http://jbcp.shahed.ac.ir 10.22070/JBCP.2024.19560.1183

Open Access Research Article

Journal of Basic and Clinical Pathophysiology
Print ISSN: 2322-1895, **Online ISSN:** 2345-4334
Vol. 12, No. 2, October 2024

A Semiannual Publication of
Shahed University Press

Protective effect of *Nigella sativa* phytochemical thymoquinone in a mouse model of brain injury induced by potassium dichromate

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Abstract

Background and Objective: Potassium dichromate, which is a form of hexavalent chromium, has been demonstrated to induce toxicity associated with oxidative stress in humans and animals. Thymoquinone (TQ) isolated from *Nigella sativa* has a wide spectrum of activities such as antioxidant, anti-inflammatory and anticancer effects. This study aimed to evaluate the protective effect of thymoquinone in an animal model of brain damage caused by potassium dichromate in the mouse.

Materials and Methods: In this study, mice were divided into 5 groups: control, control treated with 5 mg/kg thymoquinone, potassium dichromate, and potassium dichromate treated with 1 or 5 mg/kg thymoquinone. To induce neuronal injury, 0.5 mg/kg potassium dichromate was intranasally and daily administrated for two months. Thymoquinone was given orally and daily for seven weeks. After two months, activity of catalase (CAT), level of malondialdehyde (MDA), glutathione (GSH) and nitrite (Nit), and number of neurons in the parietal cortex were evaluated. Data analysis was conducted using one-way ANOVA and Tukey post-test with $p<0.05$ as significant.

Results: Potassium dichromate significantly lowered activity of CAT and elevated levels of MDA and Nit, reduced GSH level and decreased neuronal density in the parietal cortex. Treatment with 5 mg/kg thymoquinone significantly increased activity of CAT and reduced MDA level, elevated level of GSH and also prevented reduction of neuronal density in the parietal cortex.

Conclusion: Thymoquinone administration can lower oxidative stress and brain injury following potassium dichromate.

Keywords: Potassium dichromate, Thymoquinone, Oxidative stress, Parietal cortex

1. Introduction

Brain damage is the cause of death and disability of people under the age of 40 all over the world, which annually causes disability of 150 to 200 people per million. Millions of people receive emergency medical treatment for brain injuries each year, while 1.5 million people die (1). Brain damage shows a series of cognitive disorders that cause disturbances in attention, executive function and memory, which are among the most common neurological disorders (2). Heavy metal pollution has increased worldwide due to industrial activities. Heavy metals such as mercury, cadmium, chromium (potassium dichromate), lead and platinum are an occupational and environmental hazard. Chromium (Cr) is used in industries and many workers are exposed to its toxicity. The main route of exposure to this metal is inhalation. It can also be transmitted through other ways such as drinking contaminated water or direct skin contact. Cr(VI) is the most toxic form due to its high oxidation potential and high solubility (3, 4). The brain is vulnerable to these factors because it contains large amounts of unsaturated fatty acids that are damaged by reactive oxygen species during chromium exposure (5, 6).
The use of antioxidants is considered as a therapeutic

Submitted: 03 September 2024**, Revised:** 01 Octorber 2024, **Accepted:** 06 Octorber 2024
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approach in neurological diseases. Oxidative damage is involved in the pathology of neurological disorders. Antioxidants act as defense systems against oxidative stress and inhibit metal-induced toxicity (7). Thymoquinone (TQ), the predominant compound in black seed volatile oil, has a wide range of beneficial effects. Thymoquinone has liver protective, anti-inflammatory, antioxidant and anti-cancer effects. These beneficial effects of TQ support the use of this natural compound as a drug with a wide range of medical applications (8-12). The aim of this study was to determine the protective effect of thymoquinone in an animal model of brain damage caused by potassium dichromate in the mouse.

2. Materials and Methods

2.1. Animals

In this study, 40 NMRI male mice in the weight range of 20-24 grams were used. They were divided into five groups: 1) control, 2) control receiving 5 mg thymoquinone, 3) potassium dichromate, and 4 and 5) potassium dichromate groups receiving thymoquinone with a dose of 1 and 5 mg/kg. The animals were purchased from Pasteur Institute (Tehran, Iran) and kept in the animal house of Shahed University. During the study period, the animals were kept on 12 hours of light and 12 hours of darkness, in special cages for keeping laboratory animals made of transparent plastic, at a temperature of 21 to 23 degrees Celsius, with sufficient and free access to water and food. All experiments were performed according to the international guidelines for the maintenance of laboratory animals and between 8:00 AM and 3:00 PM.

