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Oxidative Stress Caused by Lithium Exposure in the *Carassius auratus* (goldfish) Liver Tissue

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ABSTRACT

Lithium is a therapeutic agent widely used in the treatment of some psychiatric disorders. The aim of this study was to investigate the effects lithium-induced oxidative stress in liver tissue. In this study, *Carassius auratus* fish was used as a model organism and total 20 control fish and 28 experimental fishes were divided 4 subgroups randomly. Lithium chloride at a concentration of 50 mg/L was added to the glass tank of the experimental group. Fish were placed in two separate glass tanks, 20 in the control group and 28 in the experimental group. Lithium chloride at a concentration of 50 mg/L was added to the glass tank, which is the experimental group. At the 24th, 48th, 72nd and 96th hours of the study, 5 samples from the control group and 7 samples from the experimental group were included in the study in four different time periods. At the end of the study, it was determined that the level of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and acetylcholinesterase(AchE) decreased in the group with lithium exposure, while the levels of Malondialdehyde (MDA) increased. It was determined that oxidative stress occurred in lithium exposure.

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INTRODUCTION

In the first industrial use of lithium element, it was intended to be used in the content of heat-resistant glass, ceramics, aircraft construction and other materials. Due to the increasing demand for greener technology, there has been rapid growth in the use of lithium batteries for laptop computers, mobile phones and especially for electric car batteries (El-Tekreti et al. 2022). While the general lithium demand was 7% per year, today the demand for lithium usage has reached 20% per year (Shahzad 2017). Lithium is ideal for use in battery applications as it has the highest electrical output per unit weight of any battery material.Therefore, the use of lithium batteries, the consumption of rechargeable lithium-ion and lithium polymer batteries is increasing. In this case, the rate of lithium waste in environmental waste has increased rapidly (Meng 2021). In addition, lithium is a medication that has been used for bipolar disorders (BD). BD treatment requires long-term and high-level lithium intake (Claire et al.2021).As a result of various anthropogenic activities, lithium can be included in the food chain of humans by polluting the aquatic environment. In some studies, it has been determined that high lithium exposure will have some toxicological effects in living organisms (Aralve Vecchio-Sadus 2008).

Goldfish is considered appropriate to be used in studies due to its advantages such as its suitability for the examination of physiological processes, its anatomical structure and being economical. The liver of fish is more sensitive to various environmental factors compared to mammals. Therefore, it is a preferred model to examine the relationship between the liver and environmental factors (Blanco et al. 2018).

Free radicals formed in the body through natural metabolic pathways are eliminated by antioxidant systems. There is a balance between free radicals and antioxidants. If this balance occurs in the form of an increase in oxidants, it is called oxidative stress (Acaroz 2019). Oxidative stress may result from increased free radical formation or lack of antioxidant activity due to endogenous and exogenous sources (Pizzioni et al. 2017). The level of malondialdehyde (MDA) is served as a reliable biomarker of lipid peroxidation (LPO) and usually served as a marker of LPO (Acaroz 2018) . Enzymes are affected from ROS and LPO production so: SOD and CAT are involved in the protection of cells against oxidative damage. Superoxide dismutase (SOD) and catalase (CAT) are antioxidant enzymes that play an essential role against oxidative stress(Acaroz 2019).Oxidative stress plays an important role in many pathogenic mechanisms. To detect the level of antioxidant response Enzyme levels such as SOD, CAT and glutathione peroxidase (GSH-Px) are used as sensitive biomarkers (Özdek et al. 2020).

The enzyme acetylcholinesterase (AchE) has a role in the membranes of erythrocytes as well as the nervous system (El-Tekreti et al.2022) It plays a role in the functions of nitric oxide released from erythrocytes and endothelial cells of vessels (Bakhtiari 2012). Thus, the importance of the enzyme in the pathogenesis of some diseases and in the physiopathological processes of various drugs that affect the erythrocyte membrane is revealed. Since there are few studies on these mechanisms, new research is needed (Bakhtiari 2012). Thus, changes in some AchE levels can be considered as an important component in membrane disorders and blood diseases.

