Thymoquinone Improves Lead-Induced Hematotoxicity in Rats

Aymen Mabrouk*
Laboratory of Histology and Cytogenetic, Faculty of Medicine, University of Monastir, Monastir 5019, Tunisia

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.

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).

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, antiadipic, 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 [(C₆H₄O₆)₂Pb. 3H₂O] 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.
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/mm³)   | 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 (10³/mm³)    | 10.46±0.61       | 10.55±0.57      | 12.27±0.55*, **  | 10.48±0.44****    |
| PLT (10³/mm³)    | 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). 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).

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.

ACKNOWLEDGMENTS

This study was supported by funds allocated to the Research Unit of Genetic, Genotoxicity and Childhood Illness (UR 12 ES 10) by the Tunisian Ministry of Higher Education and Scientific Research. The author sincerely thanks Prof. Mohsen Hassine (Hematology...
Department, Fattouma Bourguiba University Hospital of Monastir, Tunisia), Prof. Hassen Ben Cheikh (Laboratory of Histology and Cytogenetic, Faculty of Medicine of Monastir, Tunisia), and Prof. Mohsen Sakly (Laboratory of Integrative Physiology, Faculty of Sciences of Bizerte, Tunisia) for their help.

Statement of conflict of interest
The author have declared no conflict of interest.

REFERENCES

Abdel-Moneim, A.M., El-Toweissy, M.Y., Ali, A.M., Awad Allah, A.A.M., Darwish, H.S. and Sadek, I.A., 2015. Curcumin ameliorates lead (Pb(2+))-induced hemato-biochemical alterations and renal oxidative damage in a rat model. Biol. Trace Elem. Res., 168: 206-220. https://doi.org/10.1007/s12011-015-0360-1

Ahamed, M. and Siddiqui, M.K.J., 2007. Low level lead exposure and oxidative stress: current opinions. Clin. Chim. Acta, 383: 57-64. https://doi.org/10.1016/j.cca.2007.04.024

Aksu, D.S., Didin, M. and Kayikci, F., 2012. The protective role of polyphenols on blood cells in rats exposed to lead. Rev. Romana Med. Lab., 20: 233-243.

Ali, F., Singh, K., Rani, S., Ahirwar, V. and Khan, S., 2010. Effect of ascorbic acid against lead (Pb) toxicity. Int. J. Pharm. Sci. Res., 1: 81-85.

Ambali, S.F., Angani, M., Shittu, M. and Kawu, M.U., 2011. Hematological changes induced by subchronic co-administration of chlorpyrifos and lead in Wistar rats: alleviating effect of vitamin C. Pharm. Sin., 2: 276-284. https://doi.org/10.4061/2011/214924

Amin, H.A.M., Arihan, O. and Ragbetli, M.C., 2017. Effect of thymoquinone administration on erythrocyte fragility in diethylnitrosamine administered rats. J. cell. Biotechnol., 3: 1-7. https://doi.org/10.3233/JCB-179008

Ashour, T.H., 2014. Hematocin and anti-anemic effect of thymoquinone against phenylhydrazine-induced hemolytic anemia in rats. Res. J. Med. Sci., 8: 67-72.

Ashour, T.H., 2015. Thymoquinone therapy improves hyperglycemia, erythrocyte indices, erythropoietin production and erythrocyte osmotic resistance in rat model of streptozotocin-induced diabetes. Br. J. Med. Med. Res., 5: 350-361. https://doi.org/10.9734/Bjmmr/2015/13409

Assi, M.A., Hezmee, M.N.M., Haron, A.W, Sabri, M.Y.M. and Rajion, M.A., 2016. The detrimental effects of lead on human and animal health. Vet. World, 9: 660-671. https://doi.org/10.14202/vetworld.2016.660-671

Badary, O.A., 1999. Thymoquinone attenuates ifosfamide-induced Fanconi syndrome in rats and enhances its antitumor activity in mice. J. Ethnopharmacol., 67: 135-142. https://doi.org/10.1016/S0378-8741(98)00242-6

Badary, O.A., Taha, R.A., Gamal el-Din, A.M. and Abdel-Wahab, M.H., 2003. Thymoquinone is a potent superoxide anion scavenger. Drug Chem. Toxicol., 26: 87-98. https://doi.org/10.1081/DCT-120020404

Basha, D.C., Bashir, S.S. and Reddy, G.R., 2012. Lead-induced cardiac and hematological alterations in aging Wistar male rats: Alleviating effects of nutrient metal mixture. Biogerontology, 13: 359-368. https://doi.org/10.1007/s10322-012-9380-9

Caracci, A., Catalano, A., Lauria, G., Sinicropi, M.S. and Genchi, G., 2016. Lead toxicity, antioxidant defense and environment. Rev. environ. Contam. Toxicol., 238: 45-67. https://doi.org/10.1007/398_2015_5003