2.2. Experimental procedure

After killing the animals in the carbon dioxide chamber, the brain tissue was separated from the body and each brain was divided into two halves. 7 samples on the right side were used for biochemical tests and 6 samples on the left side were used for histological studies. The samples of the right half of the brain were immediately transferred to a -70 °C freezer and the left side to containers containing 10% formalin.

After weighing the samples from the right side of the brain, a certain amount of cold Tris hydrochloride buffer (pH 7.4) at a concentration of 150 mM was added to them separately and homogenized (5%) for 1 minute with a homogenizer at 7000 rpm and the homogenized solution was centrifuged at 4°C to prepare the supernatant. After centrifugation, the clear supernatant solution was separated from the sediments on the bottom of the container, the bottom sediment part was discarded, and the clear supernatant solution was used for further measurements.

2.3. Assay of malondialdehyde (MDA)

Measuring the level of MDA is based on a method founded on the reaction of thiobarbituric acid (TBA), which is carried out at boiling temperature. In this method, malondialdehyde or similar substances react with thiobarbituric acid and create a pink color whose maximum optical absorption is at a wavelength of 532 nm. The reagent contained trichloroacetic acid thiobarbituric acid. Optical absorption was read at a wavelength of 532 nm in a spectrophotometer. The standard curve was also prepared based on tetraethoxypropane dilutions and the optical absorptions obtained from the samples were adapted to the standard curve.

2.4. Measurement of glutathione concentration

In order to measure the concentration of glutathione, the supernatant was reacted with Tris buffer, methanol, (pH=8) and DTNB (2, 2-dithio-bis-nitrobenzoic acid). Optical absorption was read using a spectrophotometer at a wavelength of 412 nm.

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2.5. Assay of catalase activity

This enzyme is one of the main hydrogen peroxide neutralizing enzymes. Therefore, it is considered as an antioxidant enzyme. In this research, the specific catalase activity assay kit of Kiazist company (Iran) was used. In this experiment, the reagent contained methanol, hydrogen peroxide, periodate, and Purpald. Absorbance was read at a wavelength of 540 nm.

2.6. Nitrite level measurement

The nitrite level represents the amount of nitric oxide produced, which was measured based on the Griess reaction. In this test, equal volumes of tissue lysate and Griess reagent (0.1% N-(1-naphthyl) ethylenediamine-dihydrochloride and 1% sulfanilamide in 5% phosphoric acid) were mixed together and kept for 10 minutes. Optical absorption was measured at a wavelength of 540 nm. The results were given as microg/mg protein.

2.7. Histochemical assessment

Brain blocks were kept in 10% formalin solution. After paraffin embedding, 5 µm sections were prepared and stained with H&E protocol. Quantitative assessment was performed using a computer-assisted image acquisition and analysis system. Neuronal density in the parietal cortex was measured. To count the number of neurons in the parietal cortex, the slices of this area were examined at the infundibular level. The number of neurons was counted at 100 X magnification. For each animal, counting was done for four slices that were at least 50 µm apart, and for each group counting was done for 6 animals.

2.8. Statistical analysis

All data in this study are shown as means ± standard error. Analysis was made in GraphPad Prism version 9.3 (GraphPad Software Inc., USA). Significant differences were found out using one-way analysis of variance and Tukey's multiple range test. Level of statistical significance was $p<0.05$.

3. Results

By measuring malondialdehyde (MDA) level in the brain as a marker of lipid peroxidation (Fig. 1), it was found that in the control group treated with thymoquinone 5 mg/kg, there was no obvious and significant difference compared to the control group. In the potassium dichromate group, there was a significant increase in brain MDA compared to the control group ($P<0.001$). In the potassium dichromate group treated with thymoquinone 5 mg/kg, a significant decrease of this index was observed compared to the potassium dichromate group ($P<0.001$).

