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EFFECTS OF THYMOQUINONE ON OXIDATIVE STRESS AND BEHAVIOR IN MERCURY-EXPOSED RATS

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Abstract: Mercury (Hg) is widely used in nature. It is a substance that has toxic effects even in small amounts. Thymoquinone (TQ) is the main active phenolic compound obtained from the essential oil of Nigella sativa L. (black cumin) seed. The protective effects of TQ against diseases and toxic compounds have been studied for a long time. Aim: This study aimed to investigate the effect of TQ on oxidative stress and behavior in rats exposed to mercury. In this study, 24 adult male Wistar Albino rats weighing between 250±20 g were used. 5 mg/kg Hg and 10 mg/kg TQ were given via intragastric gavage for 21 days. Animals were randomly divided into four groups. Group 1 is the Control, Group 2 TQ (10 mg/kg), Group 3 Hg (5 mg/kg), Group 4 Hg (5 mg/kg) + TQ (10 mg/kg). Open field test and forced swimming test were performed to examine locomotor activity, anxiety, and depression-like behaviors in rats. At the end of the experiment, Malondialdehyde (MDA), total nitric oxide (NO), and reduced glutathione (GSH/RSH) levels were examined in cerebral cortex and plasma. In the open field test, Hg+TQ treatment increased the number of crossings and time spent in the center ($P<0.01$). In the forced swim test, Hg+TQ treatment increased the swimming and climbing time (respectively $P<0.01$, $P<0.001$) and decreased the immobility time ($P=0.001$). In cerebral cortex and plasma, TQ treatment decreased the increased MDA and NO levels ($p=0.01$) and increased the decreased GSH/RSH levels ($P<0.01$) as a result of Hg exposure. Mercury exposure increased oxidative stress in plasma and cerebral cortex, causing anxiety and depression-like behaviors. TQ is can be used to improve some behavioral changes and reduce oxidative stress in Hg-exposed rats.

Keywords: Mercury, Behavior, Oxidative stress, Nigella sativa, Rats, Antioxidants

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1. Introduction

Mercury, which the World Health Organization (WHO) lists among the ten chemicals that cause significant public health concerns, causes damage to many tissues and organs and is considered the third most toxic element in terms of human health (Yavuz, 2020). Hg has been reported to cause behavioral and neurochemical changes in rats (Minj et al., 2012). It was also found to increase Reactive Oxygen Product (ROP) levels in cells (Zhang et al., 2021). Increased ROP in cells causes oxidative stress by disrupting the pro-oxidant/antioxidant balance. It has been determined that mercury at various doses decreases antioxidant weapons such as Glutathione (GSH) in cells and increases Malondialdehyde (MDA), which indicates lipid peroxidation in cells (Gstraunthaler et al., 1983; Nath et al., 1996; Fouda et al., 2008).

Thymoquinone (TQ) is the primary active ingredient of the essential oil obtained from black cumin seed (Nigella sativa L.). Studies have shown that TQ protects cells from inflammation and oxidative stress and has a neuroprotective effect (Fouda et al., 2008; Kuzay et al., 2022). TQ has been shown to have antioxidant effects against different toxic substances. (Akarsu and Çetin, 2022). Additionally, antioxidant and protective effects of TQ on kidney and liver tissue were demonstrated in rats exposed to mercury (Fouda et al., 2008; Owumi et al., 2025). However, in rats exposed to Hg, the oxidative stress levels and behavior of TQ in cerebral cortex and behavior were not examined. Therefore, in the present study, the oxidative stress levels and behavior of TQ in cerebral cortex and behavior were examined in rats exposed to Hg.

2. Material and Methods

In our study, 24 adult male Wistar Albino rats weighing 250±20 g (12-13 weeks old) were acquired from the Animal Experiment Laboratory. This study was supported by Institutional Scientific Research Projects. The Project number is TIP.A3.24.011.

2.1. Chemicals

HgCl2 (Tekkim, Türkiye) and TQ (CAYMAN Chemical, USA) were used in this study.

2.2. Exposure to Mercury

Rats were given mercury (II) chloride (HgCl2) by intragastric gavage at 5 mg/kg for three weeks. In their preclinical studies, Minj et al., reported that Hg caused neurologic impairment (Minj et al., 2021).

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2.3. Experimental Design

The total duration of the study was 22 days. 5 mg/kg Hg and 10 mg/kg TQ were dissolved in tap water and administered via intragastric gavage for 21 days. Animals were randomly divided into four groups (n=6 for each group). Group 1 is the Control, Group 2 TQ (10 mg/kg), Group 3 Hg (5 mg/kg), Group 4 Hg (5 mg/kg) + TQ (10 mg/kg). The control group received only tap water to induce gavage stress. Chemicals were freshly prepared every day and administered in a total volume of 1 ml/kg (Fouda et al., 2008; Minj et al., 2021; Kuzay et al., 2022). TQ was given half an hour before Hg. The experimental protocol is summarized in Figure 1.