Çaylak, E., Aytekin, M. and Halifeoğlu, İ., 2008. Antioxidant effects of methionine, alpha-lipoic acid, N-acetylcysteine and homocysteine on lead-induced oxidative stress to erythrocytes in rats. Exp. Toxicol. Pathol., 60: 289-294. https://doi.org/10.1016/j.etp.2007.11.004

Danai, G.H. and Karami, M., 2017. Protective effect of thymoquinone against diazinon-induced hematotoxicity, genotoxicity and immunotoxicity in rats. Environ. Toxicol. Pharmacol., 55: 217-222. https://doi.org/10.1016/j.etap.2017.09.002

Darakhshan, S., Bidmeshki Pour, A., Hosseinizadeh Colagar, A. and Sisakhtnezhad, S., 2015. Thymoquinone and its therapeutic potentials. Pharmacol. Res., 95-96: 138-158. https://doi.org/10.1016/j.phrs.2015.03.011

El-Sayed, W.M., 2011. Upregulation of chemoprotective enzymes and glutathione by Nigella sativa (black seed) and thymoquinone in CCl4-intoxicated rats. Int. J. Toxicol., 30: 707-714. https://doi.org/10.1177/1091581811420741

Flora, S.J.S., Flora, G.J.S. and Saxena, G., 2006. Environmental occurrence, health effects and management of lead poisoning. In: Lead chemistry, analytical aspects, environmental impact and health effects (eds. J.S. Casas and J. Sordo). 1st edition. Elsevier Publication, Amsterdam, The Netherlands. pp. 158-228. https://doi.org/10.1016/B978-044452945-9/50004-X
Flora, S.J.S., Mittal, M. and Mehta, A., 2008. Heavy metal induced oxidative stress and its possible reversal by chelation therapy. *Indian J. med. Res.*, **128**: 501-523.

Flora, S.J.S., Pande, M. and Mehta, A., 2003. Beneficial effect of combined administration of some naturally occurring antioxidants (vitamins) and thiol chelators in the treatment of chronic lead intoxication. *Chem. Biol. Interact.*, **145**: 267-280. https://doi.org/10.1016/S0009-2797(03)00025-5

Gani, M.U., Siddiqui, M.S.I., Islam, K., Ahmed, S., Rashid, M.H., Moonmoon, S. and Mostoafa, M., 2017. Study on hematological alterations in experimental lead toxicosis in long evans rats. *Malays. J. Vet. Res.*, **8**: 11-18.

Gunturu, K.S., Nagarajan, P., McPhedran, P., Goodman, T.R., Hodsdon, M.E. and Strout, M.P., 2011. Ayurvedic herbal medicine and lead poisoning. *J. Hematol. Oncol.*, **4**: https://doi.org/10.1186/1756-8722-4-51

Gurer, H. and Eréal, N., 2000. Can antioxidants be beneficial in the treatment of lead poisoning? *Free Radic. Biol. Med.*, **29**: 927-945. https://doi.org/10.1016/S0891-5849(00)00413-5

Harzallah, H.J., Grayaa, R., Kharoubi, W., Maaloul, A., Hammami, M. and Mahjoub, T., 2012. Thymoquinone, the *Nigella sativa* bioactive compound, prevents circulatory oxidative stress caused by 1,2-dimethyldihydrazine in erythrocyte during colon postinitiation carcinogenesis. *Oxid. Med. Cell. Longev.*, **2012**.

Hossain, M.A., Akanda, M.R., Mostoafa, M. and Awal, M.A., 2014. Ameliorative effects of dried garlic powder (*Allium sativum*) on hematological parameters against lead (Pb) intoxication in broiler chickens. *Pharmacologia*, **5**: 110-119. https://doi.org/10.5567/pharmacologia.2014.110.119

Ismail, M., Al-Naqeep, G. and Chan, K.W., 2010. *Nigella sativa* thymoquinone-rich fraction greatly improves plasma antioxidant capacity and expression of antioxidant genes in hypercholesterolemic rats. *Free Radic. Biol. Med.*, **48**: 664-672. https://doi.org/10.1016/j.freeradbiomed.2009.12.002

Kamalala, S.K., Srilatha, C., Anjaneyulu, Y., Chandra Sekhara Rao, T.S., Sreenivasulu, D. and Pidugu, A.P., 2011. Hematobiochemical changes of lead poisoning and amelioration with *Ocimum sanctum* in Wistar albino rats. *Vet. World*, **4**: 260-263. https://doi.org/10.5455/vetworld.4.260

