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Thymoquinone Improved Lead-Induced DNA Damage and Oxidative Stress in Rat Brain

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ABSTRACT

Lead (Pb) is a pervasive industrial and environmental pollutant that seriously impairs the central nervous system, primarily by disrupting the redox balance. The current research was conducted to explore the possible beneficial action of thymoquinone (TQ), the main active component in *Nigella sativa* seed volatile oil, against brain oxidative stress and DNA damage caused by Pb. Wistar adult male rats were treated with TQ (5 mg/kg/day, *per os*) and/or Pb (2000 ppm of Pb acetate in drinking water) for five weeks. Results showed that Pb exposure significantly increased metal content, malondialdehyde concentration and DNA damage (assessed by comet assay), but significantly decreased the level of reduced glutathione and the activities of catalase, glutathione peroxidase, and superoxide dismutase in the brain tissue. These detrimental effects Pb-induced, except tissue metal accumulation, were significantly improved by TQ supplementation. In conclusion, our findings suggested that TQ might be a promising therapeutic alternative in Pb neurotoxicity.

1. INTRODUCTION

Lead (Pb) is an omnipresent heavy metal that affects virtually all bodily systems, primarily the central nervous system (Sanders et al., 2009). Within the brain, the damage induced by Pb in the cerebellum, hippocampus, and prefrontal cerebral cortex can lead to various neurological disorders, such as nerve damage, behavioral problems, mental retardation, and possibly schizophrenia, Parkinson and Alzheimer diseases (Liu et al., 2013). The main molecular mechanism proposed in Pb neurotoxicity is oxidative stress, a disturbance in antioxidant/prooxidant balance (Liu et al., 2014).

The cellular antioxidant defense arsenal includes enzymatic and non-enzymatic components, primarily superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) and reduced glutathione (GSH). Under oxidative stress, excess reactive oxygen species (ROS) oxidatively attacks the various critical biomolecules like membrane lipids, nucleic acids and proteins, thus causing cellular, tissue, organ and system damage (Matović et al., 2015). Lipid peroxidation (LPO), the major consequence of oxidative stress, consists of an alteration of the cellular membrane phospholipids polyunsaturated fatty acids (Matović et al., 2015).

Pb is known as a genotoxic agent; it induces chromosomal aberrations, sister chromatid exchanges, micronuclei formation, DNA-DNA and DNA-protein cross-links, and DNA single- and double-strand breaks (García-Lestón et al., 2010).

Medicinal plants are nowadays very important in the pharmaceutical industry field, as they contribute to the production of at least a third of the current drugs (Bent, 2008). Thymoquinone (TQ) (2-isopropyl-5-methyl-1,4-benzoquinone), the major active ingredient in *Nigella sativa* seed essential oil, has several medicinal benefits, such as anti-cancer (El-Far, 2015), anti-inflammatory (Taka et al., 2015), anti-hypertensive (Azzubaidi et al., 2015), anti-diabetic (El-Ameen et al., 2015) and analgesic effects (Amin and Hosseinzadeh, 2016). The strong antioxidant ability is also an interesting property of TQ (Darakhshan et al., 2015). TQ can be used as an effective therapeutic alternative since its systemic toxicity is low and its biological activity is high (Darakhshan et al., 2015).

Since Pb damages the brain through oxidative stress, the use of antioxidants will be effective in the

case of Pb cerebral hazards. Few research have been devoted to the beneficial effect of TQ against the brain toxicity of Pb (Radad et al., 2014). Thus, the current study aimed to evaluate the possible impact of TQ on Pb brain damage in rats using tissue metal accumulation, oxidative stress markers and DNA strand breaks.

2. MATERIALS AND METHODS

2.1. Chemicals

TQ (2-isopropyl-5-methyl-1,4-benzoquinone) and Pb acetate trihydrate [$(C_2H_3O_2)_2Pb.\ 3H_2O$] were obtained from Sigma-Aldrich Chemical Co. (St. Louis, Missouri, USA). The other chemicals utilized were of the highest quality.

2.2. Animals

The thirty-two Wistar male adult (4-months-old) healthy rats (200-230 g) used in this study were purchased from the Tunisian Society of Pharmaceutical Industries (SIPHAT). Animals housed in plastic cages (not chemically contaminated) were allowed access to standard diet and water *ad libitum*, and were maintained under ventilation system, with 55% humidity, in natural light/dark cycle, and at 22 ± 3°C. The present research was carried out according to the European regulation (Directive 86/609/EEC) for the laboratory animal's use and care, and with the approval of the Institutional Bioethics Committee.