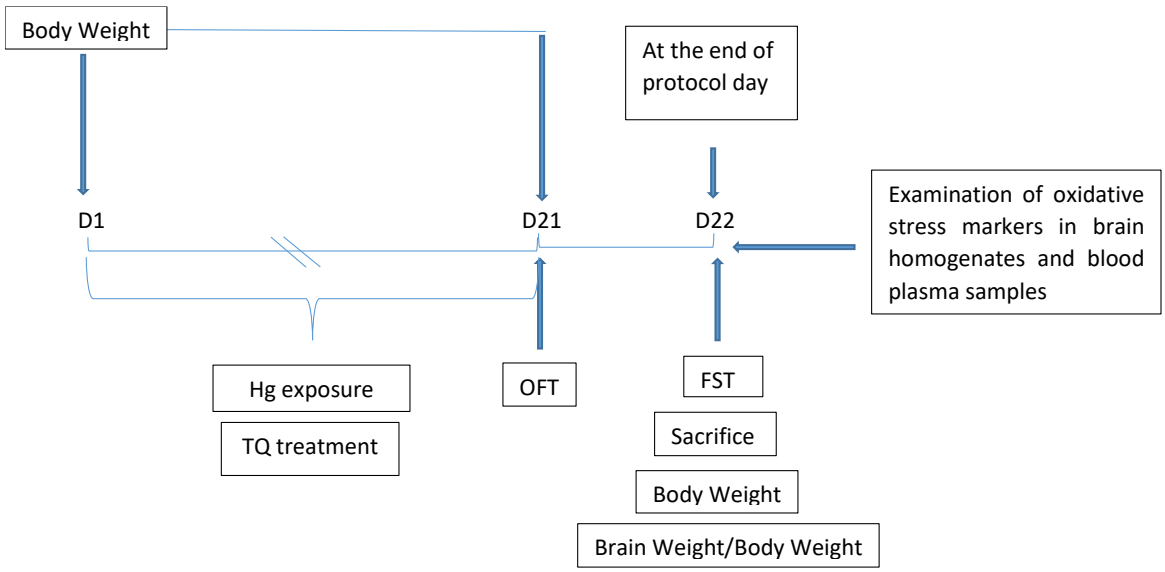

Figure 1. Timeline depicting the sequence of experimental events. D indicates day of experiment. Rats were tested in the forced swim test (FST) on day 22 and in the open field test (OFT) on day 21. Body weight was taken on day 1 and day 22. Brain weight / Body weight was taken on day 22.

2.4. Measurement of Body Weight

Rats were weighed on days 1 and 22 of the study.

2.5. Evaluation of the Relative Brain/Body Weight Ratio

Hg exposure can cause toxic effects on the brain. Therefore, on the 22nd day of the study, the brain weights of the rats were measured to establish a relationship between brain-body weight losses. To examine the change in absolute brain weight, the ratio of brain weight/body weight was calculated and this formula was used. Brain weight / Body weight x 100 (Shandilya et al., 2022).

2.6. Behavior Parameters

2.6.1. Forced swim test (FST)

FST was performed on day 22 to assess depression-like behavior in animals. It evaluates an animal's resilience to water stress. One day before, a 15-minute pre-test was performed to prevent acute stress in rats. After the pretest, rats were individually exposed to 30 cm of water at 25°C±1°C in cylindrical containers with a diameter of 15 cm and a height of 50 cm for 5 minutes. During the test period, swimming, climbing, and immobilization times were recorded with a video camera (Kuzay et al., 2022).

2.6.2. Open field test (OFT)

OFT was performed on the 21st day to assess locomotor activity and anxiety-like behavior in animals. In a 90 cm x 35 cm white Plexiglas arena divided into 24 units with a black strip on the floor, the individual movements of each rat were recorded by video camera for 5 minutes. The period spent in the center and periphery of the open field and the number of crossings were recorded (Brocardo et al., 2012). Two hours after the treatment, the behavioral tests of each rat were measured.

2.7. Evaluation of Oxidative Stress Markers

On day 22, all rats were sacrificed under im. 5mg/kg Rompun + 45mg/kg Ketamine anesthesia by removing blood from their hearts. The cerebral cortex separated from the rats were stocked at -80°C until the day of the study. The blood is centrifuged (at 2000-3000 RPM) for approximately 20 minutes at 4°C. (NUVE NF 800R, Türkiye). The supernatant is carefully collected and kept at -80°C until used.

2.7.1. Determination of plasma MDA levels

Plasma lipid peroxide levels were estimated by the method as per Kurtel et al. (1992). The supernatants were added into 1 ml of a solution with 15% (wt/vol) tricarboxylic acid, 0.375% (wt/vol) thiobarbituric acid, and 0.25 N HCL following the centrifugation of aliquots (0.5 ml). Protein precipitate was eliminated through

centrifugation and the supernatants were placed in glass test tubes with 0.02% (wt/vol) butylated hydroxytoluene with the aim of avoiding further peroxidation of lipids in the preceding steps. Next, the samples were heated at 100°C in a boiling water bath for 15 min, cooled, and centrifuged to eliminate the precipitant. The absorbance of each sample was decided at 532 nm. (Spectrostar Nano, Germany). The expression of lipid peroxide levels was achieved with regards to MDA equivalents by employing an extinction coefficient of 1.56 x 105 mol^{-1} (Kurtel et al., 1992).

2.7.2. Determination of plasma NO levels

NO levels were estimated by the method as per Miranda et al., The supernatants were deproteinized with 0.3 M NaOH and 5% (w/v) $ZnSO_4$, centrifuged at 14 000 rpm for 5 min, and supernatants were utilized for the assays. Experiments were carried out at room temperature. Nitrate standard solution was consecutively diluted. After loading the plate with samples (100 µl), vanadium III chloride (VCl3) (100 µl) was added to each well and this was quickly followed by addition of Griess reagents, sulphanilamide (SULF) (50 µl) and N-(1-naphtyl) ethylenediamine dihydrochloride (NEDD) (50 µl). After incubation (usually 30-45 min), samples were measured at 540 nm by ELISA reader. (Miranda, Espey, Wink, 2001).