In the present study, it was aimed to investigate the effects of lithium on oxidative stress regulators in different time periods in the liver tissue of *Carassius auratus* as a model organism. For this purpose, the effect of lithium, which is used in some treatments or frequently exposed to living things in nature, on oxidative stress indices in goldfish liver at different times was determined.

MATERIALS AND METHODS

Fish and Experimental Design

Carassius auratus fish used as model organisms were obtained from licensed commercial (Tetra Goldfish, Germany) aquarists. The composition of commercial feed used in feeding fish consists of fish and fish derivatives, cereals, yeasts, vegetable protein extracts, molluscs and crustaceans, oils and fats, algae, various sugars (Oligofructose 1%). The content of the feed is presented in Table 1.

Table 1. *Ingredients in Fish Feed*

Crude protein	Crude fibre	Crude fat	Moisture Content	Vitamin D3	manganese (II) sulphate monohydrate	zinc sulphate, monohydrate	Iron (iron(II) sulphate, monohydrate)
42,0%	2,0%	11,0%	6,5%	1820 IU/kg	17 mg/kg	10 mg/kg	(),7 mg/kg

Fifty-six fish (1.80 ± 0.05 g) were taken and randomly distributed into two (10 L) glass tanks. The fish were kept for one week to adapt. The feeding of the fish was carried out with commercial feed twice a day, in the morning and in the evening. The fish are in a natural photoperiod at 25.0 ± 1ºC and a continuous air stone. For the experimental study, one of the aquariums was used as the control group and the other as the experimental group. Model organism fish were divided into aquariums, 28 for the Lithium-exposed group and 20 for the control group.

Lithium exposure

Lithium chloride at a dose of 50 mg/L was added to the aquarium of the lithium-treated group and its concentration remained unchanged for 96 hours.

Lethal dose determination was determined according to Liu et al. (2018). At 24, 48, 72 and 96th hours after lithium application, 7 fish from the lithium group and 5 fish from the control group were included in the sampling. While sampling, the fish were taken into anesthesia medium containing MS222 (0.1 g/L) and liver tissues were taken. It was stored at -18 ºC until the day of analysis.

Biochemical analyzes

After the liver tissues taken from the experimental and control groups were homogenized, biochemical analyzes were performed on the supernatants obtained.

Liver tissue SOD and GSH-Px enzyme activities of all groups were measured according to the Randox-Ransod (West Virginia, USA) enzyme kit procedure, and measurements were made for SOD at 505 nm and for GSH-Px at 340 nm at 37º C in the autoanalyzer (USA).

CAT enzyme activity was determined by Aebi (1984)'s UV spectrophotometric method based on the breakdown of hydrogen peroxide (H2O2) by catalase, by measuring the activity at 240 nm.

After tissue homogenization, measurement of liver MDA levels performed by Placer et al. (1966).The interaction of malondialdehyde (MDA), one of the aldehyde products of lipid peroxidation, with thiobarbituric acid (TBA) based on the reaction. The resulting MDA forms a pink complex with TBA and the degree of lipid peroxidation is determined by measuring the absorbance of this solution at 532 nm with a spectrophotometer. The malondialdehyde levels of the supernatants were determined according to the concentration of the standard and the rate of absorbance.

AChE activities was determined based on the reduction of acetylthiocholine iodide at 412 nm and 37°C according to the colorimetric method of Ellman et al. (1967). In this method, thiol ester acetylthiocholine was used instead of oxy ester acetylcholine as a substrate. According to the principle of the method, acetylthiocholine was hydrolyzed by achE and the color formed as a result of the reaction of thiocholine released as a result of hydrolysis with Ellman's reagent DTNB [5,50 dithiol-bis-(2-mtrobenzoic acid)] was measured. As a result of the reaction, yellow colored chromophore TNB (5-thio 2-nitrobenzoic acid) is formed. The rate of formation (color intensity) of this yellow compound formed was determined by measuring the absorbance at 412 nm (Ellman et al. 1961).

