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EFFECT OF THYMOQUINONE AND BUTYLATED HYDROXYTOLUENE ON BEHAVIOR AND OXIDATIVE STRESS LEVEL IN RATS CHRONICALLY EXPOSED TO ETHANOL

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Abstract: The objective of this study is to examine the antioxidant activity of Thymoquinone (TQ) and Butylated Hydroxytoluene (BHT) and its effect on behavioral tests in rats chronically exposed to ethanol. The experimental groups were determined as Control, Sham, Ethanol, Ethanol+BHT, Ethanol+TQ and Ethanol+BHT+TQ. In order to create a chronic alcohol exposure model in rats, 7 g/kg ethanol was given for 4 weeks. BHT and TQ were given at a dose of 10 mg/kg for 4 weeks. Glutathione, malondylaldehyde and total nitric oxide levels were analyzed to evaluate oxidative stress in brain, stomach, liver and kidney tissue. Open field test and forced swim test were used to examine anxiety disorder and depression-like behaviors in rats. Compared with the control group, ethanol exposure increased malondylaldehyde and total nitric oxide levels and decreased glutathione levels in all tissues ($P<0.05$). BHT, TQ and BHT+TQ treatment increased glutathione levels and decreased malondylaldehyde levels in all tissues ($P<0.05$). In the ethanol-exposed group, swimming time decreased and immobility time increased, and it was determined that 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 ($P<0.05$). BHT and TQ treatment increased swimming time, decreased immobility time and caused an increase in the time spent in the center of the open field ($P<0.05$). Our findings showed that BHT and TQ contributed critically to the protection against ethanol-induced oxidative stress in tissues. BHT and TQ treatment improved behavioral tests after ethanol exposure. The most significant improvement was seen in the group that was given BHT+TQ simultaneously after ethanol exposure.

Keywords: Ethanol, Behavior, Oxidatıve Stress, Antioxidants, Rats, Nigella sativa

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1. Introduction

Ethanol is a greatly consumed organic solvent with toxic properties both the central nervous system and other systems such as the liver, kidneys and gastrointestinal tract. Around two billion people in the world consume ethanol and around eighty million people have been diagnosed with ethanol-related illnesses.

Chronic ethanol use raises the production of reactive oxygen species (ROS) in cells (Zhang et al., 2012; Reddy et al., 2014). ROS are the major mediators of oxidative stress. They reduce enzymatic antioxidants and non-enzymatic antioxidants (such as glutathione (GSH)). In addition, ROS oxidize lipids in the cell structure. Many by-products are formed as a result of lipid peroxidation and the most important of these is Malondylaldehyde (MDA). MDA reacts with many molecules in the cell and causes negative effects due to disruption of the structure (Sanpinit et al., 2022). Nitric Oxide (NO) is another biological molecule that can cause an increase in oxidative stress like ROS. At high concentrations, NO rapidly reacts with superoxide radicals and forms peroxynitrite. Peroxynitrite is cytotoxic (Şener et al., 2015).

There are studies showing that ethanol rises NO and MDA levels and reduces GSH levels in cells (Li et al., 2015; Park et al., 2021; Sanpinit et al., 2022). In addition, chronic ethanol exposure causes oxidative stress, edema, excitotoxicity, neuroinflammation, glial scarring and neurodegeneration in brain cells (Duncan et al., 2016). Oxidative stress has also been shown to accelerate telomere shortening, inflammation excitotoxicity and mitochondrial dysfunction. Oxidative stress is also considered to be included in the etiology of neuropsychiatric disorders, such as behavioral disorders associated with chronic alcohol use. The most common behavioral disorders in chronic alcohol users are depression and anxiety disorders (Tsermpini et al., 2022.) Open field tests and forced swimming tests are used to demonstrate anxiety disorder and depression-like behaviours in experimental animals (Brocardo et al., 2012).

Oxidative stress caused by ethanol can be prevented by antioxidants. With respect to the World Health Organization, about 70% to 80% of the world's

population trust traditional medicine. Herbal treatments have been the most utilized form of traditional medicine. Thymoquinone (TQ) is the very important component of the essential oil of Nigella sativa seeds. Previous studies indicate TQ has neuroprotective, anti-inflammatory and antioxidant effects (Kanter et al., 2005; Hosseini et al., 2017; Mehanna et al., 2021; Sanpinit et al., 2022).