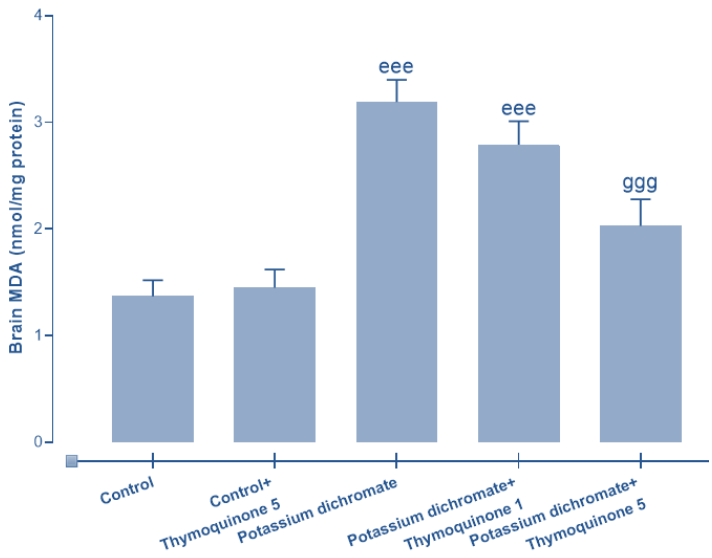

Fig. 1: Level of malondialdehyde (MDA) as an index of oxidative stress in the control and potassium dichromate treated groups
eee: $p<0.001$ compared to the control group
ggg: $p<0.001$ compared to the potassium dichromate group

By measuring level of brain nitrite (one of the NO metabolites) as an indicator of brain damage, it was found that there was no significant change by administering thymoquinone 5 mg/kg to the control group. In the potassium dichromate group, a significant increase in the amount of brain nitrite compared to the control group was evident (P<0.001), but in the potassium dichromate group treated with thymoquinone 5 mg/kg, there was no significant difference in the amount of nitrite compared to the potassium dichromate group.

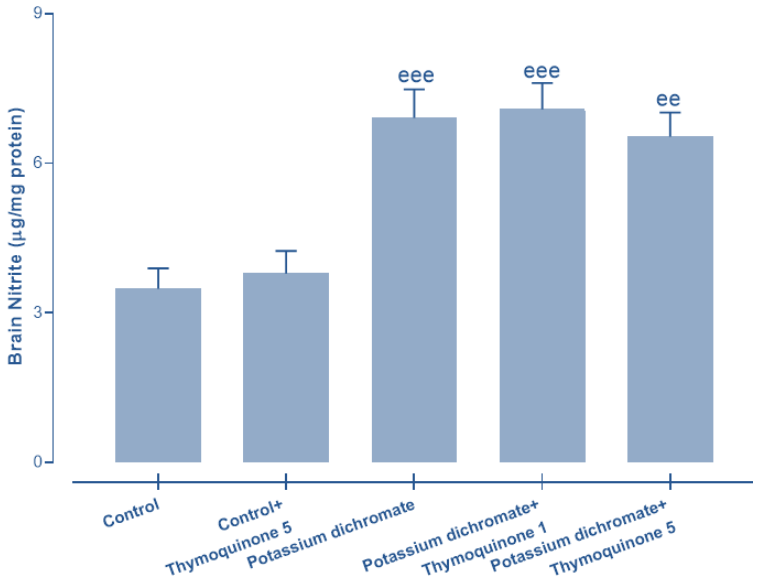

Fig 2: Nitrite level as an index of oxidative stress in the control and potassium dichromate treated groups

eee: p<0.001 compared to the control group

ee: p<0.01 compared to the control group

By measuring brain glutathione (GSH) as an indicator of cell antioxidant activity, it was found that there is no significant difference in the control group receiving thymoquinone at a dose of 5 mg/kg compared to the control group. In the group receiving potassium dichromate compared to the control group, a clear and significant decrease in glutathione was obtained compared to the control group (P<0.001). In the potassium dichromate group treated with thymoquinone at 1 mg/kg showed a significant decrease in brain glutathione compared to the control group (P<0.001). There was a significant decrease in the potassium dichromate group treated with thymoquinone 5 mg/kg compared to the control group (P<0.05). On the other hand, brain glutathione in the potassium dichromate group receiving thymoquinone at a dose of 5 mg/kg, there was a statistically significant increase versus the potassium dichromate group (P<0.05).

