



Zinc alleviates maneb-induced kidney injury in adult mice through modulation of oxidative stress, genotoxicity, and histopathological changes

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Abstract

Zinc is one of the important essential trace minerals to human health due to its antioxidant properties. The present study was conducted to elucidate its potential protective role against maneb-induced nephrotoxicity. For this purpose, animals were randomly divided into four groups of six each. Mice of group I (negative controls) have received daily 0.5 ml of distilled water, a solvent of maneb. Mice of group II (MB) have received 30 mg/kg bw of maneb daily by intraperitoneal way. Mice of group III (MB + Zn) have received the same dose of maneb as group II, along with ZnSO₄ (30 mg/kg bw) daily. Mice of group IV (Zn), considered as positive controls, have received the same dose of ZnSO₄ as group III daily. Our results revealed that ZnSO₄ co-administration to maneb-treated mice decreased kidney levels of malondialdehyde, hydrogen peroxide, protein carbonyls, and advanced oxidation protein products; the levels of non-enzymatic antioxidants like vitamin C, glutathione, and metallothionein. It recovered the alteration of antioxidant enzyme activities (catalase, superoxide dismutase, and glutathione peroxidase) and attenuated DNA fragmentation. Furthermore, this essential trace element was also able to alleviate kidney biomarkers' alterations by lowering plasma levels of creatinine, urea, uric acid, and lactate dehydrogenase. In addition, the histopathological changes induced by maneb were improved following zinc administration. Our results indicated that zinc might be beneficial against maneb-induced renal oxidative damage in mice.

Keywords Zinc · Maneb · Kidney · Oxidative damage · Mice

Introduction

Essential trace minerals play a pivotal role in the normal development and the protection of human body and health

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(Prashanth et al. 2015). Most of them mediate crucial biochemical reactions by acting as enzyme cofactors or catalysts. In particular, zinc (Zn) is a micronutrient, as well as an essential trace mineral obtained from the diet. It is essential for a wide range of biological activities except when used at high doses (Salgueiro et al. 2000). In trace amounts, this mineral is important in cellular function because it is an integral component of many proteins, including metalloenzymes, structural proteins, and transcriptional factors (Zhou et al. 2007). Many studies have revealed that Zn has antioxidant properties and acts by several different mechanisms. Indeed, Zn binds to thiol groups of biomolecules, allowing their stabilization against oxidation (Bettger 1993). Moreover, it antagonizes transition metal-catalyzed reactions by competing with redox active metals, such as iron and copper, for binding to cell membranes as well as to some proteins (Powell 2000). Zn is also a co-factor of copper zinc superoxide dismutase (Cu/Zn-SOD), which transforms superoxide anion radicals (O₂⁻) to the less harmful species: oxygen (O₂) and hydrogen peroxide (H₂O₂), as

reported by Mariani et al. (2008). This mineral has been proven to upregulate the nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor regulating the expression of genes encoding the antioxidant molecules superoxide dismutase (SOD), glutathione S-transferase (GST), heme oxygenase 1 (HO-1), and glutathione (GSH) (Cortese et al. 2008; Smith and Loo 2012; Jarosz et al. 2017). Besides, Zn has been demonstrated to increase the expression of the antioxidant enzyme catalase (Tate Jr et al. 1997). In addition, this trace element can retard oxidative processes through inducing the biosynthesis of metallothioneins, cysteine-rich proteins which can bind pro-oxidant metals, such as cadmium, and provide thiol groups to scavenge reactive oxygen species (ROS) (Dondero et al. 2005). Interestingly, previous experimental studies on animal models have confirmed Zn efficiency in protecting against oxidative injury, associated with ROS-generating xenobiotics exposure, to a variety of tissues and organs, including the kidneys, and this effect was related to the antioxidant capacity of this mineral. For instance, Ghabaee et al. (2017) have reported that Zn administration to rats during gestation and lactation protects against arsenic-induced oxidative stress in kidney tissue. Likewise, Babaknejad et al. (2016) have demonstrated that Zn treatment reverses the toxic renal alterations induced by cadmium in rats. Zn showed also an ameliorating effect against oxidative stress induced by the pesticide fipronil in the renal tissue of rats (Swelam et al. 2017). In this context, it is well documented that pesticides, in general, have numerous negative effects including dermatological, respiratory, gastrointestinal, neurological, carcinogenic, reproductive, and endocrine changes (Mnif et al. 2011; Sanborn et al. 2007; Thakur et al. 2014). Among pesticides, maneb, a manganese-containing dithiocarbamate fungicide, is extensively employed in agriculture to treat a variety of crop pathologies, because of its low acute toxicity and short environmental persistence. At large amounts, maneb produces adverse effects in non-target organisms, including human beings, like headache, nausea, weight loss, confusion, respiratory paralysis, and even death (Edwards et al. 1991). It has been demonstrated that maneb acts on the thyroid gland (Mallem et al. 2007) as well as on the central and peripheral nervous systems (Domico et al. 2006) and possesses genotoxic effects (Bertini et al. 2000). Moreover, maneb exerts an antithyroid effect due to ethylene thiourea (ETU), its main metabolite, which also causes liver toxicity. Recently, we have demonstrated the beneficial role of vanillin against maneb-induced oxidative stress, DNA damage, and liver histological changes in mice (Sefi et al. 2019). According to Jaballi et al. (2017), maneb used at graded doses (1/8, 1/6, 1/4, and 1/2) of LD50 led to oxidative stress generation, provoking renal cell damages due to lowered defense systems' capacities.