Butylated hydroxytoluene (BHT) is a phenol-derived lipophilic organic compound used as an antioxidant. Its antioxidant activity has been proven and is a product used in the food industry to extend the life of packaged foods. However, its effect on cells in biological systems is still being studied (Hamelink et al., 2005).

When we look at the literature, although there are a few studies showing that TQ has antioxidant effects in ethanol exposure, there are no studies examining the antioxidant effect of BHT in ethanol exposure. Additionally, there is no study examining the effects of TQ and BHT on behavior during ethanol exposure.

Therefore, the objective of this study was to examine the impacts of TQ and BHT on oxidative stress levels in the brain, stomach, liver and kidney tissues and behavioral tests of rats exposed to chronic ethanol.

2. Materials and Methods

2.1. Animals

In the study, 36 adult male Wistar Albino rats weighing between 250±20 g were used. Each rat was housed in separate cages in a 12-hour light-dark cycle at 24±2 ºC and fed with standard rat chow and tap water ad libitum.

2.2. Experimental Design

The study design is given in Table 1. All treatments were performed 5 days a week for 4 weeks. The control group was fed with normal rat feed and tap water in the cage. No treatment was performed. The sham group was created to study gavage-induced stress.

Table 1. Experimental design

Group	Number of rats	Explanations
C	n=6	No treatment was performed
S	n=6	Received tap water
E	n=6	Received ethanol
E+B	n=6	Received ethanol and BHT
E+T	n=6	Received ethanol and TQ
E+B+T	n=6	Received ethanol and BHT and TQ

C= control, S= sham, E= etanol, E+B= etanol+BHT, E+T= etanol + TQ, E+B+T= etanol+BHT+TQ

2.3. Chronic Alcohol Exposure

In light of the information obtained from the literature, ethanol given at doses of 3 g/kg and higher causes chronic effects. In this study, in order to create a chronic alcohol exposure model, rats were given approximately 40% ethanol (Sigma Aldrich, USA) at a dose of 7 g/kg by intragastric gavage 5 days a week for 4 weeks (Özcan and Mengi, 1998; Pal et al., 2022).

2.4. Treatment

10 mg/kg BHT (ZAG Chemistry, Türkiye) dissolved in ethanol and 10 mg/kg TQ (CAYMAN Chemical, USA) dissolved in tap water were given by intragastric gavage immediately after ethanol administration for 4 weeks, 5 days a week (Aksoy et al., 2015; Fouad and Jresat, 2015; Hosseini et al., 2017). The drugs were make ready newly every day. It was injected in a volume of 1 ml/kg. Experiments were carried out at 8:00 - 10:00 in the morning.

2.5. Behavioral Studies

Forced swimming test (Fst): To examine the severity of depression, we made a changed Fst, according to the already defined method. A 15-minute pre-test was performed on the rats to prevent acute stress. One day after the pre-test, each rat was floated separately in a 30 cm water-filled container at 25°C±1°C for 5 minutes. (In cylindrical containers with a diameter of 15 cm and a height of 50 cm). Along the test period swimming, climbing and immobility time was registered by a video camera (Kuzay et al., 2022).

Open field test (Oft): To evaluate the spontaneous locomotor activity of the rats, the individual movements of each were registered with a video camera for 5 minutes in a white plexiglass arena with a diameter of 90 cm x 35 cm, whose floor was seperated into 24 units with black stripe. The strips that animals crossed with their four paws were recorded as the number of crossings. The time spent in the center and periphery the open area was also recorded (Brocardo et al., 2012). Two hours after the treatment, behavioral tests of each rat was measured. Figure 1 presents the timeline of behavioral testing.

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 28 and in the open field test on day 29. Body weight (BW) was taken on day 0 and day 30.

2.6. Determination of Oxidative Stress Levels

At the end of 1 month, all rats were sacrificed under intra muscular Rompun (5mg/kg) + Ketamine (45mg/kg) anesthesia by removing blood from their hearts. Brain, stomach, liver and kidney tissues were frozen in liquid nitrogen and stored at -80 °C until the day of the study. GSH, MDA and total NO levels were investigated to evaluate oxidative stress. GSH levels were determined by the Modified Ellman method, MDA levels by

Thiobarbituric acid reactive substance formation, and NOx levels by the Griess method (Ayka et al., 1985; Gilbert, 2000; Miranda et al., 2001; Hassanien et al., 2015).

2.7. Statistical Analysis

Data were examined using the Statistical Package for Social Sciences 15.0 software program and offered as mean±standard deviation (SD). Comparisons between groups were made using one-way analysis of variance followed by post hoc Tukey tests. Paired Samples T test was utilized for the assessment of body weight.

