

ORIGINAL ARTICLE

Thymoquinone ameliorates lead-induced suppression of the antioxidant system in rat kidneys

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Objective: Alteration of the antioxidant status in the kidneys may be related to lead (Pb) intoxication. The present study aimed to investigate the possible beneficial effect of thymoquinone (TQ), the major active ingredient of the volatile oil of *Nigella sativa* seeds, on Pb-induced renal antioxidant defense system impairment.

Methods: A total of thirty two healthy adult male Wistar rats were randomly divided into four equal groups as follows: a control group, which received no treatment; a Pb group, which was exposed to 2,000 ppm of Pb acetate in drinking water; a Pb-TQ group, which was cotreated with Pb plus TQ (5 mg/kg/day, *per os*); and a TQ group receiving only TQ. All treatments were applied for five weeks.

Results: TQ alone did not induce any significant changes in the antioxidant defense system. By contrast, Pb exposure significantly decreased reduced glutathione level and superoxide dismutase, glutathione peroxidase, catalase, and glutathione reductase activities in the renal tissue. Interestingly, supplementation with TQ significantly improved the affected antioxidant parameters.

Conclusion: Our data are the first to provide evidence on the protective effect of TQ against Pb-induced renal antioxidant capacity impairment and suggest that this component might be a clinically promising alternative in Pb nephrotoxicity.

Keywords: *heavy metals; thymoquinone; antioxidant parameters; nephrotoxicity; rat*

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Lead (Pb) is a very toxic, non-essential heavy metal. It is present in storage batteries, water pipes, plastic, glass, ceramics, paints, insecticides, and leaded gasoline. Exposure to Pb is impossible to avoid because it is ubiquitous and persistent in the environment. Pb poisoning has been reported since the discovery of Pb thousands of years ago, and it remains a major health issue worldwide. Air, water, soil, food, and consumer products are the major routes of human exposure to Pb. The current approved treatment for Pb poisoning is to administer chelating agents that form an insoluble complex with the metal and remove it from tissue. Nevertheless, these chelating agents have no effect on low levels of exposure and are incapable of removing metal from intracellular sites; in addition, they cause many undesirable side effects (1).

After absorption, Pb accumulates primarily in the kidneys (2), making it a critical target organ for Pb toxicity. Accumulated Pb in kidneys impairs the endogenous antioxidant defense system by inhibiting the main antioxidant enzymes (superoxide dismutase [SOD], glutathione peroxidase [GPX], catalase [CAT], glutathione reductase [GR], and glutathione S-transferase [GST]). It also depletes reduced glutathione (GSH), the most important intracellular non-enzymatic antioxidant (3).

Medicinal plants nowadays are an important source of drug synthesis and at least a third of the current drugs are derived from plants (4). The seeds of *Nigella sativa* are known to have healing potential and have been used for more than 2,000 years as folk medicine in the Middle East and Far East for a wide range of diseases (5). They contain more than 30% fixed oil and 0.40–0.45% (w/w) volatile oil (6).

Thymoquinone (TQ) (2-isopropyl-5-methyl-1,4-benzoquinone), first extracted by El-Dakhakhny (7), is the main active and most abundant component (18.4–24%) of the essential oil of *N. sativa* seeds (8). It is structurally homologous with the coenzyme Q, which is an important antioxidant in the electron transport chain. The compound can be readily synthesized in gram quantities by oxidation of thymol with hydrogen peroxide (H₂O₂) (9). TQ oral administration is followed by slow absorption but relatively faster elimination (10). It could lead to biotransformation due to the metabolizing activity of liver enzymes such as DT-diaphorase (a quinone reductase), which catalyzes the reduction of TQ into a dihydrothymoquinone (11). This metabolite exhibits antioxidant properties stronger than those of TQ and similar to those of Trolox, which is considered a standard antioxidant compound (12). In rats, the LD₅₀ of TQ was 794.3 mg/kg and 57.5 mg/kg for oral and intraperitoneal administration, respectively (13). TQ has various pharmacological effects such as antihypertensive (14), anticancer (15), antidiabetic (16), anti-inflammatory (17), and analgesic properties (18). TQ is also reported to possess strong antioxidant properties (19). The high biological activity and low systemic toxicity of TQ make it a promising alternative to conventional therapeutic drugs (19).