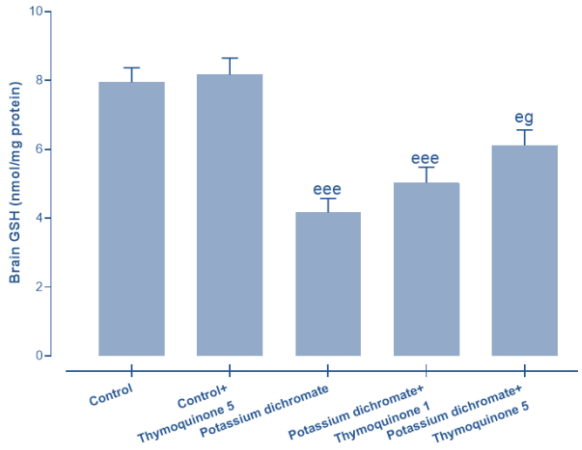

Fig 3: Level of brain glutathione as an indicator of cell antioxidant activity in the control and potassium dichromate treated groups
eee: p<0.001 compared to the control group. e: p<0.05 compared to the control group. g: p<0.05 compared to potassium dichromate group

By measuring the level of brain catalase activity as an indicator of antioxidant activity (hydrogen peroxide-decomposing enzyme), it was found that there was no significant difference in the control group receiving thymoquinone at a dose of 5 mg/kg compared to the control group. In the group receiving potassium dichromate compared to the control group, a significant decrease in catalase activity was obtained compared to the control group (P<0.01). In the potassium dichromate group treated with thymoquinone 1 mg/kg, a significant decrease in brain catalase activity was obtained compared to the control group (P<0.05). Also, in the potassium dichromate group treated with thymoquinone 5 mg/kg, a significant increase of this index was observed compared to the potassium dichromate group (P<0.05).

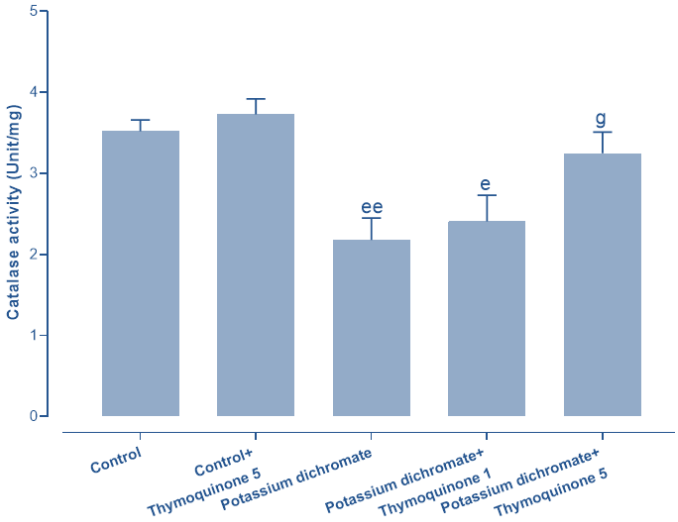

Fig 4: Level of brain catalase activity as an indicator of antioxidant activity in the control and potassium dichromate treated groups
ee: p<0.01 compared to the control group. e: p<0.05 compared to the control group. g: p<0.05 compared to potassium dichromate group

By counting the number of neurons in the parietal cortex area at the infundibular level using Nissl (Cresyl violet) staining, it was found that in the control group receiving thymoquinone at a dose of 5 mg/kg, there was no significant difference compared to the control group. A significant and obvious decrease in the number of neurons per unit area was obtained in the potassium dichromate group compared to the control group ($P<0.01$). The same significant decrease in the number of neurons per unit area was obtained in the potassium dichromate group treated with thymoquinone 1 mg/kg ($P<0.05$). There was also a significant increase in the number of neurons in the potassium dichromate group treated with thymoquinone 5 mg/kg compared to the potassium dichromate group ($P<0.05$).

