. Conversely, a significant weight gain was recorded in the AC-CP treated rats when compared to the CP-treated rats. Moreover, a significant decrease of relative kidney weight of the CP-treated mice was noticed versus that of the control mice at $p < 0.05$. The relative kidney weights revealed significant recovery ($p < 0.05$) in CA-CP treated mice when compared to the CP-treated mice (Figure 1).

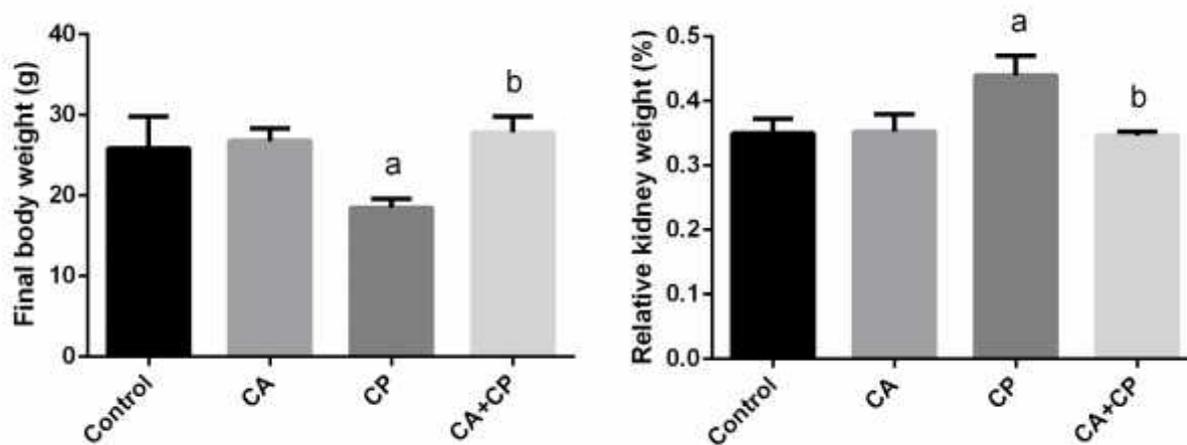


Figure 1: Effects of cinnamic acid (CA) on body weight and relative kidney weight of mice treated with cisplatin (CP). Values are means ± SEM (n=7). ^a $p < 0.05$, significant change with respect to **Control**; ^b $p < 0.05$, significant change with respect to **CP** for Duncan's post hoc test.

Normal control animals have revealed clear cut hepatic lobules separated by interlobular septa, transversed by portal vein (Figure 2a). The CP-induced hepatic damage is characterized by dispersed areas of necrotic hepatocytes, inflammatory cellular infiltration as well as cytoplasmic vacuolation and degeneration of hepatocytes (Figure 2c). Treatment of mice with CA largely prevented CP-induced histopathological changes in the liver as indicated by a reduction in inflammatory cellular infiltration and hepatocytic damages (Fig. 2d). These histological abnormalities are coincided with significant increase in activity of ALT, AST and bilirubin level (Figure 3), while treatment with CA significantly restored ALT and AST levels to normal values ($p < 0.05$) in group 4 "combined group".