3. Results

3.1. Forced Swimming Test (Fst)

In comparison to the control group, the ethanol group displayed a characteristic depressive-like behavior such as less climbing, less swimming, and prolonged immobility in Fst (P<0.05). TQ and TQ+BHT cure caused an important decrease in immobility time and a rise in climbing time and swimming time in Fst compared to the rats receiving only ethanol (P<0.05). BHT treatment caused an important decrease in immobility time and a rise in swimming time in Fst compared to the rats receiving only ethanol (P<0.05) but climbing time was not statistically significant (P>0.05) (Figure 2, 3, 4).

3.2. Open Field Test (Oft)

There was an increase time spent in the periphery and a decrease in the number of crossings and time spent in the center in the Ethanol group when compared to the control group (P<0.05). BHT cure induced reduction time spent in the periphery and an increase time spent in the center compared to the rats receiving only ethanol (P<0.05). BHT treatment did not produce a statistically important change in the number of crossings (P>0.05). TQ and TQ+BHT cure caused a significant decrease time spent in the periphery and a rise in the number of crossings and time spent in the center compared to the ethanol group (P<0.05) (Figure 5, 6, 7).

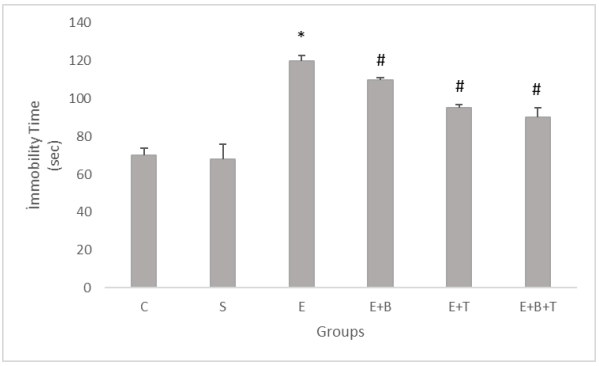

Figure 2. Immobility time (sec). The values are means±SD; n = 6, * P<0.05 Significant differences with C and S groups; # P<0.05 Significant differences with E group. C= no treatment was performed, S= received tap water, E= received ethanol, E+B= received ethanol and BHT, E+T: received ethanol and TQ, E+B+T=received ethanol and BHT and TQ.

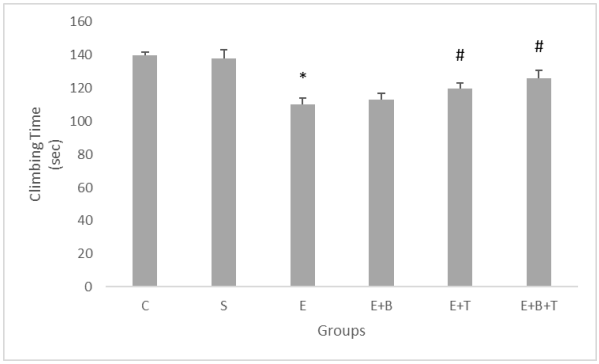

Figure 3. Climbing time (sec). The values are means±SD; n = 6, * P< 0.05 Significant differences with C and S groups; # P<0.05 Significant differences with E group. C= no treatment was performed, S= received tap water, E= received ethanol, E+B= received ethanol and BHT, E+T= received ethanol and TQ, E+B+T= received ethanol and BHT and TQ.

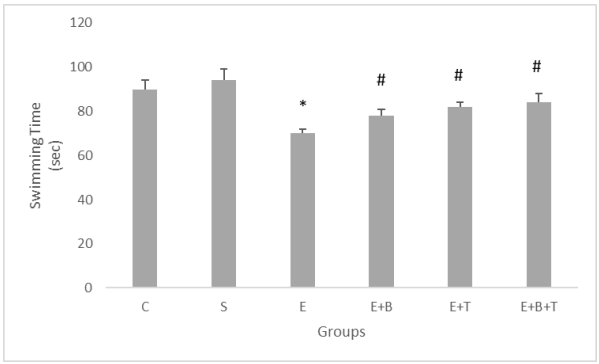

Figure 4. Swimming time (sec). The values are means±SD; n = 6, * P<0.05 Significant differences with C and S groups; # P<0.05 Significant differences with E group. C= no treatment was performed, S= received tap water, E= received ethanol, E+B= received ethanol and BHT, E+T= received ethanol and TQ, E+B+T= received ethanol and BHT and TQ.