Statistics

Statistical analyses were performed using SPSS statistical analysis package. The values obtained as a result of the analyzes were expressed as mean±standard error. For multiple comparisons of values from different sampling areas, ANOVA followed by Tukey's test was used to reveal the difference. The difference between the values was made according to 0.05.

RESULTS

The results of the presented study are given in the liver tissue MDA, CAT, SOD, GPx, AchE levels Figure 1-5 and table 1 of the control and experimental groups.

In the experimental group exposed to lithium, the level of MDA, a product of liver lipid peroxidation, increased statistically significantly compared to the control group. ($P < 0.05$) It was observed that SOD, CAT, GPx levels, which are antioxidant enzymes, decreased gradually in different time periods in the groups exposed to lithium ($P < 0.05$). Acetylcholine esterase enzyme levels decreased statistically in the experimental group compared to the control group.

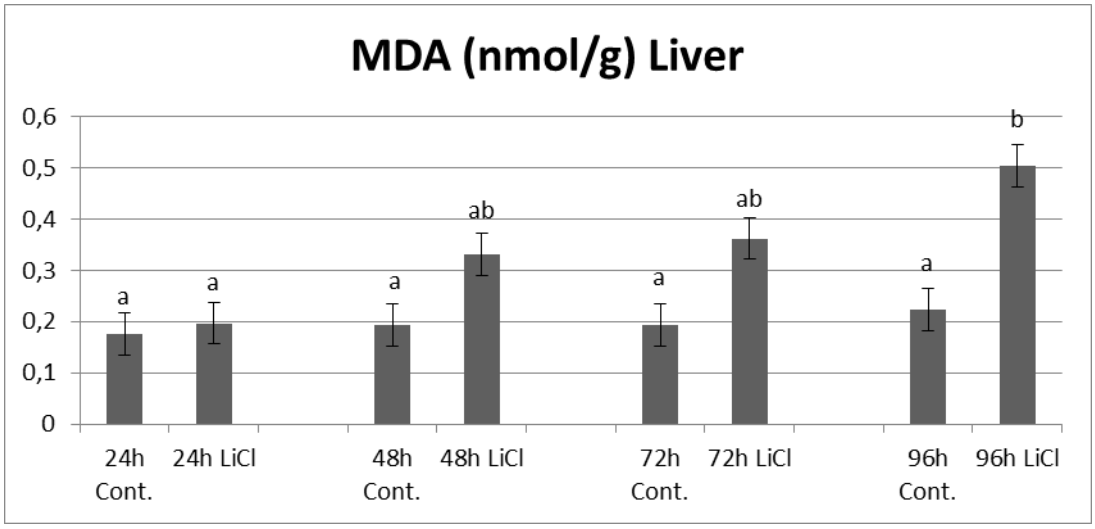

Figure 1. *The effects of lithium on MDA levels in Carassius auratus liver tissue ($p < 0.05$).*