To our knowledge, no investigations have reported the potential ability of Zn to alleviate maneb toxicity in the kidney. Therefore, the aim of the present work was focused, for the first time, on the renoprotective role of Zn against maneb-induced kidney damages in adult mice.

Materials and methods

Animals

Seven-week-old male adult mice of Swiss strain, with an initial body weight of 30 ± 1 g, were obtained from the Central Pharmacy (SIPHAT, Tunisia). They were kept in a controlled temperature (22 ± 2 °C), humidity (40%), and photoperiod (12 h light-dark cycle). Mice were acclimated for 2 weeks prior to testing. They had free access to a commercial standard pellet diet (SNA, Sfax, Tunisia) and tap water. The content of Zn in the standard diet corresponded to 65 mg/kg of pellet according to the manufacturer of Animal Nutrition Society (SNA, Sfax, Tunisia). All the experimental procedures were performed according to the International Guidelines for Animal Care (Council of European Communities 1986). The test protocol was approved by the Ethical Committee of Sciences Faculty of Sfax, with 1204 as an ethics approval number.

Experimental design

After acclimatization, animals were randomly divided into four groups of six each. Mice of group I, considered as negative controls, have received daily by intraperitoneal way 0.5 ml of distilled water, a solvent of maneb. Mice of group II (MB) have received 30 mg/kg bw of maneb daily by intraperitoneal way. Mice of group III (MB + Zn) have received the same dose of maneb as group II, along with ZnSO₄ (30 mg/kg bw) daily. Mice of group IV (Zn), considered as positive controls, have received the same dose of ZnSO₄ as group III daily. Maneb dose and the manner of administration, used in the current study, were chosen on the basis of the previous findings of Yadav et al. (2012). Concerning zinc, we have used the dose 30 mg/kg bw, according to Ding et al. (2016).

After the treatment period of 10 days, mice were fasted overnight and then sacrificed by cervical decapitation to avoid stress condition. Blood samples were collected from the trunk into heparinized tubes and centrifuged at $2200 \times g$ for 10 min. Plasma samples were drawn and stored at -80 °C until analysis. Kidney tissues were dissected out, cleaned from adipose tissue, and weighed. Some samples were rinsed, homogenized in ice-cold phosphate buffer (0.1 M; pH 7.4), and centrifuged. The resulting supernatants were collected and kept at -80 °C until biochemical analysis. Other samples were either fixed in 10% buffered formalin solution and embedded in paraffin for histological examination or taken for DNA assay.

Biochemical analysis

Protein quantification

Total protein content in kidney homogenates was estimated according to the Lowry et al. (1951) method, and bovine serum albumin was used as a standard.

Kidney lipid peroxidation

The kidney content in malondialdehyde (MDA), index of lipid peroxidation, was spectrophotometrically determined at 532 nm by Draper and Hadley (1990) method, and the values were expressed as nmoles MDA/mg of protein.

Hydrogen peroxide measurement

Measurement of hydrogen peroxide (H_2O_2) in the kidney was carried out by the ferrous ion oxidation xylenol orange method (Ou and Wolff 1996). Values were expressed as nmoles/mg of protein.

Protein carbonyl content

Kidney protein carbonyls (PCO) content was determined according to the method of Reznick and Packer (1994). The carbonyl content was determined based on the molar extinction coefficient of DNPH ($= 2.2 \times 10^4 \text{ cm}^{-1} \text{ M}^{-1}$) and expressed as nmoles/mg of protein.

Kidney advanced oxidation protein product

Kidney advanced oxidation protein product (AOPP) levels were assayed according to the method described by Kayali et al. (2006). For each sample, AOPP concentration was calculated using the extinction coefficient $261 \text{ cm}^{-1} \text{ mM}^{-1}$ and the results were expressed as nmoles/mg of protein.

Kidney non-enzymatic antioxidant (GSH and vitamin C) levels

Reduced glutathione (GSH) levels in the kidney tissue were analyzed at 412 nm by Ellman (1959) method modified by Jollow et al. (1974), and the values were expressed as nmoles/mg of protein.

Kidney ascorbic acid (vitamin C) content was determined by dinitrophenyl hydrazine method, according to Jacques-Silva et al. (2001). The data were presented as nmoles/mg of protein.

Metallothionein content in kidney

Kidney metallothionein (MT) content was estimated based on the method described by Viarengo et al. (1997) and modified

by Petrovic et al. (2001). Absorbance was measured at 412 nm. The data were expressed as μmoles of GSH/mg of protein.

Kidney enzymatic antioxidant activities

Catalase (CAT) activity in the renal tissue was determined at 240 nm following the Aebi (1984) method, and the values were expressed as $\mu\text{moles } H_2O_2 \text{ consumed/min/mg}$ of protein.

Superoxide dismutase (SOD) activity was analyzed at 560 nm according to Beauchamp and Fridovich method (1971), and the values were expressed as U/mg of protein.

Glutathione peroxidase (GPx) activity was assayed by following the method described by Flohe and Gunzler (1984). Enzyme activity was expressed as nmoles of GSH oxidized/min/mg of protein.

Assays of creatinine, urea, and uric acid levels in plasma

Creatinine, urea, and uric acid plasma levels were quantified spectrophotometrically using commercial diagnostic kits (Ref 20151, 20143, 20091), purchased from Biomaghreb.

Determination of lactate dehydrogenase activities in plasma and kidney

Plasma and kidney activities of lactate dehydrogenase (LDH) were estimated using a commercially available reagent kit purchased from Biomaghreb (Ref: 20012) and they were expressed as units/L and units/g of tissue, respectively.