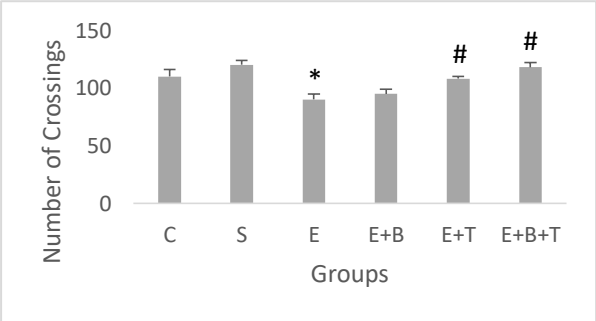

Figure 5. Number of crossings. The values are means±SD; n = 6, * P<0.05 Significant differences with C group; # P<0.05 Significant differences with E group. C= no treatment was performed, S= received tap water, E= received ethanol, E+B= received ethanol and BHT, E+T= received ethanol and TQ, E+B+T= received ethanol and BHT and TQ.

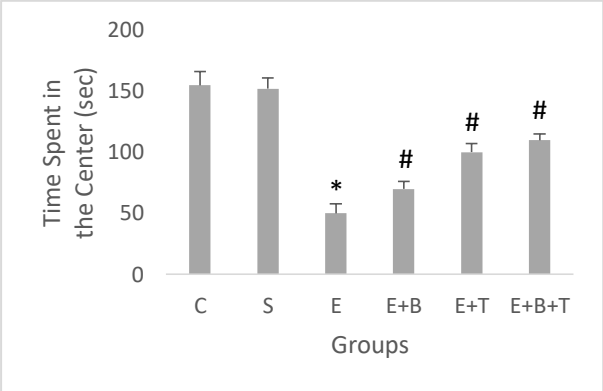

Figure 6. Time spent in the center (sec). The values are means±SD; n = 6, * P<0.05 Significant differences with C and S groups; # P<0.05 Significant differences with E group. C= no treatment was performed, S= received tap water, E= received ethanol, E+B= received ethanol and BHT, E+T= received ethanol and TQ, E+B+T= received ethanol and BHT and TQ.

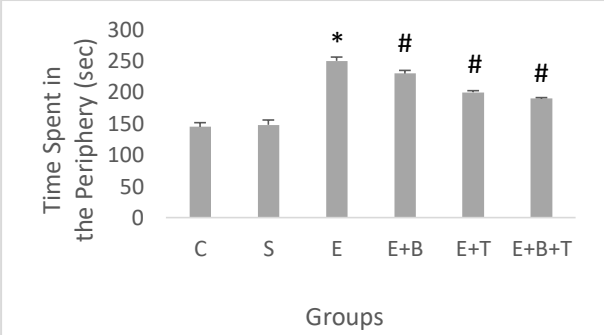

Figure 7. Time spent in the periphery (sec). The values are means±SD; n = 6, * P<0.05 Significant differences with C and S groups; # P<0.05 Significant differences with E group. C= no treatment was performed, S= received tap water, E= received ethanol, E+B= received ethanol and BHT, E+T= received ethanol and TQ, E+B+T= received ethanol and BHT and TQ.

3.3. Body Weight

At the end of the 30th day, there was a rise in body weight in the control and sham groups (P<0.05). Additionally, it was determined that there was a statistically important rise in body weight of groups TQ and TQ+BHT treatment (P<0.05). There were no important alteration in body weight in Ethanol group and BHT cure group (P>0.05) (Figure 8).

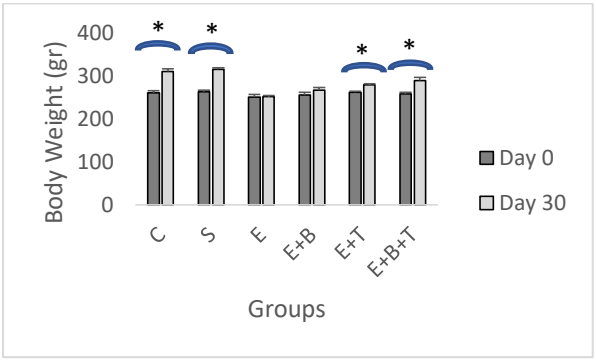

Figure 8. Measured body weight on the 0th day and the30th day. The values are means±SD; n = 6. * P< 0.05. C= no treatment was performed, S= received tap water, E= received ethanol, E+B= received ethanol and BHT, E+T= received ethanol and TQ, E+B+T= received ethanol and BHT and TQ.

