Cytotechnology (2016) 68:279–289
DOI 10.1007/s10616-014-9779-z

ORIGINAL RESEARCH

Synergistic protective role of ceftriaxone and ascorbic acid against subacute diazinon-induced nephrotoxicity in rats

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Received: 1 April 2014 / Accepted: 31 July 2014 / Published online: 24 August 2014

Abstract Diazinon (DZN) is a synthetic organophosphrus acaricide and insecticide widely used for veterinary and agricultural purposes. However, its animal and human exposure leads to nephrotoxicity. Our experimental objective was to evaluate protective effects of ceftriaxone and/or ascorbic acid—vitamin C against DZN-induced renal injury in male Wistar albino rats. DZN-treated animals revealed significant elevation in serum biochemical parameters related to renal injury: urea, uric acid and creatinine. DZN intoxication significantly increased renal lipid peroxidation, and significant inhibition in antioxidant biomarkers including, reduced glutathione, glutathione peroxidase, superoxide dismutase, catalase and total antioxidant capacity. In addition, DZN significantly reduced serum acetylcholinestrase level. Moreover, It induced serum and kidney tumor necrosis factor-α level. Both ceftriaxone and vitamin C protect against DZN-induced serum as well as renal tissue biochemical parameters when used alone or in combination along with DZN-intoxication. Furthermore, both ceftriaxone and vitamin C produced synergetic nephroprotective and antioxidant effects. Therefore, it could be concluded that ceftriaxone and/or vitamin C administration are able to minimize the toxic effects of DZN by its free radical-scavenging and potent antioxidant activity.

Keywords Diazinon · Nephrotoxicity · Ceftriaxone · Vitamin C · Antioxidant · Kidney · Rats

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Introduction

Pesticides are used extensively throughout the world for control of agricultural, veterinary and domestic insect pests and disease vectors in order to enhance the food production and maintain human and animal health. The wide-spread use of pesticides in agricultural programs and public health has caused serious ecological problems and potential health hazards, including severe acute, subacute and chronic cases of poisonings of humans and animals and therefore, are main reasons of concern (Ranjbar et al. 2002; Abdel-Daim and Halawa 2014; Abdel-Daim et al. 2014).

Among pesticides are organophosphorous insecticide, which have fully replaced the chlorinated hydrocarbons since 4 decades. The main advantage of the organophosphorous compounds is their short-term persistence in the ecosystem and low cumulative action (Zavon 1971). Although the organophosphates have been replaced by pyrethroid pesticides within the last 10–15 years, there is still a very intensive use of the organophosphorus insecticides (Salem and Olajos 1988).

Diazinon [phosphoric acid, *O*,*O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyridinyl)] phosphorothioate is an organophosphorus insecticide used worldwide in veterinary, agricultural, and domestic practice to control ticks, fleas, lice, flies, and other insect pests of domestic animals, ornamental plants, and food crops (Larkin and Tjeerdema 2000). Toxic effects of DZN on target and non-target organisms are mainly due to the inhibition of acetylcholinesterase (AChE) activity, which is a crucial enzyme for normal nervous tissue physiology. DZN induces its toxicity by binding its oxygen analog to the neuronal enzyme AChE, leading to accumulation of the endogenous neurotransmitter; acetylcholine in neurons and effectors organ (Larkin and Tjeerdema 2000). Moreover, it disrupts mitochondrial membrane transport in rat liver and affects cytochrome P450 system in human liver (Sams et al. 2004). Furthermore, DZN toxicity induced oxidative damage resulting in hematological disorders, cardiotoxicity, hepatotoxicity, nephrotoxicity, neurotoxicity, and both female and male reproductive toxicity (Al-Attar and Abu Zeid 2013; Razavi et al. 2013; Elmazoudy et al. 2011; ElMazoudy and Attia 2012).

The cells overcome oxidative stress by either removal of the damaged nucleotides and products of the lipid peroxidation or directly scavenging free radicals via endogenous enzymatic and non-enzymatic antioxidants (Madkour and Abdel-Daim 2013; Abdel-Daim et al. 2013; Azab et al. 2013).

Ceftriaxone (CTX) is a broad-spectrum antimicrobial cephalosporin, resistant to beta-lactamases, with potent activities against gram-negative and gram-positive microorganisms (Neu et al. 1981). It is effective against *Streptococcus pyogenes*, *Streptococcus feacalis*, *Streptococcus pneumonia*, *Haemophilus influenza*, *Brucella melitensis*, and *Neisseria gonorrhoeae* (Neu et al. 1981). CTX acts by inhibition of transpeptidase enzymes which are responsible for the final step in bacterial cell wall synthesis and has significant stability against beta-hydrolysis (Neu 1985). Other non-antibiotic actions of CTX on animal studies demonstrated that it could ameliorate morphine tolerance (Rawls et al. 2010b), and dependence (Rawls et al. 2010a), diabetic hyperalgesia (Gunduz et al. 2011) and neuropathic pain (Liu et al. 2010). Moreover, CTX attenuate cyclosporine A (Yilmaz et al. 2011), tobramycin (Beauchamp et al. 1994), isepamicin (Yoshiyama et al. 1998), and cadmium-induced (Dwivedi et al. 2012) nephrotoxicity in rats.