2.7.3. Determination of plasma RSH levels

The GSH levels were determined by the method as per Kurtel et al., 0.5 ml of each sample was blended with 1 ml of a solution with 100 mM Tris–HCl (pH 8.2), 1% sodium dodecyl sulfate, and 2 mM EDTA. Next, the mixture was incubated for 5 min at 25°C and centrifuged to eliminate any precipitant. 5,5-dithiobis (2-nitrobenzoic acid)/DTNB 0.3 mM was added to each reaction volume and incubated for 15 min at 37°C. The absorbance of each sample was 412 nm (Kurtel et al., 1992).

2.7.4. Determination of cerebral cortex MDA levels

Lipid peroxidation was quantified by measuring the formation of thiobarbituric acid reactive substances (TBARS). Samples were homogenized in ice-cold trichloroacetic acid (1 g tissue in 10 ml 10% trichloroacetic acid) in a tissue homogenizer (DAIHAN Scientific, Korea). Following centrifugation of the homogenate at 3,000 rpm for 10 min. 750 µl of supernatant was added to an equal volume of 0.67% (m/v) thiobarbituric acid and heated at 100°C for 15 min. The absorbances of the samples were measured at 535 nm. Lipid peroxide levels are expressed in terms of MDA equivalents using an extinction coefficient of 1.56 × 105 mol/cm (Casini et al., 1986).

2.7.5. Determination of cerebral cortex NO levels

NO levels were measured by Griess assay (Miranda, Espey, Wink, 2001). Prior to NO determination, the tissues were homogenized in five volumes of phosphate buffer saline (pH 7.4) and centrifuged at 2,000 g for 5 min. NaOH 0.25 ml, 0.3 M was added to 0.5 ml of the supernatant. After incubation for 5 min at room temperature, 0.25 ml of 5% (w/v) ZnSO4 was added for deproteinization. This mixture was then centrifuged at 14,000 rpm for 5 min and supernatants were used for the assays. A nitrate standard solution was serially diluted. After loading the plate with samples (100 µl), addition of vanadium III chloride (VCl_3) (100 µl) to each well was rapidly followed by addition of Griess reagents, sulphanilamide (SULF) (50 µl) and N-(1-naphtyl) ethylenediamide dihyrochloride (NEDD) (50 µl). After the incubation at 37°C (usually 30-45 min), samples were measured spectrophotometrically at 540 nm.

2.7.6. Determination of cerebral cortex GSH levels

The total GSH levels were determined by the Ellman method with some modifications (Aykaç et al., 1985). Briefly, samples were homogenized in ice-cold trichloroacetic acid (1 g tissue in 10 ml 10% trichloroacetic acid) in a tissue homogenizer. After centrifugation of the homogenates at 3,000 rpm for 10 min, 0.5 ml of supernatant was added to 2 ml of 0.3 M Na_2HPO_4 2 H_2O solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml 1% sodium citrate) was added and after mixing, the absorbance at 412 nm was immediately measured using a spectrophotometer at room temperature.

2.8. Statistical Analysis

Comparisons between the groups were performed using a one-way analysis of variance followed by post hoc Tukey tests. Paired Samples T-test was used to compare the initial and final measurements of body weight in the same group. The data were examined using spss 29.0 software and presented as mean ± Standard Deviation (SD).

3. Results

3.1. Impact of TQ on Body Weight in Rats Exposed to Hg

On day 22, rats exposed to Hg showed significantly lower body weight compared to both the control and TQ groups ($P<0.01$). However, co-administration of TQ with Hg (Hg+TQ group) partially reversed this weight loss, leading to a significant recovery compared to Hg-exposed rats ($P<0.05$) (Figure. 2).

3.2. Impact of TQ on Relative Brain/Body Weight Ratio in Hg Exposed Rats

When compared with the control group, no statistically significant was determined in the relative brain/body weight ratio of the TQ group ($P=0.3$). When compared with the control group and TQ group, there was a decrease in the relative brain/body weight ratio of rats exposed to Hg ($P<0.01$). When compared with Hg-exposed rats, TQ treatment given together with Hg caused an increase in the relative brain/body weight ratio ($P<0.05$) (Figure 3).

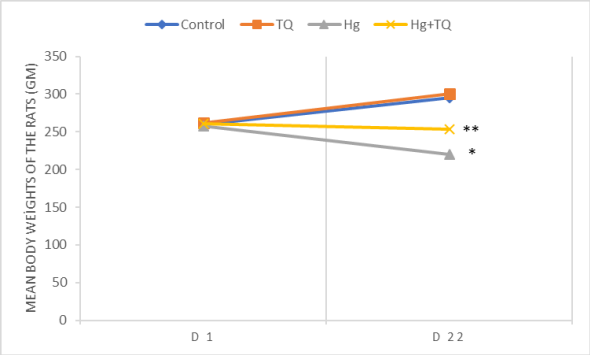

Figure. 2. Mean body weights of the rats (gm). D indicates day of experiment. Body weight was taken on day 1 and day 22. *P<0.01 Significant differences with Control and TQ groups; **P<0.05 Significant differences with Hg group. C: No treatment was performed, TQ: Received TQ, Hg: Received Hg, Hg+ TQ: Received Hg and TQ.