3.4. Oxidative Stress

Compared with the control group, it was determined that MDA and NOx levels rised and GSH levels reduced in the ethanol group's brain, stomach, liver and kidney tissues (P<0.05). In the brain tissue, when compared with the Ethanol group, there was a decrease in MDA and NOx levels and an increase in GSH levels in the BHT and BHT+TQ treatment groups (P<0.05). In the brain tissue, when compared with the Ethanol group, it was defined that there was a reduce in MDA levels and a rise in GSH levels in the TQ cure group (P<0.05), but the decrease in NOx levels was not important (p>0.05). In the stomach tissue, there was a reduce in MDA and NOx levels and an increase in GSH levels in the BHT, TQ and BHT+TQ treatment groups compared to the Ethanol group (P<0.05). In liver and kidney tissue, when compared with the ethanol group, there was a decrease in MDA and NOx levels and an increase in GSH levels in the TQ and BHT+TQ treatment groups (P<0.05). In liver and kidney tissue, when compared with the Ethanol group, it was defined that there was a reduce in MDA levels and a rise in GSH levels in the BHT cure group (P<0.05), but the decrease in NOx levels was not important (p>0.05). In the brain, stomach, liver and kidney tissues, the highest reduce in MDA and NOx levels and the highest rise in GSH levels were determened in the BHT+TQ cure group (P<0.05). The findings are given in Table 2, 3, 4, 5.

Table 2. The results of stomach tissue MDA, NO ve GSH levels

	Group (n=6)	MDA Levels (nmol/g)	NO Levels (µmol/g)	GSH Levels (nmol/g)
Stomach Tissue	C	3.7±0.1	8.2±0.6	9.4±0.6
	S	3.2±0.9	7.9±0.9	8.3±1.2
	E	8.6±0.15*	12.3±0.1*	5.6±0.9*
	E+B	6.5±0.7 #	9.6±0.7#	7.1±0.8 #
	E+T	5.33±0.9 #	10.2±0.2#	6.8±0.3 #
	E+B+T	5.21±0.7 #	9.1±0.8 #	7.2±0.1 #

The values are means±SD; n = 6. * P< 0.05 Significant differences with C group; # p <0.05 Significant differences with E group. C= no treatment was performed, S= received tap water, E= received ethanol, E+B= received ethanol and BHT, E+T= received ethanol and TQ, E+B+T= received ethanol and BHT and TQ.

Table 3. The results of liver tissue MDA, NO ve GSH levels

	Group (n=6)	MDA Levels (nmol/g)	NO Levels (µmol/g)	GSH Levels (nmol/g)
Liver Tissue	C	2.84±0.22	9.11±0.7	10.2±0.6
	S	2.76±0.3	9.3±0.4	9.8±1.3
	E	7.9±0.2*	11.9±0.7*	7.4±0.5*
	E+B	5.3±0.1 #	11.1±0.2	8.1±0.8#
	E+T	5.9±0.24 #	10.1±0.4#	8.6±0.7#
	E+B+T	5.1±0.1 #	9.8±0.1#	9.0±0.1#

The values are means±SD; n = 6. * P< 0.05 Significant differences with C group; # p <0.05 Significant differences with E group. C= no treatment was performed, S= received tap water, E= received ethanol, E+B= received ethanol and BHT, E+T= received ethanol and TQ, E+B+T= received ethanol and BHT and TQ.

Table 4. The results of brain tissue MDA, NO ve GSH levels

	Group (n=6)	MDA Levels (nmol/g)	NO Levels (µmol/g)	GSH Levels (nmol/g)
Brain Tissue	C	4.35±0.16	0.31±0.08	4.1±0.2
	S	4.6±0.26±0.20	0.33±0.07	3.9±0.05
	E	6.22±0.21 *±0.21*	0.46±0.02 *	2.6±0.08 *
	E+B	5.03±0.2 #03±0.8#	0.38±0.03 #	3.8±0.12 #
	E+T	5.42±0.15 #.42±0#	0.42±0.06	4.02±0.09 #
	E+B+T	4.85±0.46 #85±0.46	0.36±0.01 #	4.3±0.12 #

The values are means±SD; n = 6. * P<0.05 Significant differences with C group; # P<0.05 Significant differences with E group. C= no treatment was performed, S= received tap water, E= received ethanol, E+B= received ethanol and BHT, E+T= received ethanol and TQ, E+B+T= received ethanol and BHT and TQ.