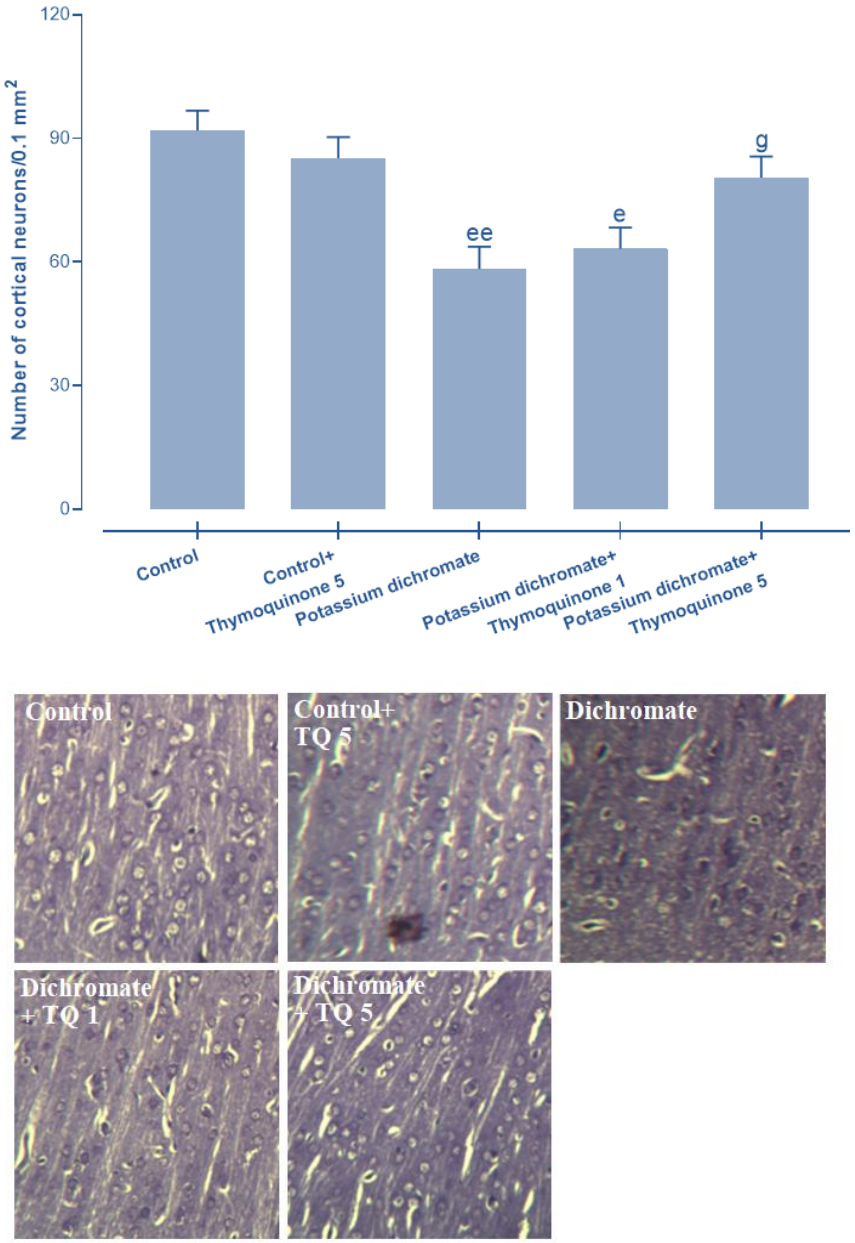

Fig 5: Number of brain neurons in the control and potassium dichromate treated groups and comparable photomicrographs. ee: $p<0.01$ compared to the control group. e: $p<0.05$ compared to the control group. g: $p<0.05$ compared to potassium dichromate group

4. Discussion

Potassium dichromate (K2Cr2O7) is one of the soluble hexavalent chromium compounds that is widely found in more than 50 different industries around the world, including pigment and textile production, leathermaking, wood processing, metallurgical and chemical industries (13). The nervous system is a commonly vulnerable organ, which results in consequences such as apoptosis when exposed to toxic metals. Extensive information is available on the cytotoxic effects of chromium on several different mammalian organs. However, little information was available on the neurological effects of hexavalent chromium (14). Previous evidence showed for the first time that hexavalent chromium accumulates in rat hypothalamus and anterior pituitary cells after oral administration and induces apoptosis, mainly due to the induction of oxidative stress (15). Dashti et al. reported in 2016 that the oxidative stress and neurotoxic effect caused by potassium dichromate in adult brain neurons is significantly higher than in immature neurons (16). There are few studies on the effects of chromium on the functional and structural integrity of the mammalian brain. Accordingly, our experimental study extends data from previous research on the brain damage effects of intranasal administration of potassium dichromate and supports the effects of oral booster doses of thymoquinone in the treatment of brain damage caused by potassium dichromate. Mammalian brain, due to its high content of unsaturated fatty acids, low antioxidant protection and high content of iron, is often the target of oxidative damage and lipid peroxidation, protein oxidation and DNA damage (17).