Ascorbic acid (AA)—vitamin C—is probably the most crucial antioxidant in extracellular fluids. It is an important vitamin and an essential component in the diet of many mammalian species. It is highly water soluble and acts as an efficient reducing agent. AA is the most effective antioxidant in preventing lipid peroxidation initiated by peroxyl radicals, and considered as a potent free radical scavenger (El-Demerdash et al. 2005; Kojo 2004). Moreover, it may restore other antioxidants such as vitamin E (Carr and Frei 1999). To our knowledge, the role of CTX and AA, alone or in combination against DZN-induced biochemical alteration of serum as well as renal lipid peroxidation and antioxidant status in rats has not been studied yet. Therefore, the present study was designed to investigate the alterations in serum biochemical parameters related renal damage as well as renal lipid peroxidation and oxidative stress induced by DZN in rats. Moreover, the role of ceftriaxone and/or vitamin C supplementation in alleviating these DZN-induced hazard effects could be evaluated.

Materials and methods

Chemicals

Diazinon (DZN); Diazinon® 60 a commercial emulsifiable formulation containing 60 % active ingredient, was purchased from Adwia Pharmaceuticals (Cairo, Egypt). DZN was diluted in deionized water for the final required concentration. Ceftriaxone (CTX) (Ceftriaxone® vial, 250 mg, 500 mg and 1 g crystalline powder) was kindly given by Sandoz-Novartis, Egypt. Ascorbic acid was purchased from Adwia Pharmaceuticals (Cairo, Egypt). All kits were purchased from Biodiagnostics Cairo, Egypt except, AChE, from (BioVision Inc., Milpitas, CA, USA) and TNF-α from Assay Designs Inc. (Ann Arbor, MI, USA). All other chemicals used in the experiment were of analytical grade.

Animals and experimental design

Fifty-six male Wistar rats, weighing 175 ± 25 g, were bought from The Egyptian Organization for Biological Products and Vaccines (Giza, Egypt). Rats were kept in a ventilated animal house of normal light–dark cycle (12 h light/dark) and temperature (25 ± 2 °C).

Table 1 Summary of different rat groups and their treatment

Group	DZN	CTX	AA
Control	−	−	−
CTX	−	+	−
AA	−	−	+
DZN	+	−	−
DZN–CTX	+	+	−
DZN–AA	+	−	+
DZN–CTX–AA	+	+	+

Diazinon 20 mg/kg body weight, daily for 4 weeks (DZN), Ceftriaxone at 100 mg/kg body weight daily for 4 weeks, 1 h before DZN dose (CTX), Ascorbic acid (vitamin C) 100 mg/Kg body weight daily, 1 h before DZN administration (AA)

Food and water were provided *ad libitum*. Experimental design and all animal handling procedures were approved by the Research Ethical Committee of the Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt (approval no. 20147). All precautions were taken into consideration to avoid animal stress.

Rats were kept for 1 week before the beginning of the experiment for acclimatization. Next, rats were randomly divided into seven different groups; 8 animals in each. Rats in the 1st group were administered normal saline and kept as control. The 2nd and 3rd groups were given CTX (100 mg/kg bw SC) (Amin et al. 2012) and ascorbic acid (100 mg/kg bw orally) (Li et al. 2010) daily for 4 weeks. The 4th group received a daily dose of DZN (20 mg/kg, SC) (Hariri et al. 2010). The 5th and 6th groups were given CTX and AA at the same dose regimen used for the 2nd and 3rd groups before DZN administration at the same dose and regimen used for the 4th group. The 7th group was given both CTX and AA 1 h before DZN administration (Table 1).

Serum collection and tissue preparation

At the end of the experiment (24 h after the DZN dose), blood samples were collected via retro-orbital plexus under light ether anaesthesia. Blood samples were left to clot at room temperature and centrifuged at 3,000 rpm for 15 min. Sera were then, separated and stored at −20 °C as aliquots for further biochemical analysis.