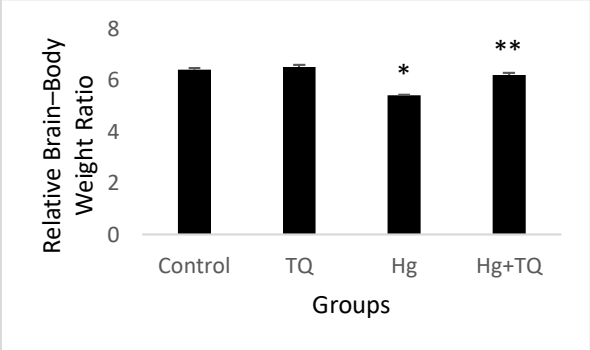

Figure. 3. Relative Brain/Body Weight Ratio. *P<0.01 Significant differences with Control and TQ groups; **P<0.05 Significant differences with Hg group. C: No treatment was performed, TQ: Received TQ, Hg: Received Hg, Hg+ TQ: Received Hg and TQ.

3.3. Behavior Parameters

Effects of TQ on Locomotor Activity and Anxiety-like Behavior in Hg Exposed Rats

On the 21st day of the study, an OFT made to evaluate locomotor activity and anxiety-like behaviors. For appraise the locomotor activity in Hg-exposed rats, the number of passes was recorded as the number of crossings that the animals crossed with their four paws. When the control group and TQ group were compared, there was no statistically significant in the number of crossings (P=0.2). When compared with the control and TQ groups, it was determined that there was a decrease in the number of crossings in rats exposed to Hg (P<0.001). When compared with Hg-exposed rats, TQ treatment given together with Hg increased the number of crossings (P<0.01).

The period spent in the center and periphery of the open field were examined to evaluate anxiety-like behaviors in rats exposed to Hg. When the control group and TQ group were compared, there was no statistically significant in the time spent in the center and periphery of the open field (P=0.3). Compared with the control and TQ groups, there was a decrease in the time spent in the center of the open field and an increase in the time spent in the periphery in rats exposed to Hg (P<0.001).

When compared with Hg-exposed rats, TQ treatment given together with Hg caused an increase in the period spent in the center of the open field and a decrease in the time spent in the periphery (P<0.01) (Figure 4A, 4B, 4C).

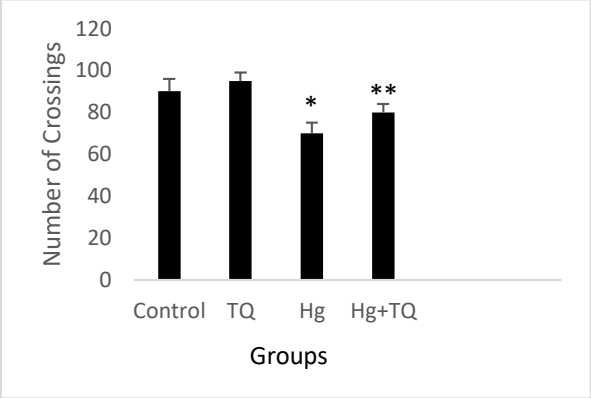

Figure. 4A. Number of crossings in the open field test. The values are means±SD; n=6. *P<0.001 Significant differences with Control and TQ groups; **P<0.01 Significant differences with Hg group. C: No treatment was performed, TQ: Received TQ, Hg: Received Hg, Hg+TQ: Received Hg and TQ.

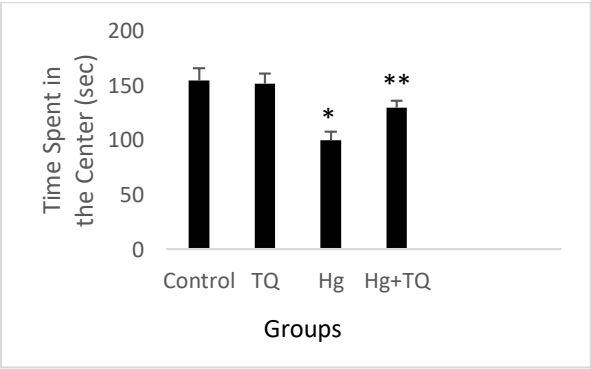

Figure. 4B. Time spent in the center (sec) in open field test. The values are means±SD; n=6. *P<0.001 Significant differences with Control and TQ groups; **P<0.01 Significant differences with Hg group. C: No treatment was performed, TQ: Received TQ, Hg: Received Hg, Hg+TQ: Received Hg and TQ.

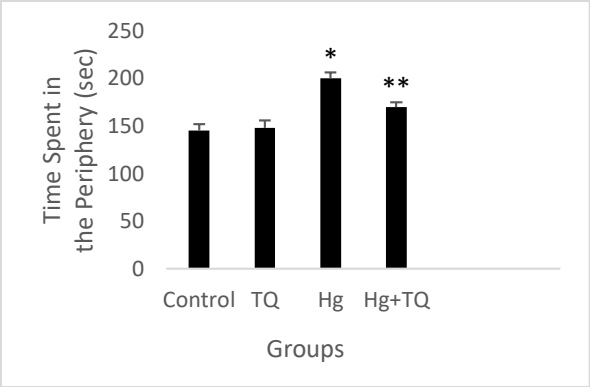

Figure 4C. Time spent in the periphery (sec) in open field test. The values are means±SD; n=6. * P<0.001 Significant differences with Control and TQ groups; ** P<0.01 Significant differences with Hg group. C: No treatment was performed, TQ: Received TQ, Hg: Received Hg, Hg+TQ: Received Hg and TQ.