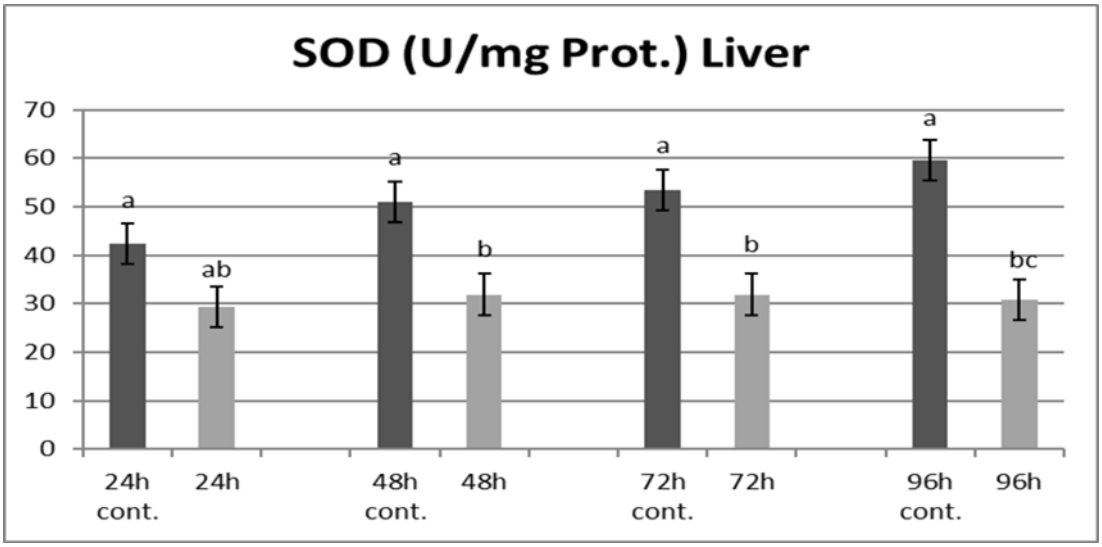

Figure 2. *SOD level in liver tissue of control and lithium group Carassius auratus ($p < 0.05$).*

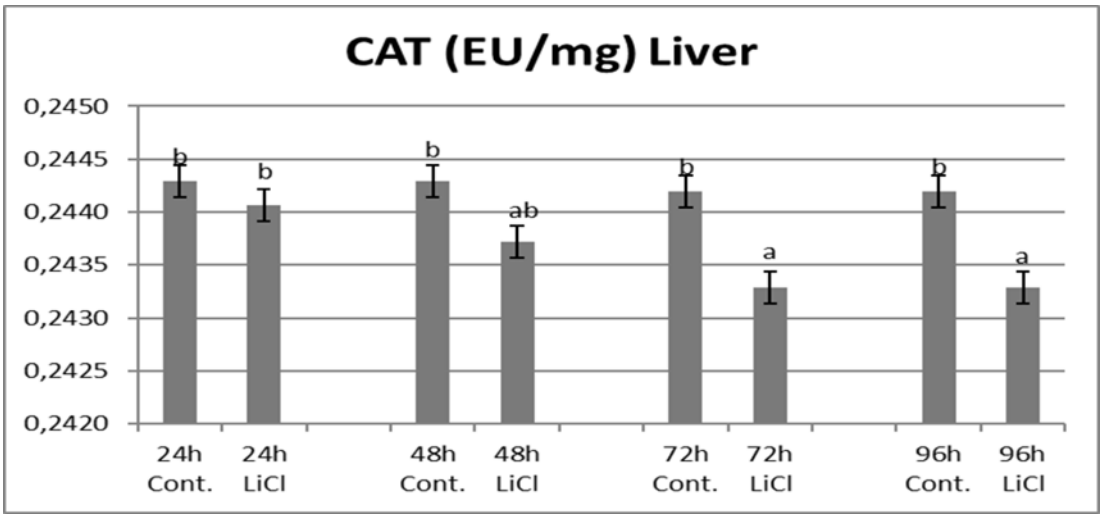

Figure 3. *Cat level in liver tissue of control and lithium group Carassius auratus ($p < 0.05$).*