After blood collection, rats were sacrificed by cervical decapitation. Kidneys were rapidly excised from each animal, trimmed of connecting tissue, and washed with 0.9 % NaCl solution and distilled water for removal of the blood. Next kidneys were blotted over a piece of filter paper and perfused with a 50 mM (sodium phosphate buffer saline (100 mM Na_2HPO_4/NaH_2PO_4) (pH 7.4) in an ice-containing medium, containing 0.1 mM ethylene-di-amine-tetra-acetic-acid (EDTA) to remove any clots and blood cells. Subsequently, renal tissues were homogenized in 5–10 ml cold buffer per gram tissue and centrifuged at 5,000 rpm for 30 min. The resulting supernatant was then transferred into Eppendorf tubes, and kept at −80 °C in a deep freezer until used for various biochemical Assays.

Serum biochemical analysis

The sera stored at −20 °C were used for estimation of serum renal injury marker products; creatinine, urea and uric acid were determined according to Larsen (1972), Coulombe and Favreau (1963) and Whitehead et al. (1991), respectively. Acetylcholinesterase (AChE) was measured according to Ellman et al. (1961).

Evaluation of tissue lipid peroxidation and antioxidant enzymes

Lipid peroxidation was evaluated by measurement of MDA content in renal tissues according to Mihara and Uchiyama (1978). Serum nitric oxide (NO) xere determined according to Green et al. (1982). The non-enzymatic antioxidant biomarker; reduced glutathione (GSH) was assessed according to Beutler et al. (1963). The enzymatic antioxidant biomarkers; superoxide dismutase (SOD) was evaluated according to Nishikimi et al. (1972), glutathione peroxidase (GSH-Px) according to Paglia and Valentine (1967) and catalase (CAT) according to Aebi (1984). In addition, total antioxidant capacity (TAC) was determined according to Koracevic et al. (2001).

Serum and kidney TNF-α assays

Serum and renal tissue TNF-α levels were measured using coomercial kits from R&D Systems (Minneapolis, MN, USA) according to the manufacturer's protocol. Briefly, in a microplate, 50 µl of the assay diluent was added to each well. Then, 50 µl of the diluted serum or renal tissue homogenate supernatant samples were added to each well and gently mixed by tapping the plate frame for 1 min then the plate was

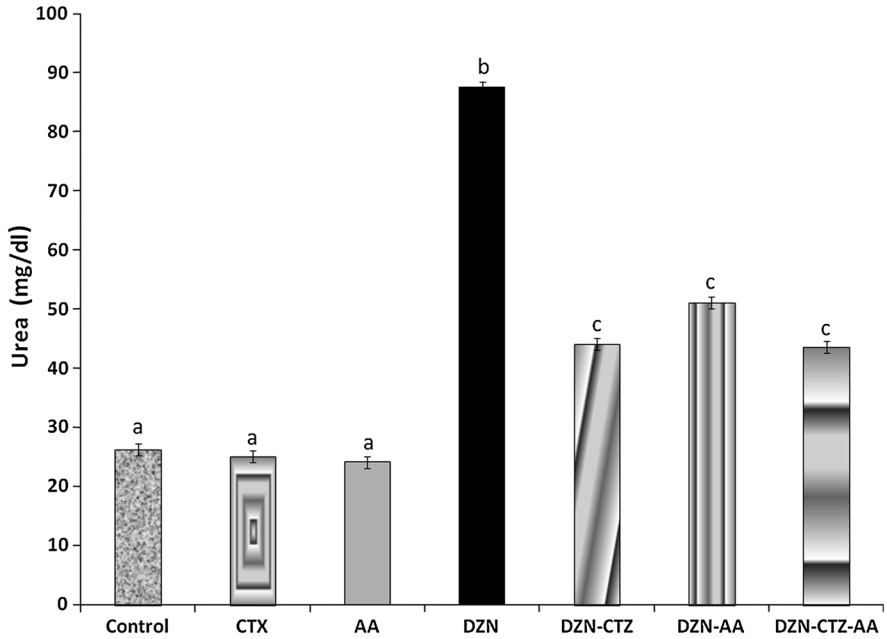

Fig. 1 Serum urea levels in the control and the different treated groups. *Different letters* mean statistical significance at $P \leq 0.05$ according to one-way ANOVA followed by Tukey's Range Test for post hoc analysis

covered and incubated at room temperature for 2 h. After incubation, each well in the plate was then aspirated and washed five times with the kit washing buffer, then 100 µl substrate solutions were added and incubated at room temperature in the dark for 30 min. The optical density was then read at 450 nm using 96-well microtiter plates ELISA reader. TNF-α level was calculated from a standard curve and multiplied by the dilution factor.

Statistical analysis

Data are presented as mean ± SE. Statistical significance of the data was analyzed using SPSS programme (Statical package for Social Science) version 16. For comparison, one-way analysis of variance (ANOVA) test and post-comparison was carried out with Tukey's Range Test for post hoc analysis. Statistical significance was acceptable at a level of $P \leq 0.05$.