Qualitative DNA fragmentation assay by agarose gel electrophoresis

Genomic DNA was isolated from the kidney tissue using a commercial kit and electrophoresed on a 1% agarose gel stained with ethidium bromide (Pure Link Genomic DNA Invitrogen ref. K 182001). The gel was then observed under ultraviolet lamp and photographed.

Histological studies

Some portions of the kidney were placed in 10% of buffered formalin solution for 48 h. The specimens were washed and dehydrated through an ascending series of ethanol. Then they were embedded in paraffin. Blocks were made, sectioned at a thickness of 5 μm , and stained with hematoxylin and eosin. The obtained slides were examined under light microscopy (Suvarna et al. 2013) and fitted with a Canon Power Shot camera (A640) to record digitally the required images for histological studies. The severity of histopathological changes (glomeruli fragmentation, Bowman's space enlargement,

hypercellularity and increased nuclear size of glomeruli, leukocytes infiltration, and bleeding area) was scored using the blind study methodology, based on the following scale: 0: no injury, 1: injury to 25% of the field, 2: injury to 50% of the field, 3: injury to 75% of the field, 4: diffuse injury.

Statistical analysis

The data of the present work were analyzed using the statistical package program Stat view 5 Software for Windows (SAS Institute, Berkley, CA). Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD) test as a post hoc test for multiple comparison tests. When comparison between two groups was required, the student unpaired *t* test was also used. All values were presented as means \pm S.D. A *p* value of 0.05 was considered as the point of minimal statistical significance.

Results

Relative kidney weight

During 10 days of treatment, no death was observed in any group; and there were no significant changes in body weights of control and treated mice. Our results showed that the relative kidney weight was significantly ($P < 0.001$) increased by 56% in MB and by 30% in MB + Zn groups, when compared to those of controls (Table 1).

Lipid peroxidation and hydrogen peroxide generation in the kidney

Administration of maneb resulted in a significant increase ($P < 0.001$) in MDA concentrations as well as in H_2O_2 levels in kidney tissues compared to those of control group. However, Zn administration to maneb-treated mice induced a significant reduction in the levels of these parameters compared to those of the maneb-treated group. Exposure of mice

to Zn alone did not induce any changes in MDA and H_2O_2 levels (Table 2).

Protein oxidative markers in the kidney

Our data revealed a significant increase ($P < 0.001$) in PCO and AOPP levels of maneb-treated mice compared to those of control group. Interestingly, co-administration of Zn with maneb significantly reduced the levels of these parameters (Table 2).

Non-enzymatic antioxidant status in the kidney

Maneb exposure caused a significant increase ($P < 0.001$) of GSH, vitamin C, and MT levels in the kidney when compared to those of control group. These modifications were alleviated following Zn administration to maneb-treated mice as indicated by a significant decrease ($P < 0.001$) of the above parameters when compared to those of MB group (Table 2).

Enzymatic antioxidant status in the kidney

In maneb-treated mice, CAT and GPx activities in the kidney were significantly decreased ($P < 0.001$) when compared to those of control group. In contrast, there was a significant increase ($P < 0.001$) in SOD activity. Co-treatment with Zn induced a total recovery in SOD and GPx activities when compared to those of maneb-treated mice. Meanwhile, CAT activity was moderately ($P < 0.05$) ameliorated. The activities of these antioxidant enzymes in kidney of mice treated only with Zn were near to those of negative controls (Fig. 1).

Biomarkers of renal toxicity

As shown in Table 3, the levels of plasma creatinine, urea, and uric acid were significantly ($P < 0.001$) increased in maneb-treated mice when compared to those of controls. In mice co-treated with Zn, a recovery of these parameters was observed.

Table 1 Final body weight, absolute and relative kidney weights in adult mice, controls and treated with maneb associated or no with Zn or with Zn alone

Parameters and treatments	Controls	MB	MB + Zn	Zn
Final body weight (g)	40.83 \pm 3.43	41.33 \pm 2.94	41.00 \pm 2.52	40.73 \pm 3.65
Absolute kidney weight (mg)	0.41 \pm 0.03	0.65 \pm 0.02 ^{***}	0.54 \pm 0.01 ^{***+++}	0.39 \pm 0.03
Relative kidney weight (mg/100 g BW)	1.01 \pm 0.11	1.58 \pm 0.13 ^{***}	1.32 \pm 0.09 ^{***++}	0.96 \pm 0.12

Values are means \pm SD for six mice in each group

MB; (MB + Zn) vs. controls: ^{***} $p < 0.001$

(MB + Zn) vs. MB: ⁺⁺ $p < 0.01$; ⁺⁺⁺ $p < 0.001$

Table 2 Kidney biomarkers and non-enzymatic antioxidants in control and treated mice with maneb, Zn or their combination

Parameters and treatments	Controls	MB	MB + Zn	Zn
MDA ^a	2.60 ± 0.22	5.51 ± 0.14 ^{***}	3.82 ± 0.09 ^{***+++}	2.65 ± 0.19
H ₂ O ₂ ^a	0.038 ± 0.002	0.093 ± 0.002 ^{***}	0.050 ± 0.002 ^{***+++}	0.040 ± 0.005
PCO ^a	5.38 ± 0.35	16.97 ± 1.18 ^{***}	6.78 ± 0.34 ^{***+++}	5.47 ± 0.46
AOPP ^a	0.04 ± 0.005	0.09 ± 0.017 ^{***}	0.04 ± 0.021 ^{***+++}	0.04 ± 0.006
GSH ^a	5.98 ± 0.46	9.18 ± 0.36 ^{***}	7.02 ± 0.28 ^{**+++}	6.29 ± 0.45
Vit C ^a	5.53 ± 0.48	7.51 ± 0.48 ^{***}	6.05 ± 0.24 ^{**+++}	5.61 ± 0.37
MT ^b	0.06 ± 0.005	0.12 ± 0.006 ^{***}	0.08 ± 0.004 ^{***+++}	0.06 ± 0.004

