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PROTECTIVE EFFECT OF THYMOQUINONE AGAINST LEAD-INDUCED ANTIOXIDANT DEFENSE SYSTEM ALTERATION IN RAT LIVER

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Alteration of the antioxidant system may be related to lead (Pb) hepatotoxicity. This study was carried out to investigate the possible beneficial effect of thymoquinone (TQ), the major active ingredient of volatile oil of *Nigella sativa* seeds, against Pb-induced liver antioxidant defense system impairment. Adult male rats were randomized into four groups: control group received no treatment, Pb group was exposed to 2000 ppm of Pb acetate in drinking water, Pb-TQ group was cotreated with Pb plus TQ (5 mg/kg/day, *per os*) and TQ group receiving only TQ. All treatments were applied for five weeks. TQ alone did not induce any significant changes in the enzymatic and non-enzymatic antioxidant status. By contrast, Pb exposure significantly decreased not only reduced glutathione level, but also superoxide dismutase, glutathione peroxidase, catalase and glutathione reductase activities in the liver tissue. Interestingly, when coadministrated with Pb, TQ significantly improved the affected antioxidant parameters. In conclusion, our results indicate a protective effect of TQ against Pb-induced liver antioxidant capacity impairment and suggest that this component might be a clinically promising alternative in Pb hepatotoxicity.

Keywords: Lead – thymoquinone – antioxidant parameters – liver – rat

INTRODUCTION

Lead (Pb) is a very toxic, non-essential heavy metal. Exposure to Pb is impossible to avoid because it is ubiquitous and persistent in the environment. After absorption, Pb accumulates in various somatic soft tissues, primarily the liver [18], making it a critical target organ for Pb toxicity. Accumulated Pb in liver impairs the endogenous antioxidant defense system not only by inhibiting the activities of antioxidant enzymes, including superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), glutathione reductase (GR), and glutathione S-transferase (GST), but also by depleting the non-enzymatic antioxidants, such as reduced glutathione (GSH) [8].

Thymoquinone (TQ) (2-isopropyl-5-methyl-1,4-benzoquinone), the main active component of the essential oil of *Nigella sativa* seeds, has various pharmacological

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effects, such as anti-hypertensive, anti-cancer, anti-diabetic and anti-inflammatory properties [7]. TQ is also reported to possess strong antioxidant properties [7]. The high biological activity and low systemic toxicity of TQ make it a promising alternative to conventional therapeutic drugs [7].

The present study was designed to investigate the potential beneficial impact of TQ oral supplementation on Pb-induced liver antioxidant defense disruption in rats.

MATERIALS AND METHODS

Chemicals

Pb acetate trihydrate [$(C_2H_3O_2)_2Pb \cdot 3H_2O$] and TQ (2-isopropyl-5-methyl-1,4-benzoquinone) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, Missouri, 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 natural light/dark cycle, with 55% humidity and under a ventilation system. 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 consisting of eight animals each and were treated for five weeks as follows: control group received tap water, Pb group received an aqueous solution containing 2000 ppm of Pb acetate (0.2%, *w/v*) [5], Pb-TQ group was cotreated with Pb (as in the Pb group) plus TQ (5 mg/kg body weight/day) and TQ group received tap water and was given TQ (5 mg/kg body weight/day) [10]. TQ was administered, as aqueous solution, by gastric tube daily between 8:00 and 9:00 a.m. At the end of the treatment period, the animals were euthanized by exsanguination through cardiac puncture under diethyl ether anesthesia.

Tissue collection

The liver was removed quickly from rats, cleared of the adhesive tissues, rinsed in ice-cold physiological saline (0.9% NaCl solution), and frozen at –80 °C until antioxidant parameters evaluation.

Biochemical assays

Liver tissue was homogenized in 10 volumes of ice-cold phosphate buffered saline (136.75 mM NaCl, 2.68 mM KCl, 10.14 mM Na_2HPO_4, 1.76 mM KH_2PO_4, pH 7.4) and the homogenates were centrifuged at 3500 rpm for 15 min at 4 °C. The supernatant fractions were collected and used in biochemical analysis.

SOD [3], GPX [20] and GR [13] activities were determined as done earlier, respectively, by using RANSOD kit, RANSEL kit and GLUT RED kit (Randox laboratories Ltd., Crumlin, UK). CAT activity was determined according to the ferrithiocyanate method of Cohen et al. [6]. The antioxidant enzyme activities were expressed as units/g of wet liver tissue. GSH level was determined spectrophotometrically by the method previously described by Ellman [9] and expressed as mg/g of wet liver tissue.

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–D, the liver activities of SOD, GPX, CAT and GR in rats receiving TQ alone were not significantly different ($p > 0.05$) from those of control group, while following Pb treatment, the activities of these enzymes were significantly decreased ($p < 0.05$) by 39.96%, 39.45%, 41.79% and 44.49%, respectively. Interestingly, TQ coadministration significantly prevented the deleterious effect of Pb on the activities of these antioxidant enzymes. In fact, in rats cotreated with Pb and TQ, liver activities of SOD, GPX, CAT and GR significantly increased ($p < 0.05$) by 47.87%, 51.13%, 57.55% and 32.76%, respectively, in relation to Pb-intoxicated rats.