Results

Serum biochemical analysis

The effects of DZN intoxication as well as the preventive effects of CTX and/or AA on serum biochemical analyses are shown in Figs. 1–4. Significant increases ($P \leq 0.05$) in serum renal injury markers (urea, uric acid and creatinine) were recorded in DZN intoxicated rats as compared to the untreated control group (333.68 %, 328.85 % and 2,412. 30 %, respectively) (Figs. 1, 2, 3, respectively). On another hand, AChE was significantly ($P \leq 0.05$) decreased in DZN intoxicated rats (55.19 %) compared to the control rats (Fig. 4).

Pre-treatment with CTX at doses of 100 mg/kg significantly ($P \leq 0.05$) reduced the serum renal injury markers: urea, uric acid and creatinine (about 58.33, 54.37 and 32.70 %, respectively), while AA pre-treatment at a dose of 100 mg/kg significantly ($P \leq 0.05$) reduced these biomarkers (about 49.67, 48.67 and 30.02 %, respectively) compared with the DZN-intoxicated group. Moreover, both CTX and AA were given in combination and significantly ($P \leq 0.05$) reduced the same parameters more than when each of them were used alone (about 29.65, 31.87 and 7.15 %, respectively) (Figs. 1, 2, 3, respectively). At the same time, pretreatment with CTX, AA or both in combination significantly ($P \leq 0.05$) increased serum AChE level (143.71, 152.83 and 179.64 %, respectively) compared with the DZN-intoxicated rats (Fig. 4).

There were no significant changes in serum markers in rats having received CTX or AA only at a dose of 100 mg/kg (2nd and 3rd groups, respectively) if compared to the normal control (1st group), indicating the safety of CTX and AA at the selected doses used in this study (Figs. 1–4).

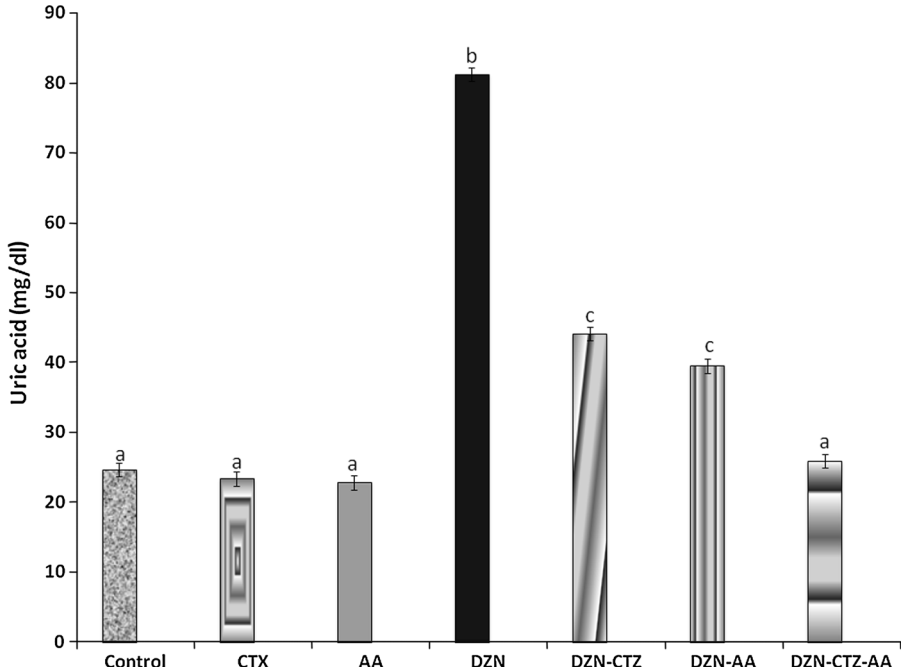

Fig. 2 Serum uric acid levels in the control and the different treated groups. *Different letters* mean statistical significance at $P \leq 0.05$ according to one-way ANOVA followed by Tukey's Range Test for post hoc analysis