Table 5. The results of kidney tissue MDA, NO ve GSH levels

	Group (n=6)	MDA Levels (nmol/g)	NO Levels (µmol/g)	GSH Levels (nmol/g)
Kidney Tissue	C	4.4±0.6	10.3±1.8	2.3±0.2
	S	4.6±0.4	10.4±0.9	2.1±0.1
	E	6.84±0.8*	12.7±0.7*	1.5±0.6*
	E+B	5.3±0.3 #	12.1±0.6	1.8±0.4#
	E+T	5.6±0.6 #	11.6±0.2#	1.7±0.7#
	E+B+T	5.2±0.5 #	11.2±1.1#	1.9±0.6#

The values are means±SD; n = 6. * P<0.05 Significant differences with C group; # P<0.05 Significant differences with E group. C= no treatment was performed, S= received tap water, E= received ethanol, E+B= received ethanol and BHT, E+T= received ethanol and TQ, E+B+T= received ethanol and BHT and TQ.

4. Discussion

In this study, the impacts of TQ and BHT on oxidative stress and behaviour were examined in rats chronically exposed to ethanol. Exposure to 7 g/kg of ethanol 5 days a week for 4 weeks importantly rised immobility time and reduced climbing and swimming time in the Fst. In the Oft, it was observed that ethanol exposure caused a rise in the time spent in the periphery and a decrease number of crossings and in the time spent in the center. There was no change in the body weight of the rats in the

measurements made after 30 days. Our results are appropriate with the results of studies in the literature. Exposure to 6.5 g/kg 22.5% ethanol once a day for 55 days in 35-day-old rats caused a decrease the number of crossings in the Oft. Researchers have reported that long-term alcohol exposure may cause damage to the cerebellar, neocortex, and motor cortex due to increased oxidative stress and inflammation (Teixeira et al., 2014). Puppies exposed to 5 g/kg 22.66% ethanol on days 4-9 after birth, It has been reported that the number of crossings decreases in the Oft and the immobility time increases in the Fst (Mazurek et al., 2021). Behavioral tests were performed on the offspring of mother rats exposed to 36% 4.3 g/kg ethanol on the 1st and 22nd days of pregnancy, on the 60th day after birth. There was a rise in the time spent in the periphery and a reduce in the time spent in the center in Oft. It was observed that there was an increase immobility time in Fst. It was stated that the body weight of pups exposed to ethanol did not differ (Brocardo et al., 2012). It has been reported that exposure to 4.8 g/kg of ethanol twice a day for 3 days caused a decrease in the number of crossings in 75–84-day old adult rats and no change in 30–37-day old adolescent rats in the Oft (Sarkar et al., 2013).

According to the Oft results in our study, 30-day cure with BHT at doses of 10 mg/kg treatment caused a rise in the time spent in the center and a decline in the time spent in the periphery. It was seen to increase the number of crossings slightly, but it was not found to be statistically significant. According to Fst, BHT treatment caused a decline in immobility time and a rise in swimming time. There was no important alteration in the body weight of the rats in the measurements made after 30 days. There is no study in the literature examining the effect of BHT treatment on behaviour. However, in a study examining the effects of BHT on locomotion and anxiety-like behaviours of zebrafish larvae. It was determined that BHT increased locomotor activity. Researchers suggest that BHT, in addition to its antioxidant activity, increases Dopamine signaling through Dopamine 2 and 3 receptors (Liang et al., 2020).

30-day cure with TQ at doses of 10 mg/kg importantly cured ethanol-induced alterations. TQ reduced the immobility time and rised the climbing and swimming time in the Fst. According to the Oft results in our study, TQ caused an increase the number of crossings and in the time spent in the center and a decline in the time spent in the periphery. It caused an increase in weight gain at the end of the 30th day. In the literature, there are no studies regarding the impacts treatment of TQ on behavioral tests in the ethanol exposure. However, there are a few studies examining the impact of TQ on behavioral tests. Our results are appropriate with the results of studies in the literature. A one dose of 40 mg/kg TQ reduced immobility time in Fst in rats induced by lipopolysaccharide stress. According to the results of Oft, it caused a decline in the time spent in the periphery and a rise in the time spent in the center. However, it did not induce any alteration in the number of crossings (Hosseini et al., 2012). In cisplatin-induced toxicity, 20 mg/kg TQ caused a rise in the time spent in the central region and the number of crossings and a decline in the time spent in the peripheral region in the Oft (Kandeil, et al., 2020). In a reserpine-induced depression model 20 mg/kg TQ treatment resulted in a rise in the number of crossings in the Oft, a decline in immobility time and a rise in swimming time in the Fst (Fahmy et al., 2020).