The influence of TQ on Pb-induced nephrotoxicity has not previously been studied. Hence, the present study is the first to investigate the potential protective effect of TQ oral supplementation against Pb-induced renal antioxidant defense disruption.

Materials and methods

Chemicals

Pb acetate trihydrate ([C₂H₃O₂]₂Pb · 3H₂O) and TQ (2-isopropyl-5-methyl-1,4-benzoquinone) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals used were of the best analytical grade.

Animals

Healthy adult (4-month-old) male Wistar rats, weighing 200–230 g, obtained from the Tunisian Society of Pharmaceutical Industries, were used in this study. The animals were housed in plastic cages (free from any source of chemical contamination) with free access to tap water (free from Pb) and standard diet. The rats were kept at 22 ± 3°C, in a natural light/dark cycle, with 55% humidity and under a ventilation system. The experiments were started after the animals were allowed to adapt to the laboratory conditions for a week. All experimental procedures in this study were in full compliance with the European Council Directive (86/609/EEC) and approved by the institutional bioethics committee.

Experimental design

After the acclimation period, the rats were randomly divided into four groups of eight animals each and were treated for five weeks as follows: the control group received tap water; the Pb group received an aqueous solution containing 2,000 ppm of Pb acetate (0.2%, w/v) (20–22); the Pb-TQ group was cotreated with Pb (as in the Pb group) plus TQ (5 mg/kg bw/day); and the TQ group received tap water and was given TQ (5 mg/kg bw/day) (23–25). TQ was administered by gastric tube daily between 8:00 and 9:00 am. At the end of the treatment period, the animals were euthanized by exsanguination through cardiac puncture under diethyl ether anesthesia.

Tissue collection

The kidneys were removed quickly from the rats, cleared of adhesive tissue, washed in ice-cold 0.9% (w/v) NaCl solution, and frozen at –80°C until assayed.

Biochemical assays

The kidney tissue was homogenized in 10 volumes of ice-cold phosphate-buffered saline (136.75 mM NaCl, 2.68 mM KCl, 10.14 mM Na₂HPO₄, 1.76 mM KH₂PO₄, pH 7.4) and the homogenates were centrifuged at 3,500 × g for 15 min at 4°C. The supernatant fractions were collected and used in biochemical analysis.

SOD (26), GPX (27), and GR (28) activities were determined in the kidney homogenates using commercial kits (Randox Laboratories Ltd., Crumlin, UK). CAT activity was determined according to the ferrithiocyanate method of Cohen et al. (29). The GSH level was determined spectrophotometrically using the method previously described by Ellman (30).

Statistical analysis

The results were expressed as mean ± SEM. Comparisons between the groups were performed by Student's *t*-test. Differences were considered statistically significant at *p* < 0.05.

Results

Antioxidant enzyme activities

As shown in Fig. 1A through D, the kidney activities of SOD, GPX, CAT, and GR in the rats receiving TQ alone were not significantly different (*p* > 0.05) from those of the control group; following Pb treatment, the activities of these enzymes were significantly decreased (*p* < 0.05) by 56.11, 58.69, 64.7, and 54.78%, respectively. Interestingly, TQ coadministration significantly attenuated (*p* < 0.05) the deleterious effect of Pb on the activities of these antioxidant enzymes. In fact, in the rats cotreated with Pb and TQ, the renal activities of SOD, GPX, CAT, and GR significantly increased (*p* < 0.05) by 70.57, 78.85, 106.5, and 73.91%, respectively, in relation to the Pb-intoxicated rats.