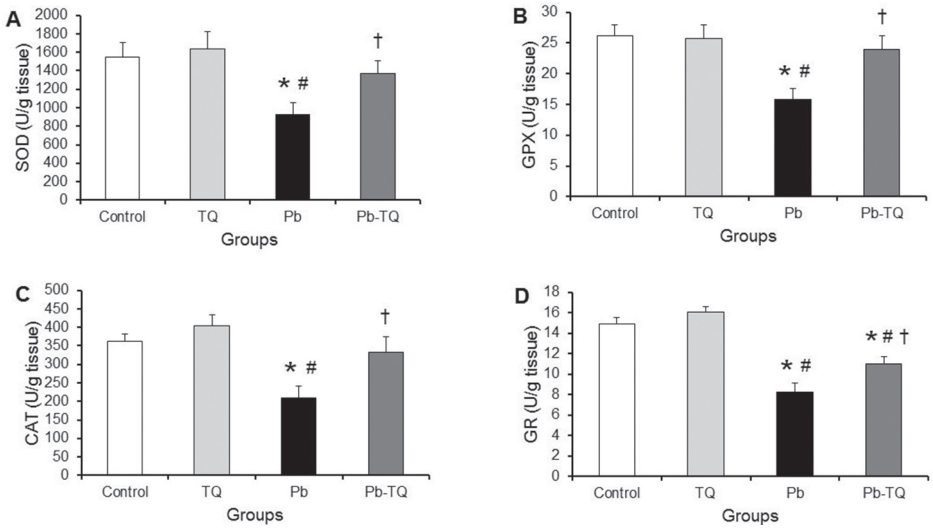

Fig. 1. Effects of lead (Pb), thymoquinone (TQ) and their coadministration on the liver activities of superoxide dismutase (SOD, A), glutathione peroxidase (GPX, B), catalase (CAT, C) and glutathione reductase (GR, D) in rats after five weeks exposure. Values are expressed as mean ± SEM ($n = 8$). Student's *t*-test: $^{*}p < 0.05$ vs control; $^{\#}p < 0.05$ vs TQ-treated rats; $^{\dagger}p < 0.05$ vs Pb-treated rats

GSH level

Results presented in Fig. 2 indicated that the administration of TQ alone had no significant effect ($p > 0.05$) on liver GSH level compared to that of the control rats. In contrast, Pb exposure caused a significant decrease ($p < 0.05$) of about 48% in the

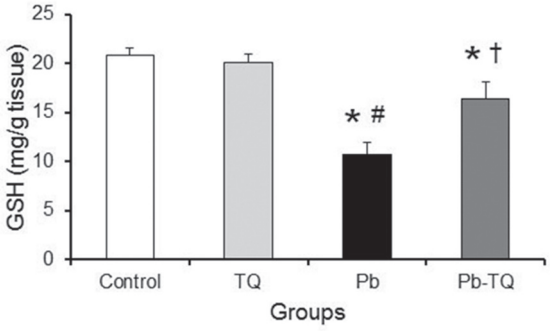

Fig. 2. Effects of lead (Pb), thymoquinone (TQ) and their coadministration on the liver level of reduced glutathione (GSH) in rats after five weeks exposure. Values are expressed as mean ± SEM ($n = 8$). Student's *t*-test: $^{*}p < 0.05$ vs control; $^{\#}p < 0.05$ vs TQ-treated rats; $^{\dagger}p < 0.05$ vs Pb-treated rats

concentration of this non-enzymatic antioxidant in relation to control animals. This adverse effect was significantly attenuated ($p < 0.05$) by 56.15% when metal-treated rats received simultaneously TQ.

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 cellular 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 liver intracellular antioxidant reserves in Pb subchronic treatment.

The metalloproteins SOD, GPX and CAT are the major antioxidant enzymes. SOD catalyzes the dismutation of superoxide anion radical ($O_2^{\bullet -}$) to hydrogen peroxide (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 liver. These results are in concordance with previous findings [8, 19].

It has been shown that Pb alters antioxidant activities by irreversible binding to functional sulfhydryl (SH) groups of several enzymes such as SOD, GPX, CAT and GR [17]. 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, the antioxidant enzymes could be a potential target for Pb toxicity [4]. The decrease in antioxidant enzyme activities may also be explained by the down-regulation of antioxidant enzyme mRNA expression [16].

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 the SH groups with reactive oxygen species (ROS), or it can be involved in the enzymatic detoxification reactions for ROS, as a cofactor or a coenzyme. In agreement with recent investigations studying the effect of Pb on rat and mice liver [15, 23], our data show that Pb treatment significantly lowered the hepatic GSH level.

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 [17]. 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 [22]. Further, the reduction of GSH synthesis can be proposed as another explanation.

In the present study, co-treatment of Pb-exposed rats with TQ significantly improved the altered antioxidant defense system in liver. Our results are in consonance with recent literature data indicating that oral supplementation of TQ (10 mg/rat/day, 30 days) protects rat liver against atherogenic suspension-induced GSH depletion and antioxidant enzyme activities reduction (SOD, GPX, CAT, GR and GST) [2]. Furthermore, Abdel-Wahab [1] reported, under the TQ effect (10 mg/kg/day, 5 weeks, *per os*), a total correction of the reduced GSH concentration and the depleted antioxidant enzyme activities (SOD, GPX, CAT and GST) in sodium fluoride-treated rat's liver. In addition, Farag et al. [12] reported that TQ (10 mg/kg/day, 28 days, *per os*) prevented reduction in liver SOD activity and GSH level provoked by subchronic treatment with cyclosporine A in rats. TQ (10 mg/kg/day, 10 days, *per os*) also reversed a hepatic decrease in CAT activity and GSH concentration in rats receiving methotrexate [11].

The restoration of liver tissue antioxidant function by TQ clearly demonstrated in the current work could be attributed to its ability to upregulate antioxidant gene expression [14, 21].

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 liver. Our findings suggest that TQ may be a clinically promising agent in Pb hepatotoxicity healing.

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