Values are means ± SD for six mice in each group

MDA malondialdehyde, PCO protein carbonyls, AOPP advanced oxidation protein products, H₂O₂ hydrogen peroxide, Vit C vitamin C, GSH glutathione, MT metallothionein

MB; (MB + Zn); Zn vs. controls: **p* < 0.05; ****p* < 0.001. (MB + Zn) vs. MB: +++*p* < 0.001

^a nmoles/mg of protein

^b μmoles GSH/ mg of protein

LDH activities in plasma and kidney

Maneb-treated mice showed a significant increase (*P* < 0.001) in the activities of LDH in plasma associated with its significant decrease (*P* < 0.001) in the kidney compared to those of controls. Co-treatment with Zn improved LDH activities in plasma and kidney (Fig. 2).

DNA fragmentation

As presented in Fig. 3, a smear ladder formation on agarose gel electrophoresis, revealing random DNA fragmentation, was observed in the kidney tissue of maneb-treated mice. Zn co-treatment decreased the smear formation. DNA damage was not detected in negative and positive control groups.

Kidney histopathological studies

Kidney of control group showed normal features of renal tubules and intact glomeruli (Fig. 4(a)). The histopathological hallmarks of maneb-induced renal injury were evident by glomeruli hypertrophy and fragmentation, Bowman’s space enlargement, hypercellularity and increased nuclear size of the glomeruli, marked leukocytes infiltration between the tubules, and a large area of hemorrhage in the interstitium (Fig. 4(c₁, d₁, d₂)). These alterations were attenuated after Zn co-treatment, indicating the reduction of the pathological changes associated with nephrotoxicity (Fig. 4(e)). Treatment with Zn alone showed a normal appearance of the kidney histoarchitecture (Fig. 4(b)).

The severity of these histological alterations was evaluated semiquantitatively in the histopathological kidney sections of control and Zn groups, and the damage scores were found to be zero. Kidney damage parameters (glomeruli fragmentation, Bowman’s space enlargement, increased cellularity and nuclear size of glomeruli, leukocytes infiltration, and hemorrhage)

for MB and MB + Zn groups were scored and results showed that injury scores for MB group were significantly higher than those for MB + Zn group (Table 4).

Discussion

There is accumulating evidence that Zn has antioxidant properties and can protect kidney against xenobiotics-induced nephrotoxicity. In the present study, we investigated, for the first time, whether Zn administration to maneb-exposed mice would potentially alleviate the adverse effects of this fungicide.

In toxicological studies, body and organ weights are the important endpoints for evaluating xenobiotics-induced organ toxicity (Crissman et al. 2004). The current study revealed that maneb exposure significantly increased the relative kidney weight without affecting the body weight of mice. This rise might be a result of the hypercellularity and the enhanced nuclear size of the glomeruli, as shown in histological kidney sections of MB group (Fig. 4(d₂)). Furthermore, El-Damaty et al. (2012) have stated that kidney enlargement and its weight increment in rats treated with pesticides could be due to the accumulation of abnormal cells. In this context, a previous study conducted by De Carvalho et al. (1989) revealed kidney toxicity, illustrated by severe glomerular lesions, in patients after unprotected use of maneb. Recently, Abolaji et al. (2017) have reported an increase in the relative kidney weight of female rats treated with two pesticides, chlorpyrifos and carbendazim.

The kidney morphological changes induced by maneb could be related to the disturbances of the normal redox state in this organ, resulting from oxidative stress installation. The latter has been recognized as a shift in the balance between oxidants and antioxidants in favor of the former, due to increased cellular levels of ROS. In such case, our results