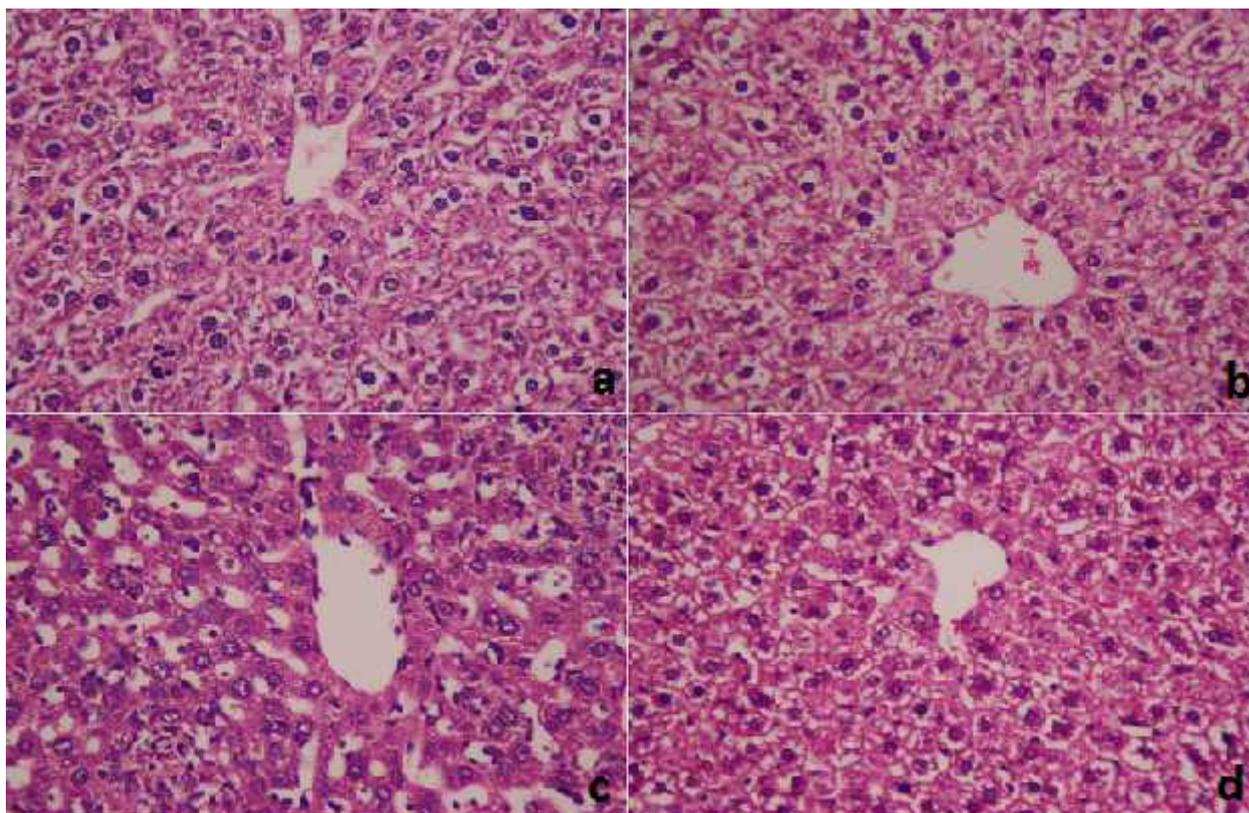


Figure (1): H&E staining showing histopathological damage induced by CP on the liver of mice and the hepatoprotective role of cinnaamic acid. **a and b)** Control and cinnaamic acid treated liver sections with normal architecture. **c)** Mice treated with cisplatin showing prominent inflammation and hepatocytic vacuolation. **c:** mice treated with the cinnaamic acid for 5 days. **d)** Treatment with cinnaamic acid pre - and post-CP showing improvement in hepatocytes (X 400).

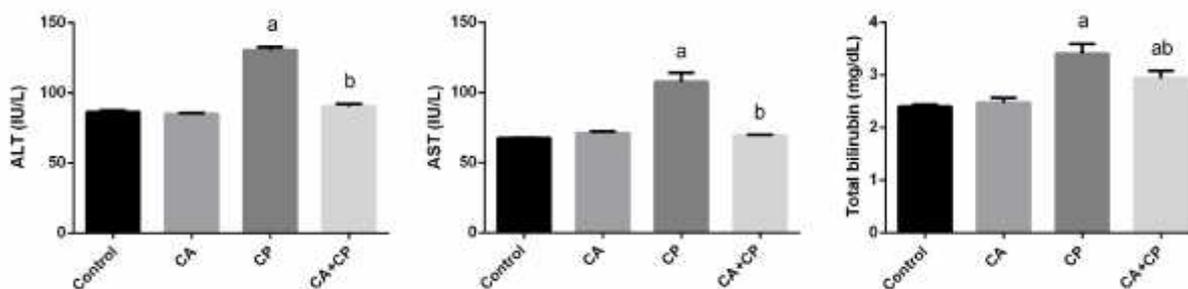


Figure 3: Protective effects of cinnaamic acid (CA) on cisplatin-induced alteration in liver function parameters of mice. Values are means \pm SEM (n=7). ^a $p < 0.05$, significant change with respect to **Control**; ^b $p < 0.05$, significant change with respect to **CP** for Duncan's post hoc test.

