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Thymoquinone Improves Lead-Induced Hematotoxicity in Rats

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ABSTRACT

The protective role of thymoquinone (TQ), the major active ingredient of volatile oil of *Nigella sativa* seeds, against the deterioration of blood indices by lead (Pb) has never been studied. Therefore, the present study was carried out to evaluate the possible beneficial effect of TQ against Pb-induced hematological changes. Adult male Wistar rats were treated with Pb (2000 ppm of Pb acetate in drinking water) and/or TQ (5 mg/kg/day, *per os*) for five weeks. Results obtained clearly showed that Pb intoxication significantly decreased the mean red blood cells, hemoglobin, hematocrit and platelets values, but significantly increased the white blood cells count. Interestingly, co-administration of TQ to the metal-treated animals corrected all the altered hematological parameters except platelets level. In conclusion, TQ can be considered for the first time as a promising therapeutic agent against Pb-induced hematotoxicity.

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INTRODUCTION

Pollution of the environment with toxic metals has increased dramatically since the beginning of the industrial revolution (Kelley, 1999). Lead (Pb) is a dangerous heavy metal which is ubiquitous in the environment (Gani *et al*., 2017). The primary route of Pb exposure in the general human population is ingestion of contaminated food and drinking water (Sharma *et al*., 2014). The International Programme on Chemical Safety of the World Health Organization has identified Pb as one of the ten chemicals of major public health concern (WHO, 2021).

One of the most sensitive targets for Pb toxicity is hematopoietic system (Carocci *et al*., 2016). Pb affects the hematopoietic system mainly by inhibiting the heme biosynthesis pathway ultimately leading to anemia (Singh *et al*., 2018). Pb has multiple hematotoxicological effects (Sharma *et al*., 2014). The adverse effects of Pb on erythrocytes have in particular been intensely analyzed because they have a high affinity for this metal and are more vulnerable to oxidative damage than many other cells (Leggett, 1993). Pb-induced oxidative stress or disruption of prooxidant/antioxidant balance in blood has been postulated to be the major mechanism of Pb associated hematotoxicity (Flora *et al*., 2003).

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Nigella sativa Linn. (black seed or black cumin) is an annual herbaceous plant in the family Ranunculaceae, native to southern Europe, north Africa and southwest Asia (Ziaee *et al*., 2012). The use of *Nigella sativa* seeds and oil in traditional remedies goes back more than 2000 years, and the herb is described as "the Melanthion" by Hippocrates and Discroides (Darakhshan *et al*., 2015). Thymoquinone (2-isopropyl-5-methyl-1, 4-benzoquinone) (TQ), is the main bioactive and most abundant component of the essential oil of *Nigella sativa* seeds (Salim *et al*., 2013). TQ has various pharmacological properties such antihypertensive, anticancer, antidiabetic, anti-inflammatory, and analgesic effects (Darakhshan *et al*., 2015). TQ is also reported to possess strong antioxidant properties (Darakhshan *et al*., 2015).

Based on the above considerations, this study was carried out to investigate for the first time the possible positive impact of TQ supplement on subchronic Pb hematotoxicity by evaluating the main hematological parameters in rats.

MATERIALS AND METHODS

Chemicals

Leas acetate trihydrate [$(C_2H_3O_2)_2Pb.\ 3H_2O$] and TQ (2-isopropyl-5-methyl-1,4-benzoquinone) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, Missouri, USA).

Animals

Thirty two healthy adult (4 months old) male Wistar rats, weighing 200-230g, 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 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 an acclimatization period, the rats were randomly divided into four groups of eight animals each and were treated for five weeks as follows: control group received tap water as the only drinking fluid, Pb group received an aqueous solution containing 2000 ppm of Pb acetate (0.2%, *w/v*) as the only drinking fluid, Pb+TQ group was cotreated with Pb (as in Pb group) plus TQ (5 mg/kg body weight/day, gavage) and TQ group received tap water as the only drinking fluid and was given TQ (5 mg/kg body weight/day) by gavage.