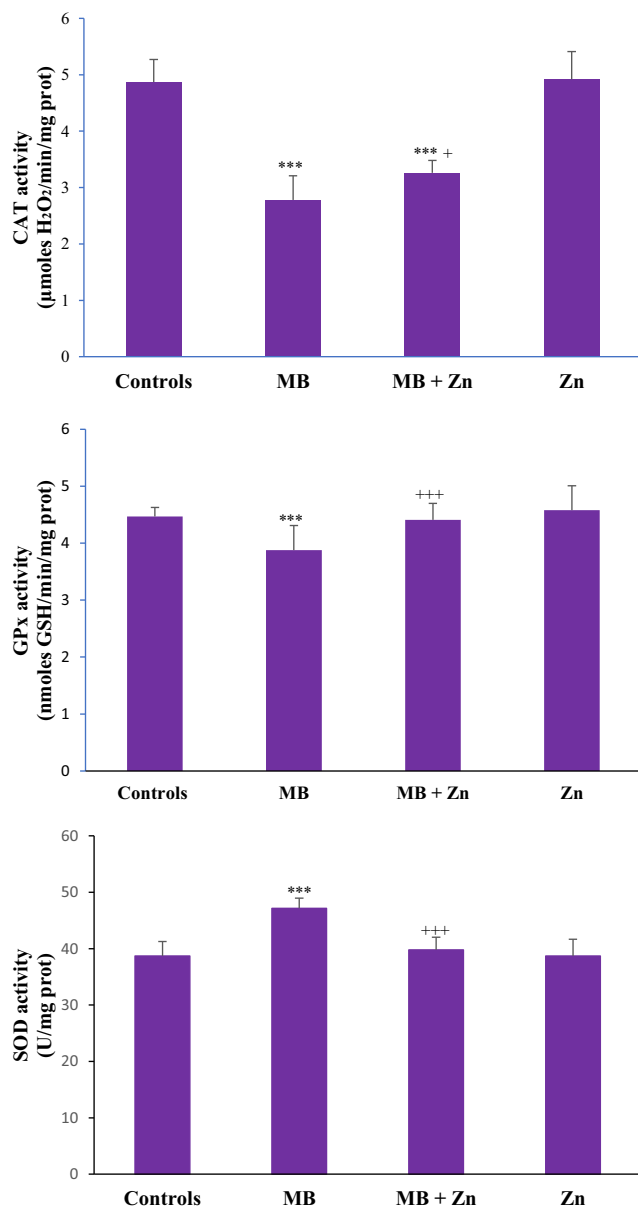


Fig. 1 Kidney enzymatic antioxidant activities (CAT, SOD and GPx) in adult mice controls and treated with maneb associated or no with Zn or with Zn alone. Values are means \pm SD for six mice in each group. MB; (MB + Zn) vs. controls: ^{***} $p < 0.001$. (MB + Zn) vs. MB: ⁺ $p < 0.05$; ⁺⁺⁺ $p < 0.001$

revealed that the concentration of malondialdehyde (MDA), the end product of lipid peroxidation, increased in the kidney of MB group. MDA has been accepted as a reliable parameter reflecting an enhanced ROS production, leading to oxidative injury to the body (Kim et al. 2012). Similarly, previous reports of Osman et al. (2011) on rats have shown an increased lipid peroxidation derived from bromuconazole exposure, a triazole fungicide. The enhanced MDA level, recorded in tissues of maneb-exposed animals, could be probably due to the excessive production of ROS caused by this fungicide leading to renal injury, as indicated in our recent study (Sefi et al.

2019) conducted in the liver. Likewise, in the present study, the generation of H₂O₂ was increased following treatment with maneb, reflecting a possible mitochondrial dysfunction induced by this fungicide. Elevated ROS production and mitochondrial dysfunction seemed to be key factors in maneb-induced toxicity, with potential secondary renal consequences. Previous studies of Todt et al. (2016) have shown that Mn/Zn ethylene-*bis*-dithiocarbamate fungicide resulted in mitochondrial dysfunction and excessive ROS production. Moreover, Jaballi et al. (2017) have demonstrated that maneb, administered to adult mice at four graded doses (1/8, 1/6, 1/4, and 1/2 of LD50), caused a significant increase in the kidney levels of MDA and H₂O₂. In the present work, the used dose of maneb (30 mg/kg bw), representing 1/50 of the fungicide LD50, was lower than those tested by Jaballi et al. (2017).

In addition to cell membrane lipids, proteins may also be altered by ROS production, especially at their labile thiol groups. Protein oxidation can be triggered directly by ROS or indirectly through the reactions with oxidative stress secondary-products (Dalle-Donne et al. 2003). In the present work, we have studied two biomarkers of oxidative protein damage, PCO and AOPP. Elevated levels of the above parameters, after maneb exposure, indicated the occurrence of protein oxidative modifications in the kidney of experimental mice. Co-administration of Zn to maneb-treated mice ameliorated MDA, H₂O₂, PCO, and AOPP levels, reflecting the antiperoxidative and free radical-scavenging properties of Zn, as has been shown previously by Goel et al. (2005). It has also been reported that this element interacts with cell membranes in order to stabilize them against various damaging effects, including those due to oxidative injuries (O'Dell 2000). Another mechanism that may also contribute to the antioxidant role of Zn is its ability to compete with transition metals like Fe and Cu, for the binding sites on the cell membrane. These two metals catalyze the production of lipid peroxides, and thereby replacement of these metals by Zn in the cell membrane could inhibit lipid peroxidation and oxidative stress condition (Barman and Srinivasan 2017). Consistent with these results, Bashandy et al. (2018) have recently reported in rats that zinc oxide nanoparticles exhibit renoprotective effects against thioacetamide, a potent hepatotoxic compound, by reducing lipid peroxidation.

Increased lipid peroxidation and oxidative stress can affect the activities of protective non-enzymatic antioxidants (Yonar and Sakin 2011). In particular, GSH and its metabolizing enzymes provide the major defense against ROS-induced cellular damage (Ozden and Alpertunga 2010). Cellular stress response is often known to involve changes in the content of thiols, which are firstly consumed during their reaction with the deleterious compounds, and then replaced through enzymatic reduction of disulfides, or by de novo biosynthesis. Two enzymes, namely glutamate-cysteine ligase (GCL, rate limiting) and glutathione synthase, are concertedly implicated in

Table 3 Creatinine, urea and uric acid levels in plasma of mice, controls or treated with maneb associated or no with Zn or with Zn alone

Parameters and treatments	Controls	MB	MB + Zn	Zn
Creatinine (μmol/l)	55.50 ± 1.87	83.83 ± 3.18 ^{***}	63.83 ± 2.63 ^{****+}	53.50 ± 3.39
Urea (mmol/l)	8.75 ± 0.66	20.98 ± 2.33 ^{***}	13.11 ± 1.33 ^{****+}	8.76 ± 0.43
BUN ^a (μmol/l)	4.08 ± 0.31	9.80 ± 1.09 ^{***}	6.12 ± 0.62 ^{****+}	4.09 ± 0.20
Uric acid (μmol/l)	221.83 ± 14.90	464.00 ± 7.48 ^{***}	346.83 ± 5.98 ^{****+}	221.50 ± 13.90