3.5. The Effect of TQ on Depression-Like Behavior in Hg-Exposed Rats

On the 22nd day of the study, a forced swim test was performed to assess depression-like behaviors. Swimming, climbing, and immobility times were recorded to examine depression-like or hopelessness behaviors. When the control group and TQ group were compared, there was no statistically significant in swimming, climbing, and immobilization times (p=0.2). When compared to the control and TQ groups, there was an increase in immobility time and a decrease in swimming and climbing time in rats exposed to Hg (P<0.001). When compared with Hg-exposed rats, TQ treatment given together with Hg caused a decrease in immobility times (P<0.001) and a rise in swimming and climbing times (Figure. 5A, 5B, 5C) (respectively P<0.01, P<0.001).

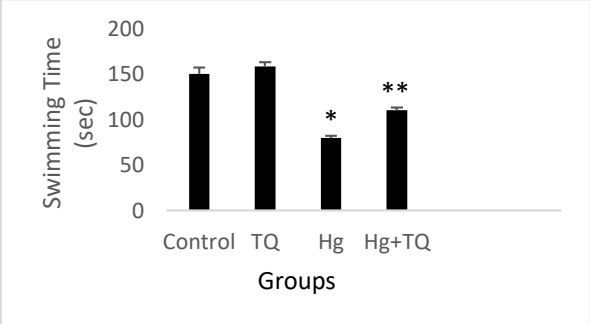

Figure. 5A. Swimming time (sec) in the forced swimming test. The values are means±SD; n = 6. * P<0.001 Significant differences with Control and TQ groups; ** P<0.01 Significant differences with Hg group. C: No treatment was performed, TQ: Received TQ, Hg: Received Hg, Hg+TQ: Received Hg and TQ.

3.4. The Effect of TQ on Oxidative Stress Markers in Rats Exposed to Hg

MDA, NO and GSH/RSH levels were analyzed to determine oxidative stress levels in cerebral cortex and plasma. When the control group and TQ group were compared, it was determined that there was no significant difference in MDA, NO and GSH levels in cerebral cortex (p=0.3). When compared to the control and TQ groups, it was determined that MDA and NO levels (respectively P<0.001, P<0.01) in the cerebral cortex of rats exposed to Hg increased, and GSH levels decreased (P<0.01). When compared with Hg-exposed rats, TQ treatment given together with Hg provided a significant improvement by increasing GSH levels in cerebral cortex and decreased MDA and NO levels (P<0.01) (Table 1).

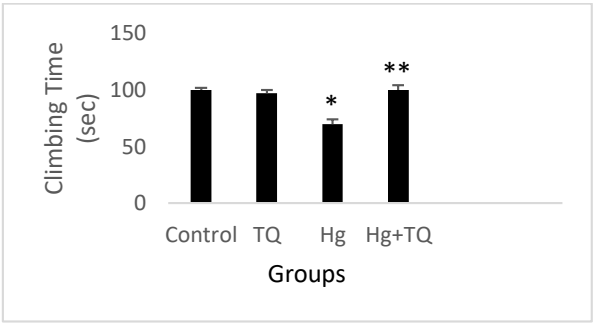

Figure 5B. Climbing time (sec) in the forced swimming test. The values are means±SD; n = 6. * P<0.001 Significant differences with Control and TQ groups; ** P<0.001 Significant differences with Hg group. C: No treatment was performed, TQ: Received TQ, Hg: Received Hg, Hg+TQ: Received Hg and TQ.

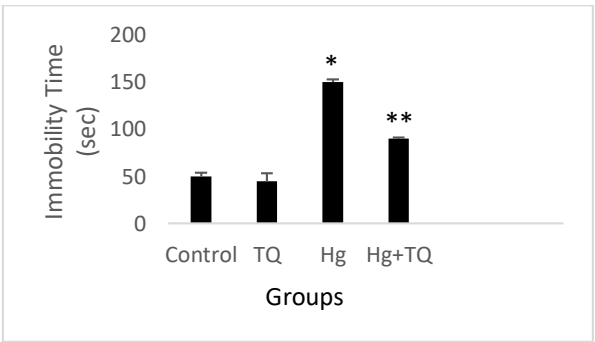

Figure 5C. Immobility time (sec) in the forced swimming test. The values are means±SD; n = 6. * P<0.001 Significant differences with Control and TQ groups; ** P<0.001 Significant differences with Hg group. C: No treatment was performed, TQ: Received TQ, Hg: Received Hg, Hg+TQ: Received Hg and TQ.

In plasma, there was no statistically significant in MDA, NO and RSH levels when the control group and TQ group were confront (P=0.8). When compared to the control group and TQ group, MDA and NO levels rised, and RSH levels reduced in the plasma of rats exposed to Hg (P<0.001). When compared with Hg-exposed rats, TQ treatment given together with Hg provided a significant

improvement by increasing RSH levels in plasma and reduced MDA and NO levels (P<0.01) (Table 2).