The renal cortex of control mice revealed a normal corpuscular and tubular histological structure (Figure 4a). In CP-treated mice, degenerative changes were noticeable observed in renal tissues. These changes were in the form of luminal dilatation with excessive cast accumulation, the renal corpuscles showed dilated capsular space with condensed and even degenerated glomerulus. Inflammatory cells infiltration and vascular congestion were noticed within the renal cortex as well as pyknotic nuclei were presented in renal tubules (Figure 4c). The kidney of mice treated with CA concurrently with CP (CA+CP group) revealed less histological damages in renal corpuscles and renal tubules. Mild tubular degeneration with luminal dilatation and inflammatory cell infiltration were seen within the renal cortex (Figure 4d). As a result of histopathological changes in mice injected with CP, the levels of uric acid, urea and creatinine were significantly elevated ($p < 0.05$) in the blood serum of mice injected with a single dose of cisplatin (5 mg/kg, I.P.). Whereas, a significant difference in the levels of the parameters mentioned above were recorded in CA-CP "combined" group when compared to the CP-treated mice. Additionally, cinnaamic acid alone did not exhibit any effect on kidney function levels parameters after 10 days of treatment in the mice (Figure 5).

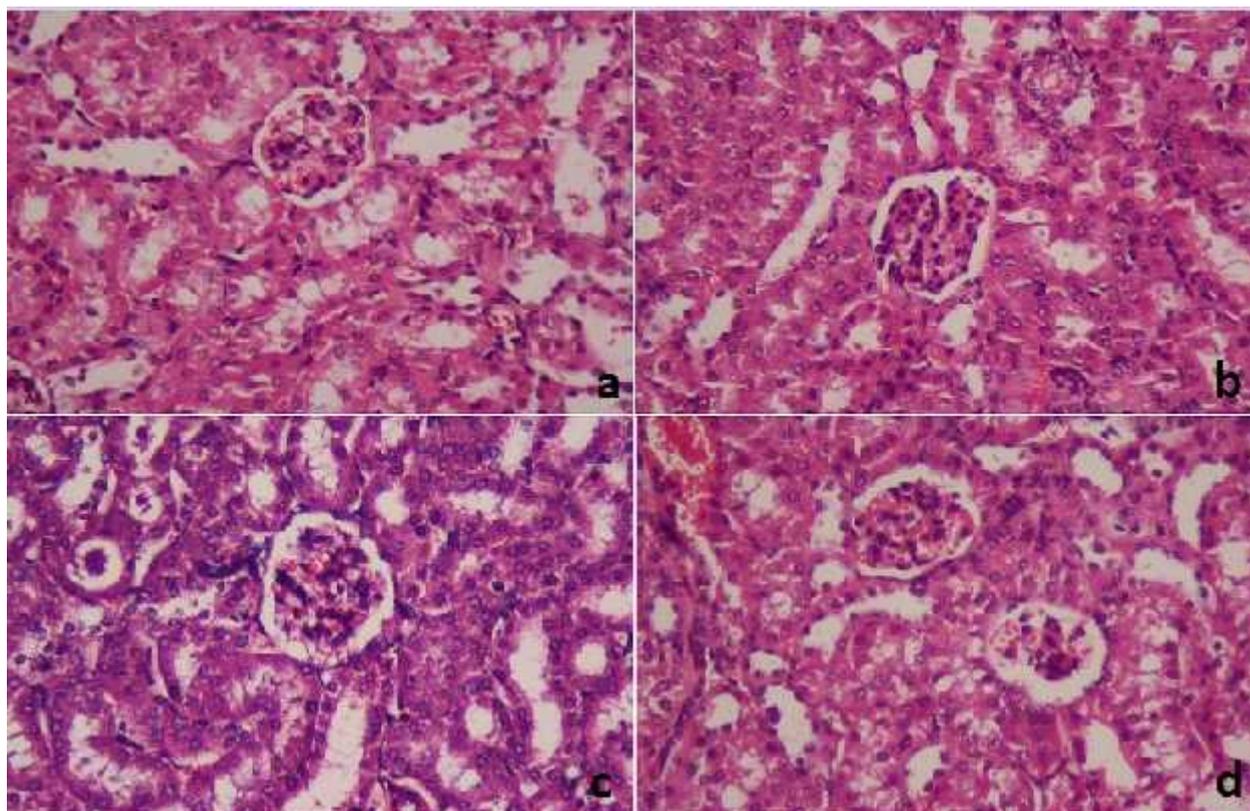