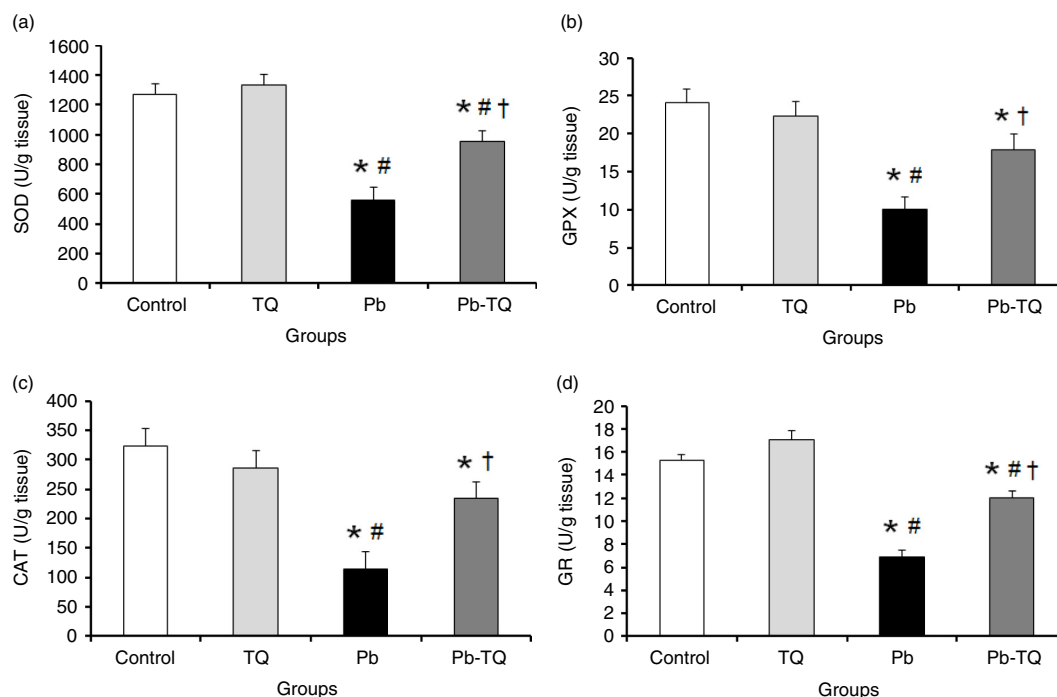


Fig. 1. Effects of lead (Pb), thymoquinone (TQ), and their coadministration on the kidney activities of superoxide dismutase (SOD, a), glutathione peroxidase (GPX, b), catalase (CAT, c), and glutathione reductase (GR, d) in rats after five weeks. Values are expressed as mean \pm SEM of eight animals. Student's *t*-test: * $p < 0.05$ versus control; # $p < 0.05$ versus TQ-treated rats; † $p < 0.05$ versus Pb-treated rats.

GSH levels

The results presented in Fig. 2 indicate that the administration of TQ alone had no significant effect ($p > 0.05$) on kidney GSH level compared to that of the control group. By contrast, Pb exposure caused a significant decrease ($p < 0.05$) of about 61.8% in the concentration of this non-enzymatic antioxidant in relation to the control rats. This effect was significantly mitigated ($p < 0.05$) by 55.16% when the Pb-treated animals simultaneously received TQ.

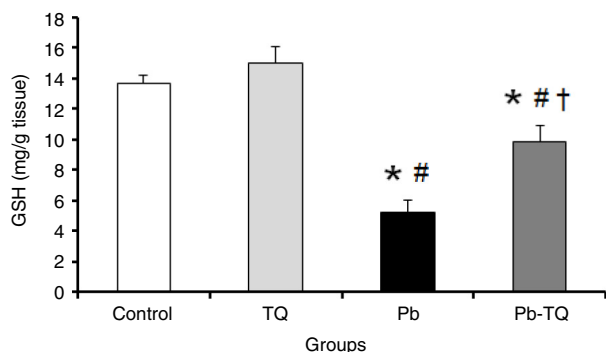


Fig. 2. Effects of lead (Pb), thymoquinone (TQ), and their coadministration on the kidney level of reduced glutathione (GSH) in rats after five weeks. Values are expressed as mean \pm SEM of eight animals. Student's *t*-test: * $p < 0.05$ versus control; # $p < 0.05$ versus TQ-treated rats; † $p < 0.05$ versus Pb-treated rats.

Discussion

Pb is a pervasive environmental and industrial pollutant with no beneficial biological role, and its toxicity continues to be a major public health problem throughout the world. Recent studies point to the potential involvement of the cell's antioxidant capacity failure in the pathogenesis of Pb poisoning, suggesting that exogenous antioxidants may play an effective protective effect. In the present study, we adopted an *in vivo* experimental animal model to investigate whether TQ could maintain renal intracellular antioxidant reserves in Pb subchronic treatment.