Table 1. The results of Cerebral Cortex MDA, NO ve GSH levels

	Cerebral cortex		
	MDA Levels (nmol/g)	NO Levels (µmol/g)	GSH Levels (nmol/g)
Control	4.43±0.18 c	0.29±0.03 c	4.2±0.08 c
TQ	4.62±0.21 c	0.27±0.01 c	4.6±0.2 c
Hg	7.5±0.12 a	0.45±0.02 a	2.7±0.03 a
Hg+TQ	5.1±0.093 b	0.3±0.01 b	3.4±0.19 b
P value	a: 0.001 b: 0.01 c: 0.03	a: 0.01 b: 0.01 c: 0.03	a: 0.01 b: 0.01 c: 0.03

The values are means±SD; n = 6. P<0.05 statistically significant. There is statistical significance between mean values shown with different letters in the same column.
a: Significant differences with Control and TQ groups,
b: Significant differences with Hg group.
C: No treatment was performed, TQ: Received TQ, Hg: Received Hg, Hg+ TQ: Received Hg and TQ

Table 2. The results of plasma MDA, NO ve RSH levels

	Plasma		
	MDA Levels (nmol/ml)	NO Levels (µmol/ml)	RSH Levels (nmol/ml)
Control	0.52±0.006 c	8.2±0.2 c	154.2±3.81 c
TQ	0.47±0.01 c	7.9±0.4 c	150.6±1.23 c
Hg	0.72±0.009 a	11.6±0.15 a	122.7±1.37 a
Hg+TQ	0.59±0.002 b	9.3±0.3 b	138.4±2.19 b
P value	a: 0.001 b: 0.01 c: 0.08	a: 0.001 b: 0.01 c: 0.08	a: 0.001 b: 0.01 c: 0.08

The values are means±SD; n = 6. P<0.05 statistically significant. There is statistical significance between mean values shown with different letters in the same column.
a: Significant differences with Control and TQ groups,
b: Significant differences with Hg group.
C: No treatment was performed, TQ: Received TQ, Hg: Received Hg, Hg+ TQ: Received Hg and TQ

4. Discussion

Experimental studies show that Hg causes damage to brain tissue looking at the literature (Minj et al., 2021; Shandilya et al., 2022). In this study, body weight and brain/body weight rate were calculated; behavioral tests were used; the levels of some oxidative stress markers in the brain and plasma were investigated to determine whether TQ has healing effects in rats exposed to Hg.

Exposure to 5 mg/kg Hg for 21 days caused a decrease in body weight and brain/body weight ratio of rats, which is also supported by the studies in the literature. In rats given 5 mg/kg Hg for three weeks, a reduction in body weight and brain weight/body weight rate was observed. The investigators reported significant demyelination with a total decrease in brain weight and shrinkage and demyelination in the basal ganglia, cortex, and hippocampus regions (Minj et al., 2021; Shandilya et al., 2022). In this study, 10 mg/kg TQ given with Hg for 21 days improved both body weight and brain/body weight ratio. In the literature, when postnatal body and brain weights were evaluated in fetuses exposed to lead in the womb, it was shown that 10 mg/kg TQ treatment produced a rise in body and brain weights. Researchers have reported that TQ is neuroprotective (Saleh et al., 2019). In rats with experimental Huntington's disease-like symptoms, 14 days of 40 and 80 mg/kg TQ suspension treatment caused a rise in body weight (Ramachandran and Thangarajan, 2016).

In this study, exposure to 5 mg/kg Hg for 21 days produced a decrease in the number of crossings in OFT, i.e., a decrease in locomotor activity in rats. In OFT, Hg exposure caused a reduction in the time spent in the center and a rise in the time spent in the periphery. This result suggests that Hg increases anxiety and causes neophobia in rats (Olczaka et al., 2011), which is in line with the studies in the literature. In rats exposed to 5 mg/kg Hg for three weeks, it was reported that there was a decrease in the number of crossings in OFT and locomotor activity and anxiety-like behaviors in rats (Minj et al., 2021; Shandilya et al., 2022). In the present study, 10 mg/kg TQ treatment increased the number of crossings and locomotor activity in OFT in rats exposed to Hg and caused improvement in anxiety-like behaviors. In 21-day-old infant rats with experimental attention deficit and hyperactivity disorder, it was found that 10 mg/kg TQ therapy for eight weeks increased locomotor activity in OFT (Abu-Elfotuh et al., 2023). TQ therapy with 5 mg/kg for three days before arsenic exposure increased locomotor activity in OFT, caused an increase in the period spent in the center of the open field and produced an anxiolytic effect (Firdaus et al., 2018). It was reported that 14 days of 40 and 80 mg/kg TQ suspension treatment increased locomotor activity in OFT in rats with experimental Huntington's disease model (Ramachandran and Thangarajan, 2018).

In this study, exposure to 5 mg/kg Hg for 21 days caused depression-like behaviors or hopelessness behaviors in rats by decreasing swimming and climbing times in FST and increasing immobility time. Studies in the literature support our results. In rats exposed to 5 mg/kg Hg for three weeks, a rise in immobility period in FST was reported (Minj et al., 2021; Shandilya et al., 2022). In the present study, in rats exposed to Hg, 10 mg/kg TQ treatment increased swimming and climbing times, decreased immobility time in FST, and caused improvement in depression-like behaviors. In rats in which experimental Huntington's disease-like symptoms were induced by Ramachandran and Thangarajan, (2016) 14 days of 10 and 20 mg/kg TQ therapy caused a reduce in immobility time in FST. In rats with an experimental depression model, 14 days of 10 mg/kg TQ treatment increased swimming and climbing and reduced immobility time (Kuzay et al., 2022). In another study in which an experimental depression model was

established, it was reported that TQ improved behavior and was effective as an antidepressant in FST (Aquib et al., 2015). These results related to behavioral tests support that TQ treatment shows neuroprotective effects by causing improvement.