In our study, TQ and BHT+TQ cure indicated better healing in comparison to BHT cure in behavioral tests. We can say that the best recovery is in the BHT+TQ cure group. There is no study in the literature on the effect of TQ+BHT combined treatment on behavioral tests.

Alcohol intake has been found to be associated with altered oxidant-antioxidant balance and cell functions. Chronic ethanol exposure has been found to cause toxic effects through ROS production and lipid peroxidation in various tissues and cells of humans and mammals (Hosseini et al., 2017). In this study, a chronic exposure model was established with 7 g/kg ethanol 5 days a week for 4 weeks. At the end of 1 month, an elevate in NOx and MDA levels and a decline in GSH levels were observed in brain, stomach, liver and kidney tissues. Our results are appropriate with the results of studies in the literature. Studies show that alcohol quickly diffuses through the blood-brain barrier and changes neurotransmission. Alcohol exposure is reported to increase MDA levels and reduce GSH levels in brain tissue. It causes impaired regeneration and neurodegeneration by activation of microglia and astrocytes (Tsermpini et al., 2022). In the study by Carol et al., it was shown that there was a large amount of neuron loss in the hippocampal cortical circuits in the coronal sections taken from the brains of rats given ethanol by gavage at a dose of 9-15 gr/kg for 4 days. The most affected regions were found to be the olfactory bulb and dentate gyrus granular cell layer. It was also found that the perirhinal, piriform and entorhinal cortex were affected (Hamelink et al., 2005).

Ethanol causes deterioration of the gastric mucosa, increasing mucosal permeability and bleeding. This leads to gastric ulcers. Leaking white blood cells (such as neutrophils) from the disrupted mucosa cause ROS production and overproduction of other inflammatory mediators. This leads to oxidative damage and cellular damage. In rats given 1 ml/kg of 80% ethanol orally for 7 days, GSH levels decreased, ROS, MDA and induced nitric oxide synthase levels increased in the stomach tissue (Sanpinit et al., 2022). Kanter et al. showed that MDA levels rised and GSH levels reduced in the stomach tissue samples of rats with gastric mucosa damage induced with 1 ml pure ethanol (Kanter et al., 2005). Furthermore, increased free radicals and ROS due to chronic ethanol use destroy gastric mucus, and increase gastric acidity and inflammatory cell infiltration. As a result, gastric ulcers are formed (Sanpinit et al., 2022; Kanter et al., 2005). When 6 mL/kg of 56% ethanol once a day for the first 4 weeks and 8 mL/kg of 56% ethanol

once a day for the next 12 weeks were given by gavage for a total of 16 weeks, MDA and ROS levels in serum and liver tissue rised and GSH levels declined (Xue et al., 2022). In the liver tissue of rats given 7 gr/kg ethanol orally for 28 days, a rise in MDA levels and a decline in GSH levels were determined (Pal et al., 2022).

The liver is the most important organ metabolizing alcohol. Alcohol metabolism in the liver disrupts lipid metabolism. It causes the production of metabolites and by-products that increase inflammatory reactions. Alcohol metabolized by cytochrome P450 2E1 in the liver cause ROS overproduction and induction of endoplasmic reticulum stress. This impairs lysosomal function and autophagy. This results in mitochondrial damage and hepatocellular death. Researchers have reported that oxidative stress triggers the CD14/TLR4 pathway in alcoholic liver disease, which causes cellular damage through the activation of macrophages and the production of inflammatory mediators such as IL1, IL-6 and TNF-. They also reported that these inflammatory factors further accelerate oxidative stress and reduce the antioxidant enzyme capacity of cells. In the liver tissue of rats given 3 and 5 mg/kg 70% ethanol daily by intragastric gavage for 28 days, an elevate in MDA levels and a decline in GSH levels were observed. Researchers suggest that antioxidants in cells are over-consumed to prevent oxidative stress caused by accelerated inflammation (Mehanna et al., 2021). In liver tissues of rats, 3 mg/kg/day ethanol exposure by gavage for 4 weeks induced an elevate in MDA levels and a decline in GSH levels (Hamelink et al., 2005). Increased oxidative stress in liver cells in chronic ethanol use causes cell membrane and mitochondrial damage, acetaldehyde accumulation, hypoxia, impairment of the immune system and iron mobilization. In alcohol-induced liver disease, steatosis, fatty liver and alcoholic hepatitis develop in the early stages. Further progression of the disease can lead to irreversible fibrosis, cirrhosis, and liver cancer (Xue et al., 2022).