Figure 4: H&E staining showing histopathological damage induced by CP on the kidney of mice and the nephroprotective role of cinnamic acid. **a and b)** Control and cinnamic acid treated kidney sections with normal renal corpuscle and renal tubules. **c)** Mice treated with cisplatin with congested shrunken and completely degenerated glomeruli, debris in the lumen of some renal tubules and pyknotic nuclei in renal tubules. **d)** Treatment with cinnamic acid pre - and post-CP showing normal renal corpuscle and renal tubule more or less like normal structure (X 400).

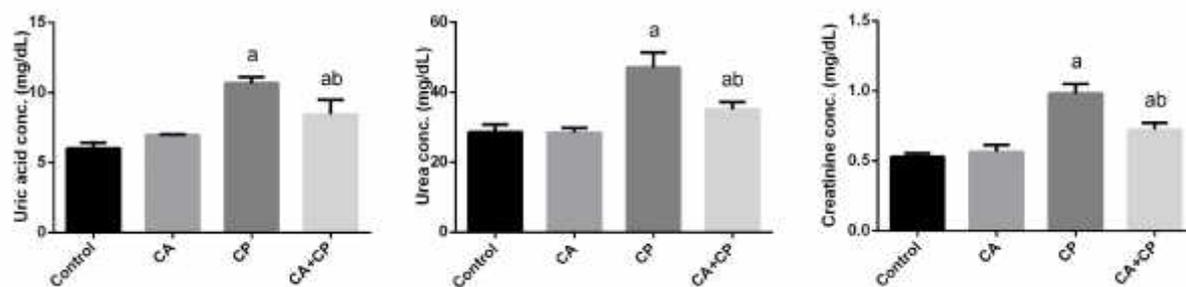


Figure 5: Protective effects of cinnamic acid (CA) on cisplatin-induced alteration in kidney function parameters of mice.

Values are means \pm SEM (n=7). ^a $p < 0.05$, significant change with respect to **Control**; ^b $p < 0.05$, significant change with respect to **CP** for Duncan's post hoc test.

Oxidative stress has been regarded as one of the underlying mechanisms of CP-induced acute liver and kidney injuries. We evaluated whether cinnamic acid (20 mg/kg) could modulate CP-induced hepatic and renal oxidative stress by measuring MDA and NO levels. CP-induced hepatic and renal oxidant/antioxidant imbalance was evident by significant increase ($p < 0.05$) in MDA and NO levels and by a significant decrease ($p < 0.05$) in GSH content in the liver and kidney tissues of animals that received CP alone compared with the control group. These changes in MDA, NO and GSH levels were attenuated by treatment with cinnamic acid as shown in Figures 6 and 7, respectively. Administration of cinnamic acid alone for 10 days increased the renal and hepatic GSH concentration as compared with the control mice.

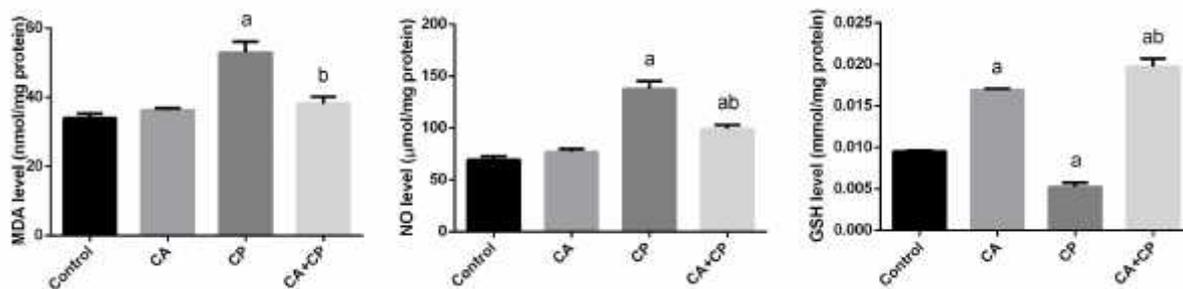


Figure 6: Effects of cinnamic acid on lipid peroxidation, nitric oxide and glutathione levels in liver of mice treated with CP.