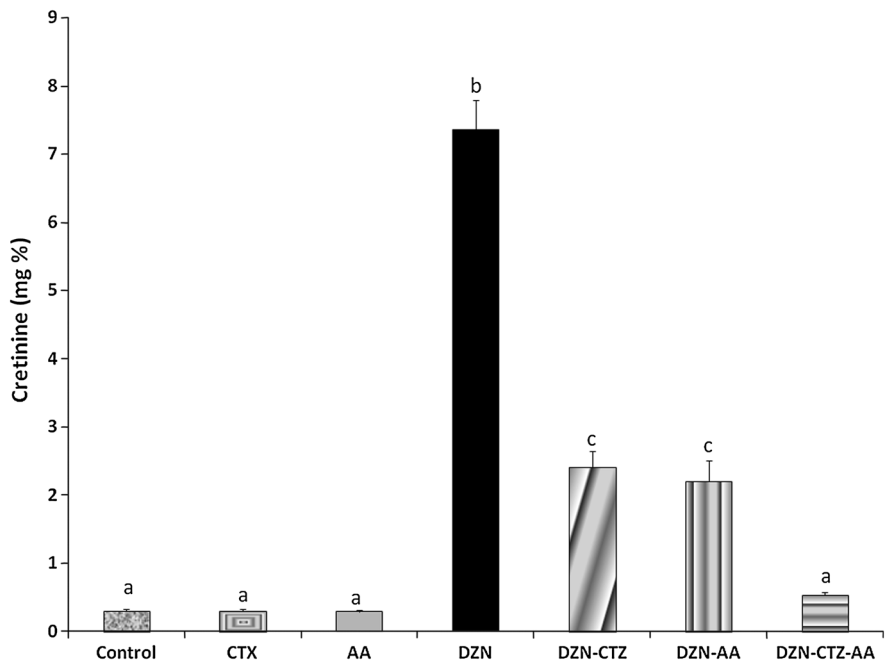

Fig. 3 Serum creatinine levels in the control and the different treated groups. *Different letters* mean statistical significance at $P \leq 0.05$ according to one-way ANOVA followed by Tukey's Range Test for post hoc analysis

Renal lipid peroxidation and antioxidant biomarkers

The effects of DZN intoxication as well as preventive effects of CTX and/or AA on renal tissue homogenate lipid peroxidation and antioxidant parameters are shown in Table 2. A significant increase ($P \leq 0.05$) in renal MDA and NO content (447.56 and 257.65 %, respectively) was observed compared to the control group. On the other hand, renal GSH, GSH-Px, SOD,

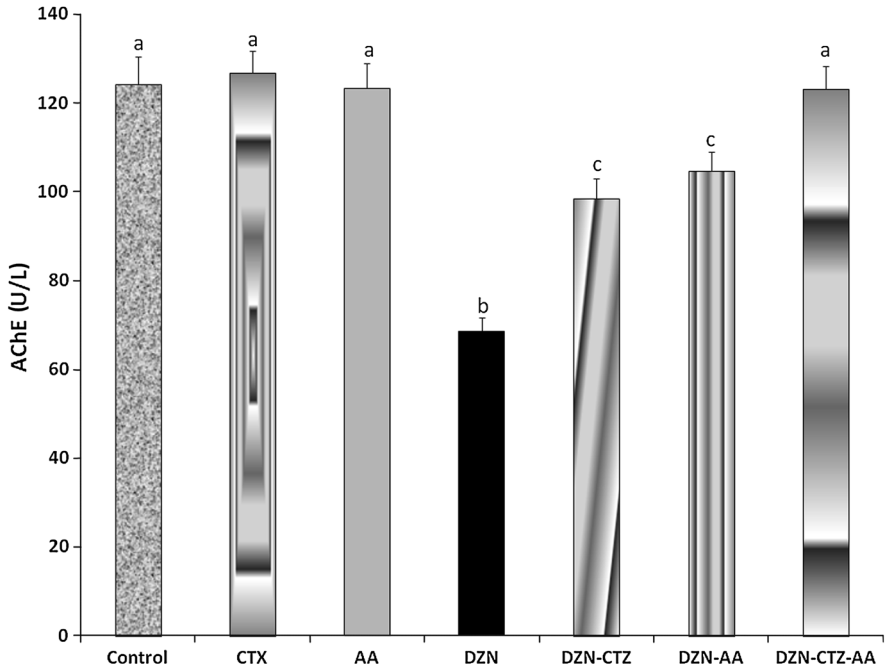

Fig. 4 Serum AChE levels in the control and the different treated groups. *Different letters* mean statistical significance at $P \leq 0.05$ according to one-way ANOVA followed by Tukey's Range Test for post hoc analysis

CAT and TAC were significantly ($P \leq 0.05$) decreased (59.06, 43.10, 38.81, 48.62 and 71.25 %, respectively). Concerning DZN-CTX group, renal MDA and NO were decreased (73.31 and 68.46 %, respectively) while GSH, GSH-Px, SOD, CAT, and TAC were increased (139.21, 171.51, 185.22,139.84 and 118.16 %, respectively) compared to the DZN-intoxicated group. Regarding to DZN-AA group, renal MDA and NO were decreased (45.62 and 63.87 %, respectively), while GHS, GSH-Px, SOD, CAT, and TAC were increased (about 154.83, 207.26, 213.34, 173.98 and 133.67 %, respectively). In addition, both CTX and AA were given in combination, significantly ($P \leq 0.05$) reduced renal MDA and NO level (26.20 and 43.67 %, respectively) and increased GHS, GSH-Px, SOD, CAT, and TAC more than when each of them was used alone (about 172.94, 234.44, 271.06, 208.13 and 147.79 %, respectively) (Table 2).