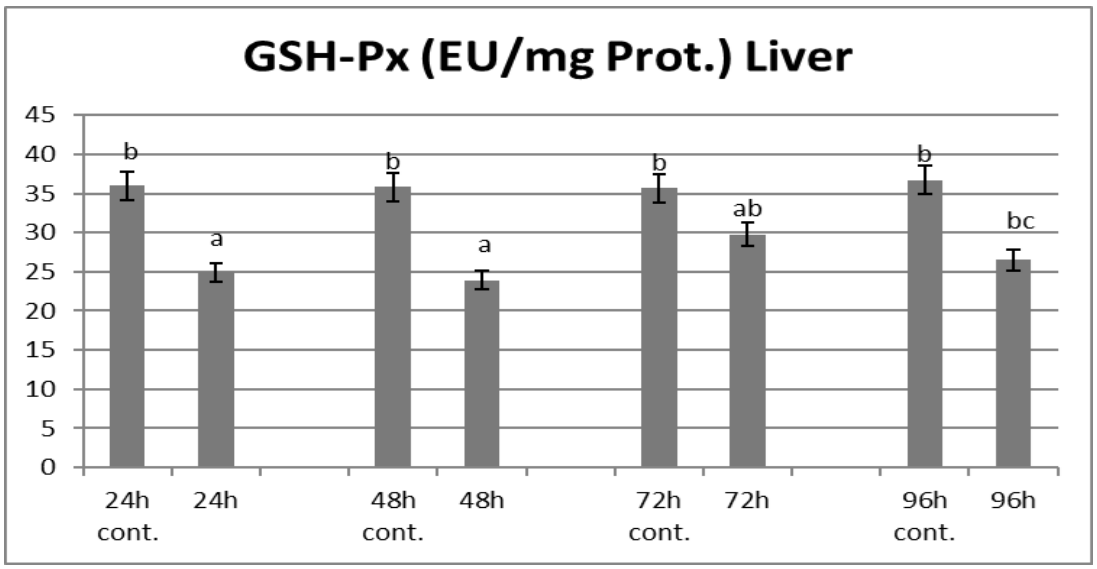

Figure 4. *GSH-Px level in liver tissue of control and lithium group Carassius auratus ($p < 0.05$).*

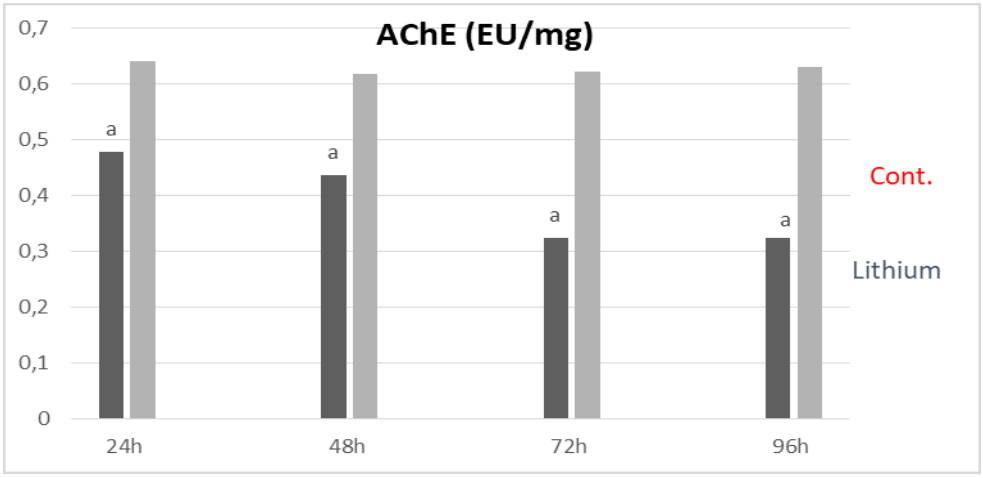

Figure 5. *AChE activity in liver tissue of all group Carassius auratus ($p < 0.05$).*

Table 1. *GSH-Px, SOD, CAT, AchE and MDA levels in liver tissue of all group Carassius auratus (p < 0.05).*

	GSH-Px (EU/mg)	SOD(U/mg)	CAT(EU/mg)	AChE(EU/mg)	MDA(nmol/g tissue)
24h cont.	42,408±10,23	42,408±10,68	0,244±0,01	0,641±0,32	0,176±0,01
24h	29,278±9,42*	29,278±8,79*	0,244±0,02*	0,477±0,26	0,196±0,01
48h cont.	50,913±12,35	50,913±11,26	0,244±0,01	0,617±0,31	0,192±0,02
48h	31,880±11,89*	31,880±9,63*	0,243±0,02*	0,436±0,29*	0,331±0,02*
72h cont.	53,427±12,47	53,427±12,96	0,244±0,03	0,622±0,31	0,192±0,02
72h	31,880±8,69*	31,880±9,63*	0,243±0,04*	0,323±0,21*	0,362±0,01*
96h cont.	59,541±14,39	59,541±12,69	0,244±0,05	0,630±0,42	0,223±0,01
96h	30,855±9,68*	30,855±9,068*	0,243±0,04*	0,323±0,21*	0,503±0,01*