Values are means ± SEM (n=7). ^ap<0.05, significant change with respect to **Control**; ^bp<0.05, significant change with respect to **CP** for Duncan's post hoc test.

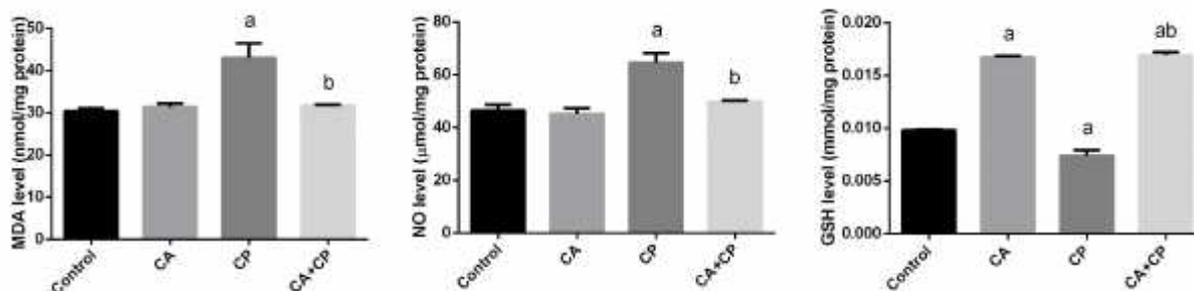


Figure 7: Effects of cinnamic acid on lipid peroxidation, nitric oxide and glutathione levels in kidney of mice treated with CP.

Values are means ± SEM (n=7). ^ap<0.05, significant change with respect to **Control**; ^bp<0.05, significant change with respect to **CP** for Duncan's post hoc test.

Discussion

CP is one of the most widely used cytotoxic therapeutic agents for the treatment of different cancers including testicular, germ cell, head and neck, bladder and lung cancers. It is an alkylating agent which at effective higher doses causes many adverse effects such as neurotoxicity, nephrotoxicity and genotoxicity [21,14]. Its chief dose-limiting side effect is nephrotoxicity, which requires a reduction of dose or discontinuation of the treatment because CP-induced nephrotoxicity can result in severe renal tubular injury leading to acute renal failure [22].

The histopathological findings showed that administration of CP-induced various degenerative changes in liver and kidney cells (nephrotoxicity and hepatotoxicity), which confirmed the biochemical evidence of the oxidative stress. In contrast, treatment with CA before and after CP obviously improved the histopathological changes induced by cisplatin. These results are in agreement with El-Sayed et al [23].

The CP concentration in proximal tubular epithelial cells is about 5 times the serum concentration. The disproportionate accumulation of CP in kidney tissue contributes to CP-induced nephrotoxicity [15]. In a study by Yao et al. [24], they suggested that inflammation, oxidative stress injury, and apoptosis probably explain part of this injury. Nephrotoxicity has been a serious problem in CP treatment and combined chemotherapy using CP and plant extracts can reduce the side effects and enhance the antitumour efficacy [25,26,21,27]. Our results revealed that administration of cisplatin to mice caused a significant elevation in serum creatinine, urea, uric acid levels, and kidney–body weight ratio. The increased urea, uric acid and creatinine levels suggest the reduction of glomerular-filtration rate [28]. These results are in accordance with previous reports of Naziroglu et al. [29], Atessahin et al. [30] and Naqshbandi et al. [31] who reported that the alterations induced by CP in the kidney functions were characterized by signs of injury, such as increase of creatinine and urea levels in plasma.

The present study demonstrated that administration of CA before and after CP treatment significantly restore hepatic renal injury and improved CP-induced alterations in serum creatinine, urea and albumin levels, and body weight and kidney–body weight ratio, i.e. improved the kidney functions. These results are in agreement with El-Sayed et al. [28] In addition, CA administration before and after CP treatment significantly improved also the liver functions (definitely ALT, AST and bilirubin levels) as shown in our results.