The metalloproteins SOD, GPX, and CAT are the major antioxidant enzymes. Their activities were used to assess antioxidant status in cells. SOD catalyzes the dismutation of superoxide anion radical (O_2^-) to H_2O_2 and O_2 . Because H_2O_2 is still harmful to cells, CAT and GPX further catalyze the decomposition of H_2O_2 to water. In the reaction catalyzed by GPX, GSH is converted into its oxidized form (GSSG), which can then be reduced back to GSH by GR. In the present study, we found that treatment with Pb for five weeks significantly decreased the activities of SOD, GPX, CAT, and GR in the rat kidney. These results are in concordance with previous findings (3, 31, 32).

It has been shown that Pb directly alters antioxidant activities by irreversible direct binding to functional sulfhydryl (SH) groups of several enzymes such as SOD, GPX, CAT, and GR (33). Because Pb interferes with the

metabolism of essential trace elements such as copper, zinc, selenium, and iron needed for proper molecular structure and enzymatic activity (2), the antioxidant enzymes could be a potential target for Pb toxicity. The decrease in antioxidant enzyme activities may be explained by the downregulation of antioxidant enzyme mRNA expression (34).

GSH is a tripeptide-containing cysteine that has a reactive SH group with reductive potency. Accordingly, GSH plays a vital role in the protection of cells against oxidative stress. It can act as a non-enzymatic antioxidant by direct interaction of SH groups with reactive oxygen species, or it can be involved in the enzymatic detoxification reactions for reactive oxygen species, as a cofactor or a coenzyme. In agreement with recent investigations studying the effect of Pb in the kidneys of rats and mice (35, 36), our data show that Pb treatment significantly lowered the renal GSH level.

As for antioxidant enzymes, Pb can damage GSH directly and/or indirectly. The reduction in concentration of GSH may be due to the high affinity of Pb to the SH groups of this tripeptide, thereby interfering with its antioxidant activity (33). Pb can also decrease the level of GSH by inhibiting the activities of GSH metabolizing enzymes, such as GR, GST, and glucose-6-phosphate dehydrogenase, by blocking their SH groups (37). Further, the reduction of GSH synthesis can be proposed as another explanation.

Despite extensive research now focusing on herbal products as alternative medicines, no evidence has been reported in the literature regarding the role of TQ against Pb-induced renal toxicity. In the present study, cotreatment of Pb-exposed rats with TQ significantly improved the altered antioxidant defense system in the kidneys.

Our results are in consonance with recent literature data indicating that oral supplementation of TQ (10 mg/kg/day, 15 days) protects rat kidneys against sodium arsenite-induced depletion of antioxidant enzyme activities (SOD, GPX, and CAT) (38). Furthermore, Farag et al. (39) reported that TQ (10 mg/kg/day, 28 days) prevented reduction in kidney SOD activity and GSH level provoked by chronic treatment with cyclosporine A, an immunosuppressant drug, and by acute renal ischemia/reperfusion in rats. In addition, Samarghandian et al. (40) reported that TQ enhances the declined renal antioxidant status in gentamicin-treated rats. TQ (10 mg/kg/day, 10 days, *per os*) also reversed a renal decrease in CAT activity and GSH concentration in rats receiving methotrexate, an anticancer drug (41).

The restoration of tissue antioxidant function by TQ clearly demonstrated in the current work could be attributed to its ability to upregulate antioxidant gene expression (42, 43).

The ability of TQ to correct the disrupted antioxidant system, as demonstrated in the present research, does not precisely mean that kidney oxidative stress can be

decreased. Numerous previous data showed that this component has powerful free radical scavenging activity (12, 14, 44–46), which may be related to the redox properties of the quinone structure of TQ molecule and to its unrestricted crossing of morphophysiological barriers to access subcellular compartments (46). The effect of TQ on endogenous antioxidants has been relatively poorly studied. Thus, in the current work we are limited to some antioxidant markers.

In conclusion, our results clearly indicate that TQ oral supplementation, at a safe dose, protects against Pb-induced cellular antioxidant defense system depletion in rat kidneys. Our findings suggest that TQ may be a clinically promising agent in Pb nephrotoxicity.

Authors' contributions

AM and HBC collected the data, and AM also analyzed the data, designed the study, and wrote the paper.

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Conflict of interest and funding

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