2.3. Experimental design

After a week of acclimatization, animals were randomly divided into four equal groups and were treated for five weeks as follows: control group receiving non treatment, Pb group received 2000 ppm of lead acetate in the drinking water (Çaylak and Halifeoğlu, 2007; Çaylak et al., 2007; Lalith Kumar and Muralidhara, 2014), Pb-TQ group receiving both Pb and TQ (5 mg/kg body weight/day) (Al-Majed et al., 2006; El-Sayed, 2011; Kurt et al., 2015) and TQ group treated with TQ (5 mg/kg body weight/day) only. TQ was given as aqueous solution by oral gavage in the morning at the same time. After experimental period, the rats were anesthetized with diethyl ether and then exsanguinated through intracardiac puncture.

2.4. Tissue collection

The rat brain was dissected out, rinsed in ice-cold saline, divided into three parts, and was maintained at -80°C until analyzed.

2.5. Pb analysis

Brain Pb levels were determined by atomic absorption spectrophotometry (Analytik Jena - novAA® 400 P AAS) at the National Institute of Research and Physical and Chemical Analysis - (INRAP) (BiotechPole Sidi Thabet, Ariana, Tunisia). Pb concentration values were given as ppm (µg/g of wet tissue).

2.6. Oxidative stress parameters determination

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) was used to homogenize the brain tissue (10%, *w/v*). The supernatants obtained after centrifugation (3500 × g, 4°C, 15 min) of the homogenates were used for oxidative stress evaluation.

The determination of brain SOD and GPX activities were performed according to the methods of Arthur and Boyne (1985) and Paglia and Valentine (1967), respectively, using commercial kits (Randox laboratories Ltd., Crumlin, UK). The activity of CAT was measured from ferrithiocyanate production (Cohen et al., 1996). These three activities were given as units/g of wet tissue. GSH content was assayed according to the spectrophotometric procedure of Ellman (1959) and was expressed as mg/g of wet tissue. The measurement of malondialdehyde (MDA), a product of LPO, was carried out according to the spectrophotometric method of Todorova et al. (2005) which is based on that of Placer et al. (1966). MDA was given as nmol/g of wet tissue.

2.7. DNA damage analysis

DNA strand breaks in brain samples were evaluated according to the Singh alkaline comet assay (Singh et al., 1988). Comet image observation was performed with fluorescence microscopy (Zeiss Axiolab). For each group, 100 randomly selected cells were acquired using Axiovision 3.1 software. The DNA damage was measured as % Tail DNA (percentage of comet tail genomic DNA) thinks to TriTek CometScore Freeware 1.6.1.13, a comet scoring software.

2.8. Statistical analysis

All data were presented as mean ± SEM. All data were evaluated by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison *post-hoc* test. $P < 0.05$ considered the differences to be statistically significant.

3. RESULTS

3.1. Pb concentration

Treatment with metal for five weeks significantly increased ($P < 0.05$) the brain Pb level compared to control group. Pb tissue accumulation was not significantly changed ($P > 0.05$) after TQ supplementation (Fig. 1).

3.2. Antioxidant enzyme activities

SOD, GPX, and CAT brain activities were significantly similar ($P > 0.05$) in the control and TQ groups (Fig. 2). In Pb-treated rats, the enzyme activities were significantly decreased ($P < 0.05$). Interestingly, TQ supplementation significantly reduced ($P < 0.05$) these metal adverse effects.

3.3. GSH content

Animals receiving TQ alone had a brain GSH concentration similar ($P > 0.05$) to that of control group (Fig. 3). In contrast, the level of this non-enzymatic antioxidant was significantly reduced ($P < 0.05$) with Pb poisoning, while administration of TQ to Pb-treated rats totally reversed ($P < 0.05$) the harmful effect of this metal.

3.4. LPO level

TQ alone had no significant effect ($P > 0.05$) on MDA brain concentration, while Pb significantly increased ($P < 0.05$) it, compared to the control rats. TQ supplementation to metal-treated animals perfectly attenuated ($P < 0.05$) the elevated brain MDA content (Fig. 4).

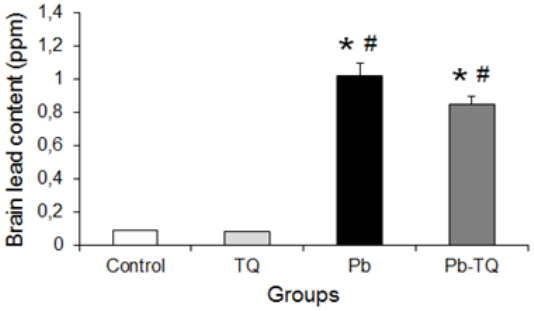

Fig. 1. Thymoquinone (TQ) had no effect on brain lead (Pb) level in rats for five-week treatment period. Each bar represents the mean ± SEM of eight rats. *, $P < 0.05$ *vs.* control; #, $P < 0.05$ *vs.* TQ (One-way ANOVA and Tukey's *post-hoc* test)