Oxidative stress has been shown to play an important role in neurotoxicity caused by Hg exposure (Barber and Shaw, 2010). In this study, exposure to 5 mg/kg Hg for 21 days caused an increase in MDA and NO levels and a decrease in GSH levels in the cerebral cortex and plasma of rats. Our findings are consistent with previous studies showing that oxidative stress plays a major role in neurotoxicity caused by Hg exposure. In rats exposed to 5 mg/kg Hg for three weeks, an increase in MDA and NO levels and a decrease in GSH levels in brain tissue were reported (Minj et al., 2021; Shandilya et al., 2022). In the present study, 10 mg/kg TQ treatment caused a reduction in MDA and NO levels and a rise in GSH/RSH levels in cerebral cortex and plasma in rats exposed to Hg. This result shows that TQ acts as an antioxidant and may reduce the risk of oxidative damage. Studies in the literature also support this result. In rats exposed to 3 mg/kg mercury, 10 mg/kg TQ treatment decreased MDA levels in kidney tissue and increased GSH levels, and was observed to cause recovery (Fouda et al., 2008). In rats exposed to 20 µg mercury for 28 days, 5 mg/kg TQ treatment increased GSH levels in kidney and liver tissue and caused recovery (Owumi et al., 2025). Elevated MDA levels in the brain tissues of fetuses and pregnant rats exposed to lead were reduced following 10 mg/kg TQ treatment. Previous studies suggest that TQ acts as a potent free radical scavenger, preserving the activity of multiple antioxidant enzymes (Saleh et al., 2019). In the hippocampus of rats given 5 mg/kg TQ three days before arsenic exposure MDA levels due to arsenic exposure decreased, and decreased GSH levels increased. It has been reported that TQ reduces the general toxic environment in the cell thanks to its free radical scavenging mechanism (Firdaus et al., 2018).

5. Conclusion

In this study, it was investigated whether TQ has a neuroprotective effect in rats exposed to Hg. Although there are studies examining the antioxidant activity of TQ in liver, kidney and plasma in mercury exposure, there is no study examining its effects on cerebral cortex and behavior. Hg caused an increase in oxidative stress by increasing MDA and NO levels and decreasing GSH/RSH levels in cerebral cortex and plasma of rats. Hg decreased body weight and brain weight/body weight rate of rats. According to behavioral test results, Hg caused decreased locomotor activity, increased anxiety, and depression-like behaviors. In Hg-exposed rats, TQ treatment decreased Hg caused oxidative stress by reducing MDA and NO levels and increasing GSH/RSH levels. It increased body weight and brain/body weight rate and improved behavioral tests. TQ may be a hoping curative drug candidate in improving behavioral and biochemical changes due to Hg exposure.

Author Contributions

The percentages of the author' contributions are presented below. The author reviewed and approved the final version of the manuscript.

	D.K.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of interest

There is no conflict of interest.

Ethical Considerations

The experimental procedures were approved by the Local Animal Care and Ethics Committee of Kırşehir Ahi Evran University, (approval date: March 18, 2024, protocol code: 68429034/04).

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References

Abu-Elfotuh K, Abdel-Sattar SA, Abbas AN, Mahran YF, Alshanwani AR, Hamdan AME, Atwa AM, Reda E, Ahmed YM, Zaghlool SS, El-Din MN. 2023. The protective effect of thymoquinone or/and thymol against monosodium glutamate-induced attention-deficit/hyperactivity disorder (ADHD)-like behavior in rats: Modulation of Nrf2/HO-1, TLR4/NF-κB/NLRP3/caspase-1 and Wnt/β-catenin signaling pathways in rat model. Biomed Pharmacother, 162: 114735. https://doi.org/10.1016/j.biopha.2023.114735

Akarsu GD, Çetin A. 2022. The effect of thymoquinone on oxidative stress parameters and apolipoprotein E in Alzheimer model in rats. Dement Geriatr Cogn Disord, 51(4): 297-309. https://doi.org/10.1159/000522644

Aquib M, Najmi AK, Akhtar M. 2015. Antidepressant effect of thymoquinone in animal models of depression. Drug Res (Stuttg), 65(9): 490-494. https://doi.org/10.1055/s-0034-1395621

Aykaç G, Uysal M, Yalçın AS, Koçak-Toker N, Sivas A, Oz H. 1985. The effects of chronic ethanol ingestion on hepatic lipid peroxide, glutathione, glutathione peroxidase and glutathione transferase in rats. Toxicol, 36: 71-76.

https://doi.org/10.1016/0300-483X(85)90128-1

Barber SC, Shaw PJ. 2010. Oxidative stress in ALS: Key role in motor neuron injury and therapeutic target. Free Radic Biol Med, 48: 629-641. https://doi.org/10.1016/j.freeradbiomed.2009.11.018

Brocardo PS, Boehme F, Patten A, Cox A, Gil-Mohapel J, Christie BR. 2012. Anxiety- and depression-like behaviors are accompanied by an increase in oxidative stress in a rat model of fetal alcohol spectrum disorders: Protective effects of voluntary physical exercise. Neuropharmacology, 62: 1607-1618. https://doi.org/10.1016/j.neuropharm.2011.10.006

Casini AF, Ferrali M, Pompella A, Maellaro E, Comporti M. 1986. Lipid peroxidation and cellular damage in extrahepatic tissues of bromobenzene-intoxicated mice. Am J Pathol, 123: 520-531.