Serum and kidney TNF-α assays

DZN intoxication significantly ($P \leq 0.05$) increased both serum and renal tissue TNF-α (566.88 and 801.24 %, respectively) compared with the control rats (Figs. 5, 6). Pretreatment with CTX significantly ($P \leq 0.05$) reduced both serum and renal TNF-α levels (43.42 and 39.83 %, respectively), at the same time AA pretreatment induced significantly ($P \leq 0.05$) reduction of both levels (about 37.47 and 33.49 %, respectively). Moreover, when both CTX and AA were given in combination they induced a more significant ($P \leq 0.05$) reduction in these levels (21.81 and 15.49 %, respectively) compared to the DZN-intoxicated rats (Figs. 5, 6).

Discussion

Reactive oxygen species (ROS) are continuously generated inside the mammalian body as a result of exposure to many exogenous chemicals, xenobiotics and drugs in our environment and/or endogenous metabolic events involving electron transport mechanism and redox enzymes (Al-Sayed et al. 2014; Eldahshan and Abdel-Daim 2014; Abdel-Daim 2014). In normal conditions, there is balance between the ROS produced and the antioxidants, as the ROS generated are neutralized by the endogenous antioxidants (Sun 1990). Deleterious effects caused by ROS occur as a result of an imbalance between the formation and inactivation of these agents leading to disturbance in normal cellular physiology and different pathological conditions (Sun 1990; Ibrahim and Abdel Daim 2015). Free radicals have been implicated in the pathogenesis of many

Table 2 Renal tissue malondialdhyde, nitric oxide and antioxidant biomarkers in the control and the different treated groups

Parameters	Experimental groups						
	Control	CTX	AA	DZN	DZN–CTX	DZN–AA	DZN–CTX–AA
MDA (nmol/g)	$3.43^{a} \pm 0.19$	$3.34^{a} \pm 0.17$	$3.30^{a} \pm 0.19$	$16.25^{b} \pm 1.27$	$11.92^{c} \pm 0.45$	$7.42^{d} \pm 0.52$	$4.26^{a} \pm 0.21$
NO (μmol/g)	$6.52^{a} \pm 0.40$	$6.37^{a} \pm 0.35$	$6.27^{a} \pm 0.43$	$16.81^{b} \pm 1.07$	$11.51^{c} \pm 0.61$	$10.74^{c} \pm 0.76$	$7.34^{a} \pm 0.36$
GSH (mg/g)	$7.07^{a} \pm 0.32$	$7.27^{a} \pm 0.37$	$7.64^{a} \pm 0.36$	$4.18^{b} \pm 0.14$	$5.81^{c} \pm 0.28$	$6.47^{ac} \pm 0.21$	$7.22^{a} \pm 0.29$
GSH-Px (mg/g)	$4.88^{a} \pm 0.30$	$5.10^{a} \pm 0.36$	$5.51^{a} \pm 0.24$	$2.10^{b} \pm 0.17$	$3.60^{c} \pm 0.24$	$4.36^{ac} \pm 0.28$	$4.93^{a} \pm 0.27$
SOD (U/g)	$18.33^{a} \pm 1.47$	$18.74^{a} \pm 1.49$	$19.55^{a} \pm 1.35$	$7.11^{b} \pm 0.45$	$13.18^{c} \pm 0.64$	$15.18^{ac} \pm 0.80$	$19.28^{a} \pm 0.92$
CAT (U/g)	$0.32^{a} \pm 0.03$	$0.33^{a} \pm 0.02$	$0.35^{a} \pm 0.02$	$0.15^{b} \pm 0.01$	$0.22^{c} \pm 0.01$	$0.27^{ac} \pm 0.01$	$0.32^{a} \pm 0.02$
TAC (μmol/g)	$1.39^{a} \pm 0.05$	$1.45^{a} \pm 0.06$	$1.49^{a} \pm 0.07$	$0.99^{b} \pm 0.05$	$1.17^{c} \pm 0.04$	$1.33^{a} \pm 0.05$	$1.47^{a} \pm 0.04$

Data are expressed as mean ± SE; ($n = 8$). *DZN* Diazinon, *CTX* ceftriaxone, *AA* ascorbic acid, *MDA* malondialdehyde, *NO* nitric oxide, *GSH* reduced glutathione, *SOD* superoxide dismutase, *CAT* catalase, *TAC* total antioxidant capacity. Within the same row, different letters mean statistical significance at ($P \leq 0.05$) according to one-way ANOVA followed by Tukey's Range Test for post hoc analysis