Pb acetate concentration (2000 ppm) and exposure duration (5 weeks) were based on previous studies (Çaylak *et al*., 2008; Aksu *et al*., 2012). Pb acetate solution was prepared in tap water (free from Pb) and replaced daily to minimize precipitation of Pb.

The oral dose of TQ (5 mg/kg/day) used in this study has been reported previously in several models of toxicity as an excellent protective daily dose in rats (Badary, 1999; El-Sayed, 2011). TQ was dissolved in warm tap water (free from Pb) (65°C), and the resulting TQ solution was cooled at room temperature before oral administration. TQ was administered by gastric tube daily between 8:00 and 9:00 a.m.

Hematological study

At the end of the treatment period, the animals were anesthetized with diethyl ether, and the blood samples were collected in EDTA tubes by cardiac puncture and were immediately used for the quantification of hematological parameters. Red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), white blood cells (WBC) and platelets (PLT) were quantified in an automatic hematological assay analyzer (Beckman Coulter, USA).

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

As shown in Table I, there were no statistically significant changes ($P > 0.05$) in the hematological profile between control and TQ groups. However, RBC number, HGB content, HCT percentage, and PLT count dropped significantly ($P < 0.05$), while WBC number increased significantly ($P < 0.05$) after Pb treatment compared to those of control.

Supplementation of TQ to Pb-exposed rats nearly returned RBC, HCT and WBC values to control levels. A significant improvement ($P < 0.05$) was also observed in HGB concentration in the Pb+TQ group compared to the Pb group, but without reaching the control level. However, TQ insignificantly ameliorated ($P > 0.05$) the reduced PLT value in metal-intoxicated animals.

DISCUSSION

Maintaining normal hematological characteristics is essential to ensure good health. In order to evaluate the beneficial effect of TQ against Pb-induced hematotoxicity, we measured the main hematological parameters in rats. In the present study, the Pb-intoxicated animals showed a significant decrease in the mean RBC, HGB, HCT and PLT values, but a significant increase in the WBC count in comparison with the control group. Our findings are in line with previous data (Ali *et al*., 2010; Karamala *et al*., 2011; Basha *et al*., 2012; Abdel-Moneim *et al*., 2015; Nikolić *et al*., 2015).

Table I. Effects of lead (Pb), thymoquinone (TQ), and their co-exposure on hematological parameters in rats after five weeks.

Items (unit)	Control	TQ	Pb	Pb+TQ
RBC (10^6/mm^3)	7.45±0.14	7.29±0.2	6.01±0.19*, **	6.8±0.3***
HGB (g/dl)	13.86±0.2	13.41±0.19	11.41±0.33*, **	12.7±0.47*, ***
HCT (%)	40.75±1.15	40.83±1.46	32.97±2.05*, **	38.6±0.91***
WBC (10^3/mm^3)	10.46±0.61	10.55±0.57	12.27±0.55*, **	10.48±0.44***
PLT (10^3/mm^3)	696±26.55	688.37±25.03	565.75±16.2*, **	600.62±28.25*, **

Data represent mean ± SEM (n=8). RBC, Red blood cells; HGB, hemoglobin; HCT, hematocrit; WBC, white blood cells; PLT, platelets. *P < 0.05 compared with control; **P < 0.05 compared with TQ; ***P < 0.05 compared with Pb (Student's *t* test).