Firdaus F, Zafeer MF, Ahmad M, Afzal M. 2018. Anxiolytic and anti-inflammatory role of thymoquinone in arsenic-induced hippocampal toxicity in Wistar rats. Heliyon, 4(6): e00650. https://doi.org/10.1016/j.heliyon.2018.e00650

Fouda AM, Daba MY, Dahab GM, Sharaf el-Din OA. 2008. Thymoquinone ameliorates renal oxidative damage and proliferative response induced by mercuric chloride in rats. Basic Clin Pharmacol Toxicol, 103(2): 109-118. https://doi.org/10.1111/j.1742-7843.2008.00267.x

Gstraunthaler G, Pfaller W, Kotanko P. 1983. Glutathione depletion and in vitro lipid peroxidation in mercury- or maleate-induced acute renal failure. Biochem Pharmacol, 32: 2969-2972. https://doi.org/10.1016/0006-2952(83)90513-4

Kurtel H, Granger DM, Tso P, Grisham MB. 1992. Vulnerability of intestinal interstitial fluid to oxidant stress. Am J Physiol, 263(4 Pt 1): G573-G578. https://doi.org/10.1152/ajpgi.1992.263.4.G573

Kustimur S, Kalkanci A, Akbulut G, Gonul B, Bulduk E, Aksakal FN, Yetkin I. 2007. The effect of vaginal candidiasis on the levels of oxidative biomarkers in plasma and tissue samples of diabetic rats. Mycopathologia, 164(5): 217-224. https://doi.org/10.1007/s11046-007-9048-3

Kuzay D, Dileköz E, Özer Ç. 2022. Effects of thymoquinone in a rat model of reserpine-induced depression. Braz J Pharm Sci, 58: e19847. https://doi.org/10.1590/s2175-97902022e19847

Minj E, Upadhayay S, Mehan S. 2021. Nrf2/HO-1 signaling activator acetyl-11-keto-β-boswellic acid (AKBA)-mediated neuroprotection in methyl mercury-induced experimental model of ALS. Neurochem Res, 46: 2867-2884. https://doi.org/10.1007/s11064-021-03374-y

Miranda KM, Espey MG, Wink DA. 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide, 5(1): 62-71. https://doi.org/10.1006/niox.2000.0319

Nath KA, Croatt AJ, Likely S, Behrens TW, Warden D. 1996. Renal oxidant injury and oxidant response induced by mercury. Kidney Int, 50: 1032-1043. https://doi.org/10.1038/ki.1996.390

Olczak M, Duszczyk M, Mierzejewski M, Meyza K, Majewska MD. 2011. Persistent behavioral impairments and alterations of brain dopamine system after early postnatal administration of thimerosal in rats. Behav Brain Res, 223: 107-118. https://doi.org/10.1016/j.bbr.2011.04.036

Owumi S, Otunla M, Akindipe P, Oluwawibe B, Babalola JA, Chimezie J, Arunsi U, Owoeye O, Oyelere AK. 2025. Thymoquinone modulates oxidative stress and inflammation, correcting mercury-induced HO-1/NRF/Trx pathway disruption in experimental rat hepatorenal system: An in vivo and in silico study. Biometals, 38: 1179-1202. https://doi.org/10.1007/s10534-025-00699-1

Ramachandran S, Thangarajan S. 2016. A novel therapeutic application of solid lipid nanoparticles encapsulated thymoquinone (TQ-SLNs) on 3-nitropropionic acid induced Huntington's disease-like symptoms in Wistar rats. Chem Biol Interact, 256: 25-36. https://doi.org/10.1016/j.cbi.2016.06.027

Ramachandran S, Thangarajan S. 2018. Thymoquinone loaded solid lipid nanoparticles counteracts 3-nitropropionic acid induced motor impairments and neuroinflammation in rat model of Huntington's disease. Metab Brain Dis, 33: 1459-1470. https://doi.org/10.1007/s11011-018-0257-6

Saleh HA, Abd El-Aziz GS, Mustafa HN, El-Fark M, Mal A, Aburas M, Deifalla AH. 2019. Thymoquinone ameliorates oxidative damage and histopathological changes of developing brain neurotoxicity. J Histotechnol, 42(3): 116-127. https://doi.org/10.1080/01478885.2019.1586344

Shandilya A, Mehan S, Kumar S, Sethi P, Narula AS, Alshammari A, Alharbi M, Alasmar AF. 2022. Activation of IGF-1/GLP-1 signalling via 4-hydroxyisoleucine prevents motor neuron impairments in experimental ALS rats exposed to methylmercury-induced neurotoxicity. Molecules, 27: 3878. https://doi.org/10.3390/molecules27123878

Yavuz CI. 2020. Çevresel cıva maruz kalımı ve sağlık etkileri. Turk J Public Health, 18(2): 204-217. https://doi.org/10.20518/tjph.710343

Zhang H, Wang S, Wang Y, Lu A, Hu C, Yan C. 2021. DHA ameliorates MeHg-induced PC12 cell apoptosis by inhibiting the ROS/JNK signaling pathway. Mol Med Rep, 24: 558. https://doi.org/10.3892/mmr.2021.12228