Kelley, C., 1999. Cadmium therapeutic agents. *Curr. Pharm. Des.*, **5**: 229-240. https://doi.org/10.1023/A:1009972120539

Khalife, K.H. and Lupidi, G., 2007. Nonenzymatic reduction of thymoquinone in physiological conditions. *Free Radic. Res.*, **41**: 153-161. https://doi.org/10.1080/10715760600978815

Khattab, M.M. and Nagi, M.N., 2007. Thymoquinone supplementation attenuates hypertension and renal damage in nitric oxide deficient hypertensive rats. *Phytother. Res.*, **21**: 410-414. https://doi.org/10.1002/ptr.2083

Kruk, I., Michalska, T., Lichszteld, K., Kladna, A. and Aboul-Enein, H.Y., 2000. The effect of thymol and its derivatives on reactions generating reactive oxygen species. *Chemosphere*, **41**: 1059-1064. https://doi.org/10.1016/S0045-6535(99)00454-3

Leggett, R.W., 1993. An age-specific kinetic model of lead metabolism in humans. *Environ. Hlth. Perspect.*, **101**: 598-616. https://doi.org/10.1289/ehp.9310159

Mansour, M.A., Nagi, M.N., El-Khatib, A.S. and Bekairi, A.M., 2002. Effects of thymoquinone on antioxidant enzyme activities, lipid peroxidation and DT-diaphorase in different tissues of mice: a possible mechanism of action. *Cell Biochem. Funct.*, **20**: 143-151. https://doi.org/10.1002/cbf.968

Nikolić, R., Krsić, N., Jovanović, J., Kočić, G., Cvjetković, T.P. and Radovanljević-Stevanović, N., 2015. Monitoring the toxic effects of Pb, Cd and Cu on hematological parameters of Wistar rats and potential protective role of lipic acid and glutathione. *Toxicol. Ind. Hlth.*, **31**: 239-246. https://doi.org/10.1177/0748233712469652

Ohyashiki, T., Kobayashi, M. and Matsu, K., 1991. Oxygen-radical-mediated lipid peroxidation and inhibition of ADP-induced platelet aggregation. *Arch. Biochem. Biophys.*, **288**: 282-286. https://doi.org/10.1016/0003-9861(91)90196-P

Patil, A.J., Bhagwat, V.R., Patil, J.A., Dongre, N.N., Ambekar, J.G., Jailkhani, R. and Das, K.K., 2006. Effect of lead (Pb) exposure on the activity of superoxide dismutase and catalase in battery manufacturing workers (BMW) of Western Maharashtra (India) with reference to heme biosynthesis. *Int. J. environ. Res. Publ. Hlth.*, **3**: 329-337. https://doi.org/10.3390/ijerph2006030041

Salim, L.Z.A., Mohan, S., Othman, R., Abdelwahab, S.I., Kamalideghan, B., Sheikh, B.Y. and Ibrahim, M.Y., 2013. Thymoquinone induces mitochondria-
mediated apoptosis in acute lymphoblastic leukaemia in vitro. Molecules, 18: 11219-11240. https://doi.org/10.3390/molecules180911219

Sayed-Ahmed, M.M., Aleisa, A.M., Al-Rejaie, S.S., Al-Yahya, A.A., Al-Shabanah, O.A., Hafez, M.M. and Nagi, M.N., 2010. Thymoquinone attenuates diethylnitrosamine induction of hepatic carcinogenesis through antioxidant signaling. Oxid. Med. Cell. Longev., 3: 254-261. https://doi.org/10.4161/oxim.3.4.12714

Sharma, B., Singh, S. and Siddiqi, N.J., 2014. Biomedical implications of heavy metals induced imbalances in redox systems. Biomed. Res. Int., 2014. https://doi.org/10.1155/2014/640754

Shaterzadeh-Yazdi, H., Noorbakhsh, M.F., Hayati, F., Samarghandian, S. and Farkhondeh, T., 2018. Immunomodulatory and anti-inflammatory effects of thymoquinone. Cardiovasc. Hematol. Disord. Drug Targets, 18: 52-60. https://doi.org/10.2174/1871529X18666180212114816

Singh, N., Kumar, A., Gupta, V.K. and Sharma, B., 2018. Biochemical and molecular bases of lead-induced toxicity in mammalian systems and possible mitigations. Chem. Res. Toxicol., 31: 1009-1021. https://doi.org/10.1021/acs.chemrestox.8b00193

WHO, 2021. International Programme on Chemical Safety: Ten chemicals of major public health concern. Available: https://www.who.int/ipcs/assessment/public_health/chemicals_phc/en/.

Ziaee, T., Moharreri, N. and Hosseinzadeh, H., 2012. Review of pharmacological and toxicological effects of Nigella sativa and its active constituents. J. med. Pl., 2: 16-42.