Values are means ± SD for six mice in each group

BUN blood urea nitrogen

^a BUN was calculated using the formula:

$$BUN (\mu\text{moles/l}) = [\text{urea} (\mu\text{moles/l})] / 2.14$$

MB; (MB + Zn) vs. controls: ^{***}*p* < 0.001

(MB + Zn) vs. MB: ⁺⁺⁺*p* < 0.001

GSH synthesis, which is both constitutive and regulated. According to Dickinson and Forman (2002), exposure to ROS-generating compounds can elevate GSH content by increasing its biosynthesis rate, as reported in our experiment after maneb exposure. Concerning ascorbic acid, the main water-soluble antioxidant vitamin able to scavenge free radicals (Molyneux et al. 2002), its increase after maneb exposure resulted probably from the re-activation of enzymatic and non-enzymatic mechanisms of vitamin C synthesis. Our results were in accordance with those of Spodniewska and Zasadowski (2008), who have found an increase of GSH

and ascorbic acid contents in the liver of rats treated for 7 days with pyrantel tartrate, an anthelmintic regimen. Moreover, a significant increase of metallothionein (MT) content was found in maneb-treated mice. In fact, the main characteristic of MT is its richness in cysteine residues (Dondero et al. 2005), which are responsible for the metal sequestration capacity of the protein. Thiol groups can also serve as scavengers of highly toxic compounds such as free radicals and reactive metabolites of xenobiotics. In our study, the stressful condition caused by the toxic effect of maneb can be prevented by an elevation of intracellular thiol groups like MT. It is proposed that one of the self-protecting mechanisms of the kidney against toxic compounds is the increase of the MT level and consequently the elevation of naturally occurring ROS scavengers in the cell. The significant improvement of renal non-enzymatic antioxidant (GSH, vitamin C, and MT) contents by Zn treatment supported its potential protective role against maneb-mediated renal injury.

In addition, other antioxidant enzymes, like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), must be considered in cellular stress condition. Such enzymes act in two steps as follows. First, SOD catalyzes the

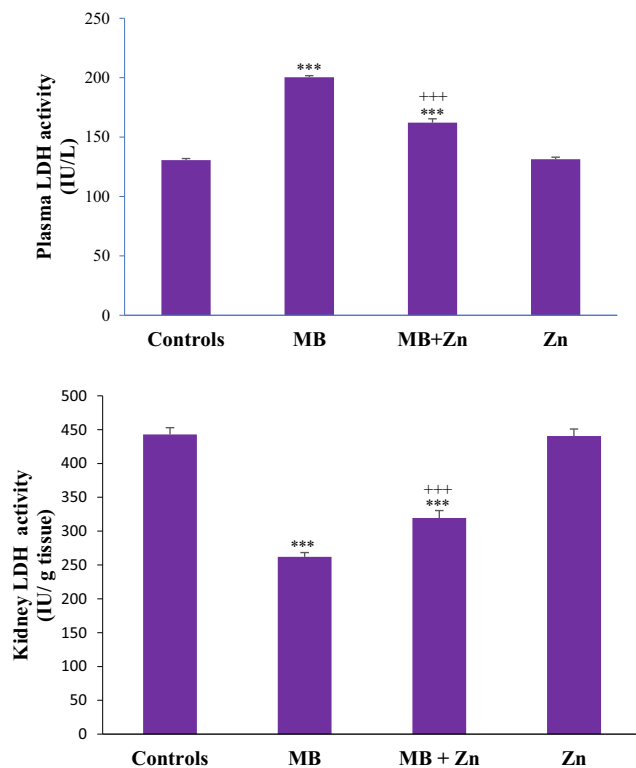


Fig. 2 Lactate dehydrogenase activities in plasma and kidney in mice controls and treated with maneb associated or no with Zn or with Zn alone. Values are means ± SD for six mice in each group. MB; (MB + Zn) vs. controls: ^{***}*p* < 0.001. (MB + Zn) vs. MB: ⁺⁺⁺*p* < 0.001