The observed decrease in RBC, HGB and HCT in Pb-exposed rats in the current work is a manifestation of anemia. Pb-induced anemia is primarily the result of both inhibition of heme biosynthesis and shortening of circulating erythrocyte life span. In addition, Pb can also induce inappropriate production of erythropoietin leading to inadequate maturation of red cell progenitors, which can contribute to anemia (Patil *et al*., 2006). As an electropositive metal, Pb has high binding affinity for negatively charged sulfhydryl groups resulting in denaturation of δ-aminolevulinic acid dehydratase (ALAD), the most vulnerable among heme biosynthesis pathway key enzymes, in erythrocytes (Gunturu *et al*., 2011). Zinc, which serves as a cofactor for ALAD, is replaced by Pb, which is another factor behind the inactivation of this enzyme (Flora *et al*., 2008). Failure of normal functioning of ALAD to convert two molecules of δ-aminolevulinic acid (ALA) into porphobilinogen decreases heme formation (Assi *et al*., 2016). In Pb poisoning, accumulated ALA substrate in erythrocytes due to ALAD inhibition induces reactive oxygen species (ROS) generation (Jomova and Valko, 2011). Superoxide anion radical and hydrogen peroxide, which are produced as a result of both ALA and ALA/oxy-HGB coupled autoxidation, can interact and generate hydroxyl radical, the most reactive ROS (Ahamed and Siddiqui, 2007). It has been reported that Pb-induced anemia results from ROS generation and subsequent erythrocyte hemolysis (Gurer and Ercal, 2000). Pb-induced hemolysis may result of ROS-generated lipid peroxidation in RBC membranes (Carocci *et al*., 2016). Pb can increase the susceptibility of RBC membranes to lipid peroxidation by altering their fatty acid compositions (Flora *et al*., 2006).

In our study, the increased WBC value in Pb group might be due to activation of the body immune system to face the toxic effect of metal (Ali *et al*., 2010). The recorded leukocytosis might also be attributed to the presence of immature WBC in blood (Hossain *et al*., 2014).

The thrombocytopenia seen in Pb-treated rats is probably caused by lysis of PLT as a consequence of lipid peroxidation affecting their membrane which is more highly vulnerable to oxidative damage than that of RBC (Ohyashiki *et al*., 1991; Ambali *et al*., 2011). Low PLT count may also be due to decreased production of thrombopoietin by the liver as Pb induces liver oxidative damage (Singh *et al*., 2018).

The beneficial effects of TQ on Pb hematological alterations that have been observed in the present work were reported in other experimental animal models of ROS generating agents. The study conducted by Harzallah *et al*. (2012) showed that intraperitoneal injection of TQ (5 mg/kg, once per week, 10 weeks) normalized the elevated PLT count and attenuated the increased WBC number in 1,2-dimethylhydrazine-treated rats. In addition, the work of Ashour (2014) indicated that oral TQ administration (15 mg/kg/day; 3, 5 and 8 days) totally reversed the deteriorating effects of phenylhydrazine on RBC, HGB and HCT values at each time point of analysis in rats. Besides, therapy with TQ supplementation (35 and 10 mg/kg/day, 28 days, *per os*) successfully protected the main hematological parameters (RBC, HGB, HCT, WBC and PLT) against streptozotocin (Ashour, 2015) and diazinon toxicity in rats (Danaei and Karami, 2017). Furthermore, TQ (4 mg/kg/day, 5 days, *per os*) showed ameliorative effects on diethylnitrosamine-induced RBC count, HGB concentration and HCT level alterations in rats (Amin *et al*., 2017).

In the current study, the protective effect of TQ therapy against Pb hematotoxicity could be attributed to its well-known strong antioxidant properties, in particular, the neutralization of Pb-overproduced free radicals (Kruk *et al*., 2000; Mansour *et al*., 2002; Badary *et al*., 2003; Khalife and Lupidi, 2007; Khattab and Nagi, 2007) and the promotion of the expression of antioxidant defense genes (Ismail *et al*., 2010; Sayed-Ahmed *et al*., 2010; El-Sayed, 2011). By preventing the erythrocyte membrane fragility responsible for hemolysis and the erythropoietin production inhibition responsible for low RBC count, TQ can also exert its anti-anemic effect (Ashour, 2015). Further, immunomodulation and anti-inflammation are probably the ways by which TQ restored the altered WBC number (Shaterzadeh-Yazdi *et al*., 2018).

CONCLUSION

The present study showed that subchronic treatment with Pb caused pronounced deterioration of RBC, HGB, HCT, WBC and PLT levels in rats. Interestingly, our results showed for the first time that oral supplementation of TQ protected significantly against Pb hematological alterations. Therefore, TQ can be considered as a promising therapeutic agent against hematotoxicity induced by Pb and could also be effective against other toxics and certain pathogenic factors. However, further studies are required to clarify the TQ mechanism involved in this hematoprotective action.

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Statement of conflict of interest

The author have declared no conflict of interest.

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