Chronic ethanol exposure rises oxidative stress in the kidneys by causing hyperacetylation of mitochondrial proteins. This leads to metabolic dysregulation and disrupted renal function. The TLR4/NF-kB signaling pathway is an important mediator of inflammation and fibrosis in kidney injury. Researchers have reported that oxidative stress is associated with this signaling pathway (Mehanna et al., 2021). In kidney tissues of rats, 3 mg/kg/day ethanol exposure by gavage for 4 weeks induced an elevate in MDA levels and a decline in GSH levels (Hamelink et al., 2005). Kidney cells are also significantly affected by ethanol. Increased oxidative stress as a result of chronic ethanol exposure inhibits tubular reabsorption. Increased cell proliferation and inflammation in the cells of the renal tubules induce abnormal thickening of the basement membrane of the glomeruli. Therefore, the ability of the kidney to regulate body fluid volume and electrolyte balance is impaired in chronic ethanol exposure (Mehanna et al., 2021).

In our study, it was determined that rats given 10 mg/kg BHT with chronic ethanol exposure decreased MDA and NO levels and rised GSH levels in the brain, stomach, liver and kidney tissues. There are studies in the literature showing the antioxidant activity of BHT (Miranda et al., 2001). However, only one study has examined the effects of BHT as a result of direct ethanol exposure. Carol et al. showed that intraperitoneal injection of 40 mg/kg BHT twice daily on days 2 to 4 of 4-day ethanol-exposed rats greatly decreased neuronal loss in the hippocampus and entorhinal cortex. Stating that the mechanisms by which alcohol causes neuronal oxidative damage are unknown and potentially involve acetaldehyde-derived alkaloidal metabolite formation or inflammatory mechanisms, the researchers reported that BHT may reduce inflammation and associated oxidative stress by blocking TNF-α activation. The researchers also measured the oxidation or reduction potential of BHT in vitro by cyclic voltammetry and showed that BHT exhibits an irreversible oxidation potential and is an antioxidant (Hosseini et al., 2017).

In our study, it was determined that rats given 10 mg/kg TQ with chronic ethanol exposure had decreased MDA and NO levels and rised GSH levels in brain, stomach, liver and kidney tissues. The studies in the literature were found to be compatible with our results. Intraperitoneal 10 mg/kg TQ treatment decreased the increased MDA levels and rised the reduced GSH levels in the kidney and liver tissues of rats exposed to 3 mg/kg/day ethanol by gavage for 4 weeks (Hamelink et al., 2005). In rats given 10 mg/kg TQ 1 hour before ingestion of 1 ml pure ethanol, it was defined that MDA levels decreased in the stomach tissue. However, there was no alteration in GSH levels (Kanter et al., 2005).

In this study, it was determined that the highest decline in MDA and NO levels and the highest rise in GSH levels in brain, stomach, liver and kidney tissues as a result of chronic ethanol exposure were found in the group in which TQ+BHT was given together. There is no study in the literature examining how TQ+BHT coadministration affects oxidative stress levels in ethanol exposure.

Our results support that TQ and BHT, which have antioxidant properties, may reduce oxidative stress caused by ethanol exposure in the brain, stomach, liver and kidney tissue. Compared to TQ alone and BHT alone, the combined use of TQ+BHT may reduce oxidative stress more. Additionally, TQ and BHT+TQ treatment against ethanol toxicity gave better results in behavioral tests. However, further research is recommended to explain the antioxidant activity of TQ and BHT and their effects on behavioral tests.

5. Conclusion

Our findings suggest that BHT and TQ critically contribute to the protection against alcohol-induced oxidative stress in brain, stomach, liver and kidney tissue. 30-day TQ and BHT treatment rised antioxidant capacity and caused healing in behavioral tests. The most

significant improvement was observed in the group in which BHT and TQ were given simultaneously after ethanol exposure. Investigating the mechanisms responsible for the antioxidant activity of BHT and TQ that may be protective against ethanol exposure will be the focus of our future research.

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

The author declared that there is no conflict of interest.

Ethical Consideration

The study was initiated after ethical approval was obtained from Animal Experiments Local Ethics Committee at its meeting (approval date: January 11, 2023, protocol code: 68429034/01).

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