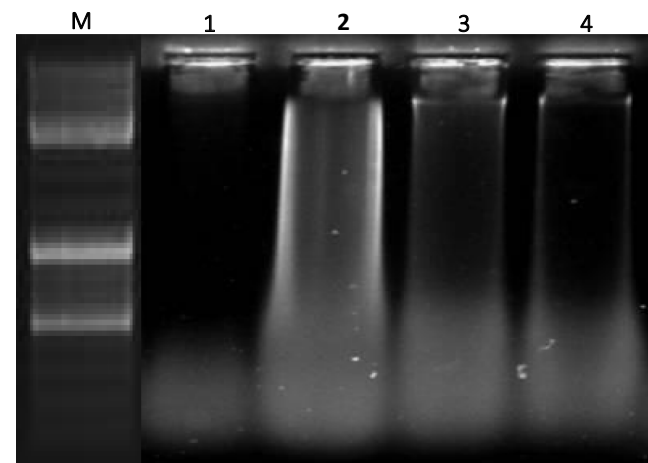
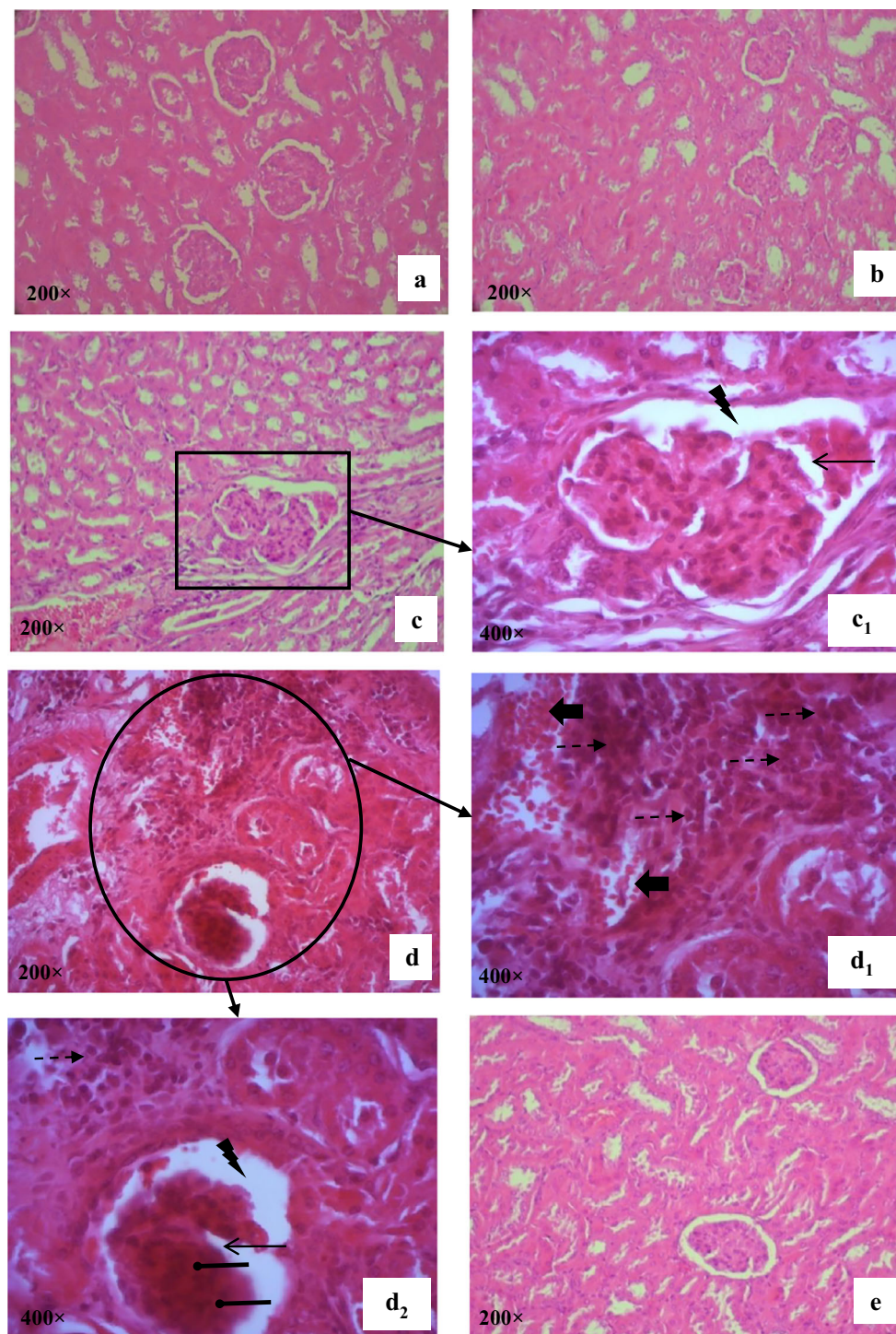


Fig. 3 Agarose gel electrophoresis of DNA in the kidney of adult mice. M marker, lane 1 controls, lane 2 MB, lane 3 MB + Zn, lane 4 Zn

Fig. 4 Kidney tissue histological aspects of adult mice; control (a), treated with zinc alone (b), maneb (c, c₁, d, d₁, d₂), and zinc along with maneb (e). Optic microscopy; H&E; magnification a, b, c, d, e: ($\times 200$) and c₁, d₁, d₂: ($\times 400$). Arrows indicate: \rightarrow Glomerular fragmentation; \blacktriangledown Bowman's space enlargement and hypertrophy; \bullet Glomerulus exhibiting a hypercellularity and an increased nuclear size; \blacksquare Hemorrhage; $---$ \rightarrow Infiltration of leukocytes



conversion of superoxide radicals into oxygen and hydrogen peroxide, which is next converted by CAT and GPx to water and oxygen (Sies 1993). In the current study, exposure to maneb decreased the activities of both CAT and GPx, and paradoxically induced a significant increase of SOD activity. Our results were in accordance with the recent study of Jaballi et al. (2017), who have reported that mice exposure to graded maneb doses (1/8, 1/6, 1/4, and 1/2 of LD50)

significantly decreases CAT and GPx activities and increases SOD in their renal tissue. In our study, Zn counteracted oxidative damages by modulating these enzymes' activities, suggesting that this bioelement could act as a potent ROS scavenger, making cells apt to preserve their integrity and function. These effects could be related to the direct and indirect antioxidant properties of Zn (Powell 2000). Indeed, this trace element is a structural component of the SOD enzyme

Table 4 Kidney injury scores in mice treated with maneb associated or no with Zn

Parameters and treatments	MB	MB + Zn
Glomeruli fragmentation	1.66 ± 0.51	0.66 ± 0.51 ⁺⁺⁺
Bowman's space enlargement	2.66 ± 0.51	0.33 ± 0.51 ⁺⁺⁺
Increased cellularity and nuclear size of glomeruli	2.83 ± 0.40	0.50 ± 0.54 ⁺⁺⁺
Leukocytes infiltration	3.83 ± 0.40	0.16 ± 0.41 ⁺⁺⁺
Hemorrhage	3.33 ± 0.51	0.33 ± 0.51 ⁺⁺⁺