*(p<0,05)

DISCUSSION

The study we presented demonstrated that lithium exposure resulted in oxidative tissue damage, as determined by elevated lipid peroxidation products and decreased levels of certain antioxidant enzymes in liver tissue. Lithium enhances oxidative damage, which is further supported by the fact that it causes a decrease in AchE activity. The levels of MDA oxidant biomarkers and GSHPx, CAT, SOD, antioxidants have been suggested to determine the presence of oxidative stress free radicals or oxidative biomarkers (Mis L et al 2018). Lithium triggers oxidative damage such as superoxide and nitric oxide by creating reactive oxygen species in the liver. These reactive products increase the lipid peroxide level in the tissues, causing tissue damage and limiting the antioxidant enzyme activities. Therefore, lithium-induced organ contributes to the pathophysiology of renal failure. (Vijaimohan et al 2010). In the current study, 50 mg/L lithium significantly reduced the total antioxidant capacity and activity of SOD, GSH-Px, and CAT in goldfish liver. Results are similar with lithium exposure studies in zebra (Liu et al. 2018), mammals (Kiełczykowska et al. 2014; Mezni et al. 2017) and carp (Jing et al.2021).

In the presented study, it was found that the MDA level in the liver tissue increased. MDA is a by product of lipid peroxidation, and this increase partially demonstrated the supportive effect of lithium on the generation of reactive oxygen species. It is consistent with the findings of studies that showed that exposure to lithium raised the concentration of reactive oxygen species (Eskandari et al. 2012; Liu 2018). MDA, which is an indicator of oxidative stress, is formed as a result of oxidation of polyunsaturated fatty acids and leads to the accumulation of lipids in the liver tissue (Lee et al. 2019). Recent studies have supported that lipid peroxidation caused by lithium contributes to tissue damage by causing damage to cell membranes (El 2022). In a study conducted to examine the erythrocyte oxidant-antioxidant status of lithium carbonate administered at different doses, it was suggested that MDA levels increased(Zhou et al. 2020). Studies have emphasized the role of these reactive oxygen metabolites in mediating the pathogenesis of liver dysfunction caused by some chemical agents (Donato 2021). Lithium toxicity can be attributed to the incidence of lipid peroxidation, which is brought on by the liver's high concentration of polyunsaturated fatty acids (Öter 2023). Due to the increase in ROS in erythrocytes, the antioxidant effect's decrease results in oxidative damage(Ilkaya 2020). According to several research, oxidative stress may play a role in toxicities brought on by lithium exposure (Ahmad et al. 2011; Toplan et al. 2013; Nciri et al. 2012; Öter 2023).

GSHPx, SOD, CAT, and GSH are the most important antioxidants in protecting erythrocytes from the destructive effects of lipid peroxidation (Mis 2021). Superoxide anions formed in erythrocytes are converted to hydrogen peroxide (H2O2) with the effect of superoxide dismutase. The resulting H2O2 is converted to H2O by reacting with GSH by glutathione peroxidase or by being converted to H2O and O2 by CAT.

SOD levels as well as decreased SOD synthesis capacity cause cells to become more sensitive to conditions such as radiation and certain drugs. In the presented study, it was suggested that the SOD enzyme level was decreased

in the liver tissues of Carassius auratus fish given high doses of lithium chloride, which damaged a detoxification mechanism against toxicity (Oruç 2010).Treatment with lithium in healthy individuals altered the SOD levels of all subjects. In another study where it was reported that lithium exposure decreased the SOD level, it was frequently stated that excessive lipid accumulation could lead to oxidative stress (Sabzi et al. 2017). The reason for the decrease in the SOD/CAT ratio after lithium treatment was interpreted as the decrease in the hydrogen peroxide level (Khairova 2012).