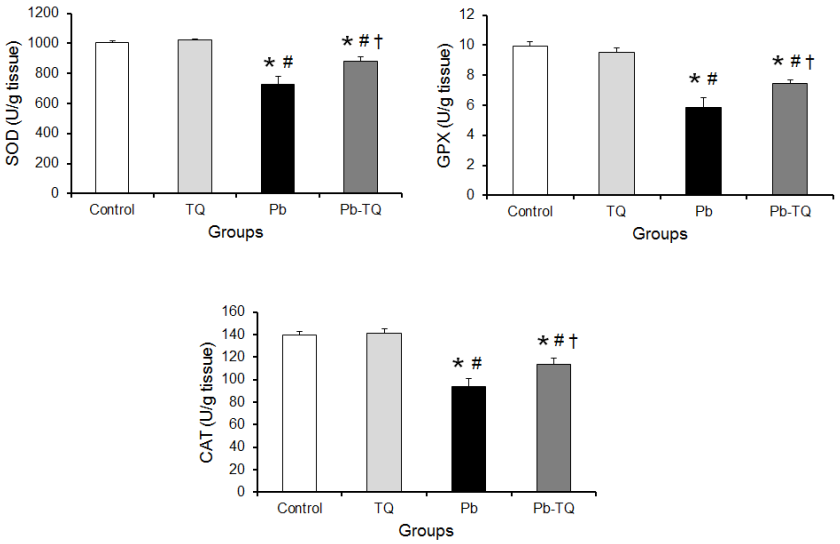

Fig. 2. Thymoquinone (TQ) protected against Pb-induced superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT) brain activities alteration in rats for five-week treatment period. Each bar represents the mean ± SEM of eight rats. *, $P < 0.05$ *vs.* control; #, $P < 0.05$ *vs.* TQ; †, $P < 0.05$ *vs.* Pb. (One-way ANOVA and Tukey's *post-hoc* test)

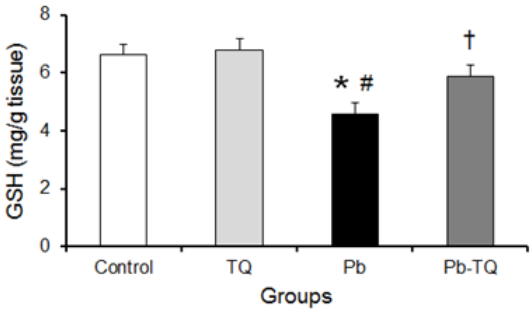

Fig. 3. Thymoquinone (TQ) protected against lead (Pb)-induced brain reduced glutathione (GSH) depletion in rats for five-week treatment period. Each bar represents the mean ± SEM of eight rats. *, $P < 0.05$ *vs.* control; #, $P < 0.05$ *vs.* TQ; †, $P < 0.05$ *vs.* Pb (One-way ANOVA and Tukey's *post-hoc* test)

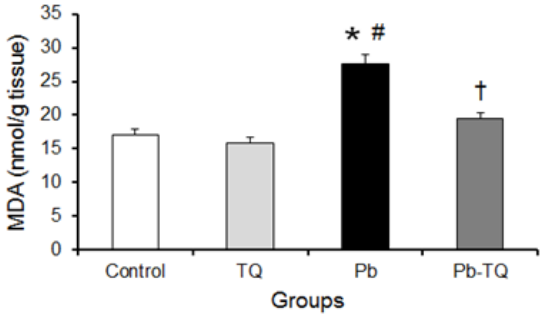

Fig. 4. Thymoquinone (TQ) protected against lead (Pb)-induced malondialdehyde (MDA) brain overproduction in rats for five-week treatment period. Each bar represents the mean ± SEM of eight rats. *, $P < 0.05$ *vs.* control; #, $P < 0.05$ *vs.* TQ; †, $P < 0.05$ *vs.* Pb (One-way ANOVA and Tukey's *post-hoc* test)

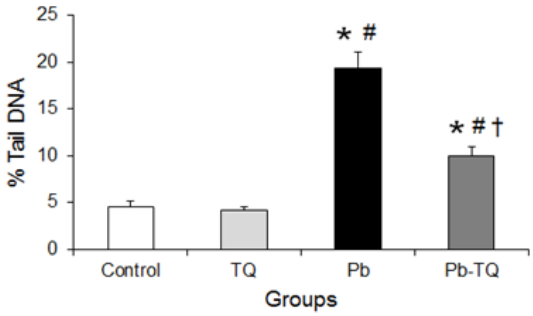

Fig. 5. Thymoquinone (TQ) protected against lead (Pb)-induced brain DNA damage (as % tail DNA) in rats for five-week treatment period. Each bar represents the mean ± SEM of eight rats. *, $P < 0.05$ *vs.* control; #, $P < 0.05$ *vs.* TQ; †, $P < 0.05$ *vs.* Pb (One-way ANOVA and Tukey's *post-hoc* test)