Values are means ± SD for six mice in each group (MB + Zn) vs. MB: ⁺⁺⁺*p* < 0.001

involved in neutralizing free radicals' attacks through converting the superoxide anion radical (O_2^-) to hydrogen peroxide (H_2O_2) (Ling et al. 2016), which is in turn removed by CAT and GPx enzymes (Raat et al. 2009). Also, Zn is able to bind directly to sulfhydryl groups, allowing their protection from oxidation (Powell 2000). Thus, we provide evidence revealing that Zn could mitigate oxidative stress damages owing to its free radical scavenging potential. Our results were in line with those of Swelam et al. (2017) who have reported that Zn supplementation mitigates fipronil-induced oxidative damage by an improvement of GPx, CAT, and SOD activities in the liver and kidney of rats.

Furthermore, kidney impairment was assessed by measuring plasma creatinine, urea, and uric acid concentrations. The current increment in these biochemical parameters proved renal dysfunction in maneb-treated mice. Creatinine and urea are waste products deriving from protein metabolism and they require to be excreted through the kidney. Consequently, an obvious elevation of these compounds, as noticed in our study, was indicative of kidney functional damage, as reported earlier by Gowda et al. (2010). These findings concurs with a similar report in a recent study of Jaballi et al. (2017). Renal injury was also confirmed in the present work by LDH activity, which decreased in kidney and increased in plasma, speaking in favor of membrane damage. Co-administration of Zn to maneb-intoxicated mice afforded a significant protection against the fungicide-induced nephrotoxicity, due to its antioxidant properties.

Regarding DNA damage of kidney tissue triggered by maneb exposure, our study demonstrated a higher intensity of DNA laddering on agarose gel electrophoresis as compared to the controls, which might be attributed to oxidative stress induced by this fungicide. Our previous study confirmed the genotoxic effect of maneb in the liver (2019). According to Bertini et al. (2000), ethylene thiourea the main metabolite of maneb, has been proven to have genotoxic effects in Wistar rats. In the present study, Zn co-administration led to a reduced DNA fragmentation induced by maneb. Thereby, Zn could protect DNA and other important molecules from oxidation, improving consequently kidney function. In fact, it exhibited DNA protection, attenuated lipid peroxidation, and decreased free radicals production indicating its strong

antioxidant power. Our results were in accordance with other studies (Varghese et al. 2009), showing that Zn reduces the oxidative DNA damage in the kidney of rats exposed to indomethacin, a non-steroidal anti-inflammatory drug.

Overall, the biochemical and molecular changes, observed in the current work following maneb exposure, were confirmed by kidney histopathological findings. The structures of glomeruli as well as renal tubules were affected after maneb treatment. In fact, kidney of mice exposed to maneb showed glomerular hypertrophy and fragmentation (Fig. 4(c₁)); and an enlargement of glomeruli Bowman's space (Fig. 4(c₁ and d₂)). Such changes in glomerular structure have been observed by Chaâbane et al. (2017) as a result of penconazole fungicide treatment in rats. Moreover, kidney sections of maneb-treated mice showed also some abnormalities in the renal glomerular structure characterized by an hypercellularity and an increased nuclear size (Fig. 4(d₂)). These changes might occur as an outcome of direct glomerular cytotoxicity and oxidative stress at the glomerular level caused by maneb. The renal glomeruli are particularly sensitive to toxicity because they have a high oxygen consumption, a vulnerable enzymatic system, and a complicated transport mechanism that may be used for toxins transport and may be damaged by such toxins during their excretion (Chevalier 2016). Similar histopathological observations have been reported in kidney of rats treated with permethrin, a pyrethroid insecticide (Nessiem et al. 2003). Maneb administration also induced a marked leukocyte infiltration between the tubules and a large area of hemorrhage in the interstitium (Fig. 4(d₁)). Scoring of kidney histological changes revealed that leukocytes infiltration was the most prominent pathological feature associated with maneb exposure. Interestingly, the reduction of histopathological changes and the low injury scores in the kidney of mice co-treated with both maneb and Zn suggested the ameliorative effect of this essential mineral.

On the basis of this work, maneb administered to adult mice at a dose of 30 mg/kg bw caused a disruption of the redox status, increased MDA and protein oxidation products' levels, induced DNA fragmentation and histopathological changes in the kidney tissue. Maneb was considered as a pro-oxidant agent. It disturbed the oxidant-antioxidant balance and induced a depletion of renal antioxidant activities.

The co-administration of Zn to maneb-treated mice attenuated the toxicity of this fungicide in mice, as objectified by the biochemical and histological improvements. The mechanisms, which contributed to its effectiveness, involved ROS quenching, attenuation of lipid and protein oxidation, prevention of DNA damage, and improvement of the antioxidant status. Thus, Zn appeared to be a promising agent against maneb-induced renal dysfunction.

Conclusion

In summary, we have shown that exposure of mice to maneb resulted in an increased renal cytotoxicity mainly through inducing lipid peroxidation and ROS production. Zn administration alleviated the toxicity rate in the renal tissue of maneb-exposed mice. Understanding the mechanism of the protective role of Zn at the cellular level may provide a rationale for its use in antioxidant therapeutic strategies against nephrotoxicity in human beings exposed to different fungicides.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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