One antioxidant enzyme, catalase, transforms H2O and O2 to protect cells from the damaging effects of hydrogen peroxide. If hydrogen peroxide is not broken down by catalase, it acts as a precursor to the hydroxyl radical, a very dangerous free radical for the body, and this radical causes permanent damage to the cell (Mis 2021). In our study, we observed a decrease in CAT level in Carassius auratus liver tissue due to oxidative stress caused by lithium chloride given at different time periods. The potential role of the presence of the CAT enzyme is related to the control of oxidative stress in aging and cells, mainly caused by H2O2. The GSH-Px enzyme reduces hydrogen peroxide (H2O2) to water in the reaction where reduced GSH is converted into oxidized glutathione (GSSG) (Mis 2022). In this study, excessive lithium chloride exposure resulted in a decrease in GSH-Px activity in liver tissue. It was noted that the amount of GSH-Px reduced in Liu's research on zebra gill tissue (Ahmad et al. 2000). Liu (2018) This decrease in antioxidant enzyme levels may be related to lithium-related side effects in organisms exposed to lithium. Similar results were found in the study conducted in different model fish for the increase of GSH-Px activation (Varga and Matkovics 1997). According to several research, the toxic effects of lithium are caused by oxidative stress (Toplan et al. 2013).

Impaired cholinergic transmission is one of the complications seen in the etiopathology of memory deficit in neuroegenerative diseases. Neurodegeneration in the frontal cortex and hippocampus regions within the brain results in impaired cholinergic transmission in two ways. First, the decrease in acetylcholine release reduces cholineacetyltransferase activity, which causes acetylcholine deficiency. Secondly, increased acetylcholinesterase activity contributes to acetylcholine shortage at the synapse by disrupting the available acetylcholine (Kangtao and Bais 2018). The use of antioxidants as neuroprotective agents is a potentially promising approach for the treatment of neurodegenerative diseases. Decreased AChE enzyme activities increase acetylcholine levels in the synaptic cleft, which is followed by cholinergic system desensitization and ultimately leads to cholinergic dysfunction (Öz et al. 2015). Taken together, however, these findings imply that lithium causes cholinergic dysfunction. It was observed that the level of acetylcholine esterase enzyme decreased in model organism fish exposed to lithium at different time periods. It has been observed that this is in parallel with previous studies on this subject.

One of the contributing factors to the decrease in AchE activity may be the increase in ROS production. Along with the increase in ROS, it has been reported that the peroxidation of the membranes increases and finally, disruptions in the functions of the cholinergic system have been reported (Melo et al.2003). In a study investigating the changes in cholinergic system enzymes in LiCI toxicity of zebrafish, it was determined that high-dose LiCI adversely affected the cholinergic system and even caused inhibition. It has been stated that neurotransmitter functions cannot be fulfilled in the purinergic and cholinergic systems (Oliveira et al. 2011).

It should be considered that lithium has important effects on metabolism and biochemical parameters. Its effect on organisms exposed to lithium is shaped by developmental stages and genetic differences. Therefore, more research by researchers is needed to understand the molecular targets of lithium. Lithium activity is affected by enzymes, receptors and cell death factors (Shaldubina et al. 2001). It has been stated that oxidative stress plays a role in the pathology of bipolar disorder. It is crucial to establish the link between lithium therapy and the development of the illness (Brüning et al. 2012).

CONCLUSIONS

Lithium has been linked to hepatic lipid accumulation in several studies. Species and exposure duration both affect lipid accumulation. Depending on the exposure time and dose of lithium used in the treatment of some diseases, antioxidant supplementation may be recommended or prevention should be taken to correct the antioxidant

system. Our study's findings demonstrate that exposure to high doses of lithium might cause oxidative stress. The fundamental process, however, is a problem that requires more research.

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ETHICAL STATEMENT

This study was approved by the ethics committee of the Animal Experimentation and Ethics Committee of Van Yuzuncu Yil University, dated 10/11/2020 (approval number :E.75241).

CONFLICT OF INTERESTS

The authors declared no conflict of interest.

AUTHORS CONTRIBUTION

Mis L, Çilingir Yeltekin A: Conducting experiment, planning the study, writing manuscript, Sama: acquisition of data, Conducting experiment.

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