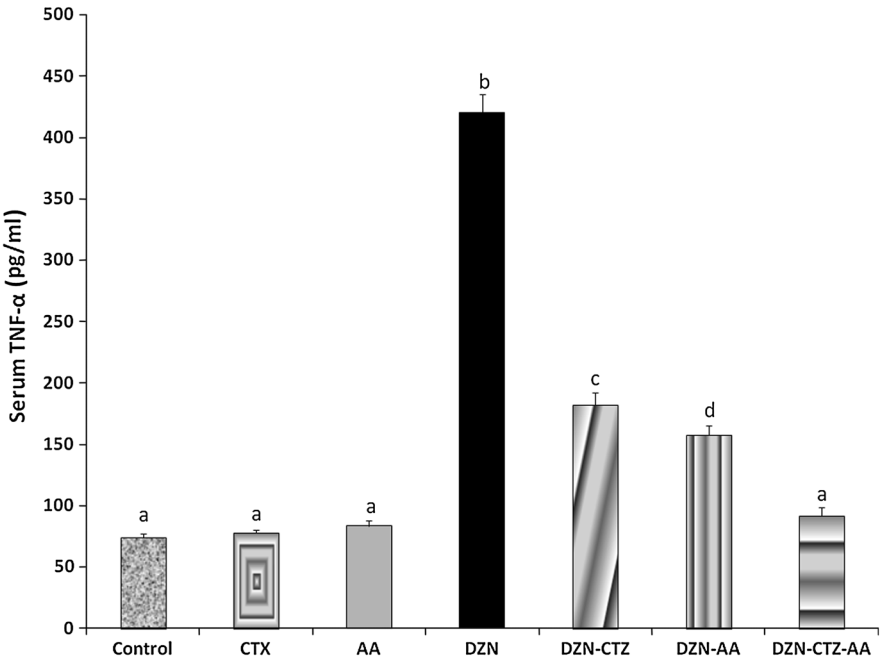

Fig. 5 Serum TNF-α levels in the control and the different treated groups. *Different letters* mean statistical significance at $P \leq 0.05$ according to one-way ANOVA followed by Tukey's Range Test for post hoc analysis

degenerative diseases such as cataract, cancer, stroke, coronary heart disease, diabetes, rheumatoid arthritis, Alzheimer's disease and ageing process (Akash et al. 2012, 2013; Abdel-Daim et al. 2010a, b; Funasaka et al. 2012; Willcox et al. 2004).

Diazinon, an organophosphrus insecticide, has been widely used in industrial, veterinary, and agriculture practice, that would be potentially an exposure risk to animals and human as well (Larkin and Tjeerdema 2000). Although several investigations about DZN toxicity have been published, little study has been performed about the preventive agents used against such a toxicity and mechanism of their ameliorative action (Larkin and Tjeerdema 2000; Al-Attar and Abu Zeid 2013; Razavi et al. 2013; Elmazoudy et al. 2011; ElMazoudy and Attia 2012).

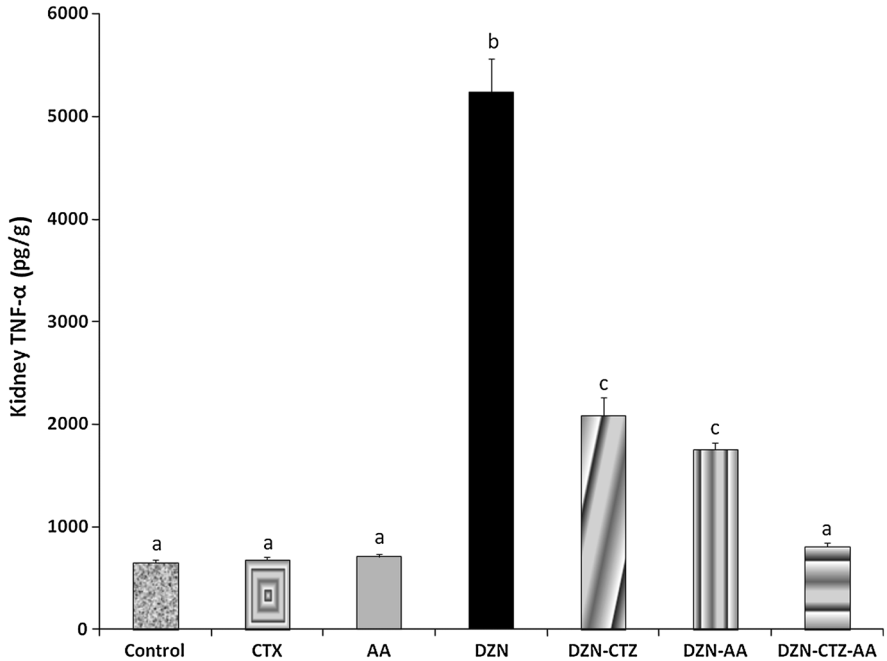

Fig. 6 Renal TNF-α levels in the control and the different treated groups. *Different letters* mean statistical significance at $P \leq 0.05$ according to one-way ANOVA followed by Tukey's Range Test for post hoc analysis