It has been reported that changes in oxidative stress biomarkers indicate the tissue's ability to cope with oxidative stress. The antioxidant enzyme catalase (CAT) acts as a defense against free radicals. This enzyme is responsible for the catalytic decomposition of hydrogen peroxide into molecular oxygen and water. Glutathione (GSH) is normally present in cells in millimolar concentrations and is known to protect the cellular system against the toxic effects of lipid peroxidation. It is also very important in maintaining the cellular redox state and its reduction is considered as an indicator of oxidative stress (17). It has been reported that most of the antioxidant enzymes are deactivated after exposure to potassium dichromate due to the direct binding of heavy metals to the active site of the enzyme or due to the displacement of metal auxiliary agents from the active sites of the enzyme. An increase in lipid peroxidation is indicated by an increase in malondialdehyde (MDA), which is the final product of lipid peroxidation (17, 18). Current results showed that treatment with potassium dichromate at a dose of 0.5 mg/kg caused oxidative stress due to a significant decrease in GSH content and CAT activity and a significant increase in MDA level compared to the control group. The results obtained for various biomarkers of oxidative stress in the present study reflect the results obtained by other studies. According to previous studies, chromium can induce oxidative stress, lipid peroxidation, DNA damage and apoptosis in different experimental models (19, 20).

It has been recommended that the consumption of antioxidants helps to ameliorate the harmful effects of oxidative stress. Thymoquinone has proven its activity as a hepatoprotective, anti-inflammatory, antioxidant, cytotoxic and anticancer chemical by activating specific mechanisms. It has been reported that oral administration of thymoquinone reduces nitrite and lipid peroxide caused by acetaminophen (21). Thymoquinone is effective in protecting rats against acetaminophen-induced hepatotoxicity, possibly through increased resistance to oxidative and nitrosative stress (21). Thymoquinone significantly reduces lipid peroxidation MDA in the hippocampus following cerebral ischemia-reperfusion injury (22). A study was designed to evaluate the potential effectiveness of natural antioxidants celastrol and thymoquinone to reduce AlCl3-induced psychomotor abnormalities and oxidative-inflammatory load in male albino rats (23). The results of this study indicated the effectiveness of thymoquinone and celastrol in reducing malondialdehyde and increasing the total antioxidant capacity and catalase enzyme activity, reducing the levels of inflammatory factors (23). Also, in mice treated with thymoquinone, the GSH content of their liver was restored and MDA production was prevented (24). In addition, thymoquinone has been shown to protect brain tissue against nitrosative stress (25). Also, thymoquinone improves brain disorders caused by sodium nitrite through various mechanisms, including reducing oxidative stress, restoring reduced glutathione concentration, reducing high levels of pro-inflammatory cytokines, restoring cytochrome c oxidase activity, and reducing brain apoptosis markers (26, 27). Another study showed that thymoquinone improved passive avoidance memory as a result of treatment (28). In addition, the results showed that thymoquinone treatment decreased the level of inflammatory cytokines (TNF-α and IL-1β) and increased the level of glutathione and anti-inflammatory cytokine (IL-10) in the remaining cells in the hippocampus (29). This study suggests that thymoquinone may have beneficial therapeutic effects on cognitive complications, oxidative stress, neuroinflammation and histology in rats. Oral administration of thymoquinone. In line with the above studies, our results showed that the treatment of animals with thymoquinone at 5 mg/kg of body weight decreased lipid peroxidation index

(malondialdehyde) and also increased the activity of catalase enzyme and increased glutathione levels. Meanwhile, the above-mentioned effects of thymoquinone at a dose of 1 mg/kg did not appear significantly.

Conclusion

In summary, the present study showed that administration of potassium dichromate increases oxidative stress and decreases antioxidant factors. Meanwhile, malondialdehyde levels decreased and catalase enzyme activity and glutathione content increased in the groups treated with thymoquinone at 5 mg/kg. According to our current study, thymoquinone did not have a significant effect on the amount of nitrite. Also, thymoquinone caused tissue recovery in the damaged brain area caused by potassium dichromate.

Acknowledgement

This research study was certified and financially supported partly by Shahed University (Tehran, Iran).

Conflict of interest statement

No conflict of interest is declared by the authors.

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