It was evident that cisplatin hepato- and nephrotoxicity occurs as a result of oxidative stress and increased generation of reactive oxygen species (ROS) such as: superoxide anion, hydrogen peroxide, and hydroxyl radicals due to the increased

activity of NADPH oxidase, xanthine oxidase, and adenosine deaminase [32]. These free radicals damage the lipid components of the cell membrane via peroxidation and denaturing its proteins, which subsequently lead to enzymatic inactivation [33]. This explains the increase of lipid peroxidation in tissues treated with CP.

Among many markers of oxidative stress, renal and hepatic MDA and NO were reported to be increased following injection of CP. As shown in Figures 5 & 6 there were significant increases ($p < 0.05$) in MDA and NO levels in the kidney and liver of animals that received CP alone compared with the control group. These results were in agreement with Bae and his colleagues [34], who demonstrated that an increase in NO due to the ability of CP to upregulate inducible nitric oxide synthase (iNOS) mRNA levels in kidney after CP injection. iNOS is able to produce large amounts of nitric oxide that, under oxidative stress conditions, can react with superoxide anion ($O_2^{\cdot-}$) to form nitrate ($ONOO^-$), an oxidant species able to modify a great number of biomolecules such as amino acids, proteins, enzymes and cofactors [15] and involved in CP-induced hepato- and nephrotoxicity.

Research into the mechanism of CP hepato- and nephrotoxicity is an important step for protection against CP side effects. One theory involves binding of CP to sulfhydryl (SH) groups in the cells, which are necessary for enzyme function and depletion of intracellular glutathione leads to tissue damage [35]. This explains the significant depletion of GSH levels in tissues of mice treated with CP that is due to the consumption of GSH in the tissues by ROS which interact with -SH groups as compared with the control group. GSH is an antioxidant preventing the damage caused by ROS. GSH is the most abundant -SH molecule in the organ tissues that can act either as a non-enzymatic antioxidant molecule by direct interaction of -SH group with ROS or it can be implicated in the enzymatic detoxification reaction for ROS, as a cofactor or coenzyme [36-37].

Cinnamic acid administration can attenuate MDA and NO increments in our investigated tissues in the combined groups. CA significantly improved the lipid peroxidation in the mice induced by cisplatin as manifested by the decreased MDA level, accompanied by the increased GSH content. These results could be attributed to the potential antioxidant effect of CA [38] and are in agreement with those obtained by Patra et al. [39] who demonstrated that CA protects mice from cyclophosphamide-induced hepatotoxicity and myelotoxicity because cinnamic acid has antioxidant effect and as a result detoxifying (scavenging) the free radicals or ROS and so the GSH doesn't get consumed via ROS. This explains the significant increment of GSH levels in tissues treated with CA-treated mice as compared with the control group.

Sova et al. [40] reported that many cinnamic acid derivatives, especially those with the phenolic hydroxyl group, are well-known antioxidants and are supposed to have several health benefits due to their strong free radical scavenging properties. It is also well known that cinnamic acid has antimicrobial activity. Cinnamic acid derivatives, isolated from plant material, propolis and synthesized, have been reported to have antibacterial, antiviral and antifungal properties. As a result this compound may inhibit lipid peroxidation by scavenging free radicals and increasing intracellular concentration of glutathione [41].

Furthermore, CA could decrease the NF- κ B activity. This explains the significant decrease of NO levels in tissues treated with CA-treated mice as compared with the control group, because CA deactivates NF- κ B which in turn activates iNOS that produces the NO [42]. Another mechanism may be considered: CA is a known Nrf2 activator that in turn deactivates the iNOS, resulting in low levels of NO produced in tissues treated with CA-treated mice as compared with the control group [43]. This explanation is in agreement with Wondrak et al. [12] and Lee et al. [44].

In conclusion: The present study demonstrated that CA provided a significant protective effect against cisplatin-induced nephrotoxicity and hepatotoxicity when administered concurrently to cisplatin and the mechanism of nephro- and hepatoprotection by CA could be due to the antioxidant, anti-inflammatory and free radical scavenging activities of the CA.

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