In the present study, the renal injuries caused by DZN may be caused by the oxidative stress resulted from free radical production. DZN intoxication significantly ($P \leq 0.05$) increased serum renal injury markers: urea, uric acid and creatinine. Moreover, it significantly ($P \leq 0.05$) reduced serum AchE level (Figs. 1–4). DZN treatment significantly ($P \leq 0.05$) increased lipid peroxidation through elevated kidney MDA level, significantly ($P \leq 0.05$) decreased kidney enzymatic; GSH-Px, SOD and CAT as well as non-enzymatic; GSH antioxidant level (Table 2). In addition, it significantly ($P \leq 0.05$) increased both serum and renal tissue TNF-α compared with the control group.

All these effects are involved in the cascade of events leading to DZN-mediated kidney oxidative stress and toxicity. This indicates that renal injuries induced by DZN is the result from oxidative stress that arises as a result of excessive ROS production, which have been known to attack various cellular molecules, including lipids and causing lipid peroxidation. The activities of antioxidant enzymes, including the enzymes involved in glutathione metabolism were also disrupted in the DZN-intoxicated group (Table 2) indicating the involvement of oxidative stress in DZN-mediated renal damage. These results are consistent with the literature and point towards the role of ROS in DZN-mediated injury and toxicity. The insecticide, diazinon was reported to increase serum urea and creatinine, urinary glucose, and renal tissue MDA, while antioxidant markers were decreased in rats and mice (Boroushaki et al. 2013; Cakici and Akat 2013; El-Demerdash and Nasr 2013). Long term administration of diazinon in rabbits induced significant oxidative DNA damage in the liver and kidney and significantly reduced plasma CAT, GSH and TAC levels (Tsitsimpikou et al. 2013). The levels of serum TNF-α, uric acid, LDH, AST, ALT were significantly increased by sub-acute administration of diazinon in rats (Hariri et al. 2010).

In the current study, the pre-administration of CTX and/or AA at a dose level of 100 mg/kg for each, reduced the serum renal injury markers. Moreover, they reduced the lipid peroxidation in kidney tissues. In addition, there were elevations of renal antioxidant enzymes and glutathione levels due to the administration of both preventive agents. Furthermore, they increased serum AChE and reduced serum and kidney TNF-α. Both CTX and vitamin C induced synergistic protective effects against DZN-induced biochemical alterations of serum and tissues (Table 2; Figs. 1–6).

These results are in agreement with many previous literatures, which examined the nephroprotective and antioxidant effects of CTX against many drugs and xenobiotics-induced nephrotoxicity (Yilmaz et al. 2011; Beauchamp et al. 1994; Yoshiyama et al.

1998; Dwivedi et al. 2012). Pre-treatment with AA might play a role in reducing the toxic effect of DZN, and its powerful antioxidant properties seem to mediate such a protective effect, indicated by the reduction of MDA as well as the elevation of GSH, GSH-Px, SOD, CAT and TAC levels in renal tissue (Nematbakhsh et al. 2012; Ashrafi et al. 2012; Rehman et al. 2012; Saleem et al. 2012).

The protective effect of CTX and/or vitamin C against DZN-induced oxidative stress in our rat model could be either directly by scavenging ROS and inhibition of lipid peroxidation and/or indirectly through the enhancement of the activities of SOD and CAT, the enzymatic free radicals' scavengers in the cells. Therefore, CTX and vitamin C could be used in combination to prevent and treat renal diseases, especially those induced by oxidative damage.

Conclusion

Oxidative stress plays a major role in DZN-induced nephrotoxicity. Antioxidants have been proven to be effective in preventing DZN-induced toxicity in many previous interventions. CTX and AA are potent antioxidants, which are reported to ameliorate the effect of many known nephrotoxic agents.

In the present study, clearly DZN exposure resulted in varying degrees of inhibition of antioxidant enzymes, induction of lipid peroxidation, and alterations of biochemical parameters of serum. CTX and/or AA pre-treatment provided near complete protection in terms of serum and tissues' biochemical changes, antioxidant enzymes activity and oxidative stress, especially when given in combination.

Acknowledgments The authors would like to thank Sandoz-Novartis, Egypt, for supplying Ceftriaxone® used in our experiment. The company had neither role in designing the experiment nor in publication process. This research received no Grant from any funding agency.

Conflict of interest The authors declare that there are no conflicts of interest.

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