3.5. DNA damage

Comet assay indicated that TQ administration did not alter brain DNA expressed in % tail DNA ($P > 0.05$), while it was significantly increased ($P < 0.05$) after Pb exposure, in comparison with control rats (Fig. 5). TQ supplementation significantly reduced ($P < 0.05$) the metal-induced DNA damage.

4. DISCUSSION

Subchronic treatment with Pb resulted in it significant brain metal deposition. The same outcome in rat's brain under Pb acetate intoxication was reported by Agrawal et al. (2015) and Hernández-Plata et al. (2015). In fact, Pb, thinks to its calcium ion substitute ability, can cross the blood brain barrier (Sanders et al., 2009). The privileged metal distribution can also be explained by the brain presence of Pb-binding non-enzyme proteins of high-affinity like metallothioneins, acyl-CoA binding protein, thymosin β4, and calcium-sensing receptor (Handlogten et al., 2000; ATSDR, 2005).

Our investigations are in line with those of Dewanjee et al. (2013), Kalender et al. (2014), Ashafaq et al. (2016) and Wang et al. (2016) and indicated that brain SOD, GPX, CAT and GSH levels were significantly depleted in Pb-exposed rats compared with control group. The enzymatic and non-enzymatic antioxidant alterations in brain can be attributed to the inactivation of their functional sulfhydryl groups by irreversible binding to Pb or by oxidation through ROS Pb-overproduced (Valko et al., 2005, Matović et al., 2015). Also it can be related to the interference of Pb with cerebral metabolism of essential trace elements needed for antioxidant enzyme activity and molecular structure, downregulation of brain antioxidant enzyme mRNA expression (Baranowska-Bosiacka et al., 2012) and to the inhibition of the activity of enzymes influencing the GSH concentration especially glutathione reductase and glucose-6-phosphate dehydrogenase (Sivaprasad et al., 2004).

According to the present study and those of Kalender et al. (2014) and Abdulmajeed et al. (2016), Pb treatment significantly stimulated rat brain LPO as shown by the increase in the MDA concentration.

The comet assay is a highly sensitive method for single- and double-strand DNA breakage detection (Lee and Steinert, 2003). Along with this, Pb treatment markedly increased the % tail DNA in

rat brain. Valverde et al. (2002) and Youbin et al. (2013) reported similar results in Pb-intoxicated mice.

Pb-induced brain LPO and DNA damage are most probably due to excess generation of ROS as a consequence of the cerebral endogenous antioxidant defense system depletion as previously shown. In this respect, a highly ROS production increase has been reported in brain of Pb orally treated rats (Pachauri et al., 2012; Liu et al., 2014).

Despite the current numerous studies looking at herbal products as an alternative medicine, our results demonstrated for the first time that TQ protected effectively against Pb-induced brain damage by improving the altered antioxidant defense system and preventing the LPO and the DNA strand breakage in brain. Our findings are in consonance with those of previous investigations showing the TQ effectiveness against free radical generating agents-induced brain oxidative stress (Abdel-Zaher et al., 2013; Sedaghat et al., 2014; Gülşen et al., 2016) and leukocyte genotoxicity (Naga et al., 2013; Al-Shdefat et al., 2014; Fouda et al., 2014).

TQ has been shown to diminish oxidative stress by strong free radical scavenging action (Kruk et al., 2000; Mansour et al., 2002; Badary et al., 2003; Khalife and Lupidi, 2007; Khattab and Nagi, 2007) and by antioxidant enzyme gene expression upregulation (Ismail et al., 2010; Sayed-Ahmed et al., 2010; El-sayed, 2011). The considerable potential of ROS neutralization may be explained by the redox properties of the quinone structure of TQ molecule and by its unrestricted crossing of morphophysiological barriers to access to subcellular compartments (Badary et al., 2003).

5. CONCLUSION

For the first time, the current findings indicated that TQ protected against subchronic Pb-induced DNA damage and oxidative stress in rat brain, without affecting tissue metal content, and open new perspectives for the clinical use of this component in Pb neurotoxicity. However, subsequent experiments are required to explore the impact of TQ in combination with chelating agents in Pb brain toxicity.

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INTEREST CONFLICT

The authors declare that they have no conflict of interest.

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