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Impact of chronic lithium treatment on brain oxidative stress and anxiety-like behaviors in rats: Dose-dependent effects

Evren Eraslan[1], Erdal Matur[1], Sibel Akyol[2], Elif Ergül Ekiz[1], İbrahim Akyazı[1], Deniz Aktaran Bala[3], Feraye Esen Gürsel[4] and Nuran Dariyerli[2]

[1] *Istanbul University-Cerrahpasa, Faculty of Veterinary Medicine, Department of Physiology, Istanbul, Turkey*
[2] *Istanbul University-Cerrahpasa, Faculty of Medicine, Department of Physiology, Istanbul, Turkey*
[3] *Istanbul University-Cerrahpasa, Vocational School of Veterinary Medicine, Food Processing Department, Food Technology Programme, Istanbul, Turkey*
[4] *Istanbul University-Cerrahpasa, Faculty of Veterinary Medicine, Department of Biochemistry, Istanbul, Turkey*

Abstract. Lithium (Li) is a mood-stabilizing drug. Although one of the potential mechanisms underlying the neuroprotective effects of lithium is related to its antioxidative effect, its mechanisms of action are not fully understood. Herein we aimed to investigate the impact of varied dosages of long-term lithium therapy on oxidative stress parameters in the brains of healthy rats, and on anxiety-like behaviors, and whether any changes in behavior can be attributed to modifications in oxidative stress levels within the brain. Thirty-two adult Wistar albino male rats were randomly assigned to four treatment groups. While the control (C) group was fed with a standard diet, low Li (1.4 g/kg/diet), moderate Li (1.8 g/kg/diet), and high Li (2.2 g/kg/diet) groups were fed with lithium bicarbonate (Li_2CO_3) for 30 days. Malondialdehyde increased, while superoxide dismutase and catalase levels decreased in the brains of the high Li group animals. In addition, anxiety-like behaviors of animals increased in the high Li group considering fewer entries to and less time spent in the open arms of the elevated plus maze test. Our findings underscore the potential adverse effects of prolonged lithium treatment, especially at doses approaching the upper therapeutic range. The induction of toxicity, manifested through heightened oxidative stress, appears to be a key mechanism contributing to the observed increase in anxiety-like behaviors. Consequently, caution is warranted when considering extended lithium therapy at higher doses, emphasizing the need for further research to delineate the precise mechanisms underlying these effects and to inform safer therapeutic practices.

Key words: Lithium bicarbonate — Oxidative stress — Brain — Anxiety-like behaviors — Rats

Introduction

Lithium is a mood-stabilizing drug that has been shown to have neuroprotective effects. Although lithium's therapeutic mechanisms are not fully understood, it has been employed in the treatment of various neurodegenerative conditions, including Parkinson's, Huntington's, and Alzheimer's disease, as well as Bipolar disorder (Camins et al. 2009; Chiu and Chuang 2011; Haupt et al. 2021; Haussmann et al. 2021)

The generation of reactive oxygen species (ROS) in the brain and oxidative stress has been suggested to play an important role in the pathology of several neuropsychiatric disorders including depression and anxiety (Shukla et al. 2011; Khairova et al. 2012). The brain is particularly

Correspondence to: Evren Eraslan, Istanbul University-Cerrahpasa, Faculty of Veterinary Medicine, Department of Physiology, Istanbul, Turkey
E-mail: eraslan@iuc.edu.tr

vulnerable to oxidative stress as it utilizes a large amount of oxygen concerning its weight and is not particularly enriched in antioxidant defenses (Cecerska-Heryć et al. 2022). Oxidative stress has been defined as an 'imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage' (Sies 1997). It has been assessed by measurement of reaction products of oxidative damage, for example, lipid peroxidation (Betteridge 2000). The free radical oxidation of polyunsaturated fatty acids is known as lipid peroxidation (Gutteridge 1995). Lipid peroxidation levels are measured as a marker of oxidative stress (Betteridge 2000). Malondialdehyde (MDA) is a compound that is derived from the peroxidation of polyunsaturated fatty acids. MDA is the principal and most studied biomarker to measure oxidative stress (Tsikas 2017; Cordiano et al. 2023). The antioxidant defense system includes enzymes such as superoxide dismutase (SOD) and catalase (CAT) which play a central role in the clearance of ROS generated by oxidation reactions and limiting tissue damage. SOD converts superoxide radicals into hydrogen peroxide (H_2O_2) and CAT breaks down H_2O_2 (Betteridge 2000; Tang and Wang 2012). Therefore, MDA and the enzymes SOD, and CAT are the major markers of oxidative stress levels (Khairova et al. 2012).

One of the potential mechanisms underlying the neuroprotective effects of lithium is related to its antioxidative stress effect which may play an important role in the therapeutic effect of lithium (Ghanaatfar et al. 2023). Mood-stabilizing drugs, including lithium were reported to increase antioxidant expression and activity in the rat brain, particularly in the hippocampus and prefrontal cortex (Tang and Wang 2012; Popović et al. 2019; Gavrilović et al. 2022). Therefore, it is conceivable that the previously demonstrated antioxidant properties of this drug are one of the potential mechanisms underlying lithium's neuroprotection and efficacy in the treatment of neurodegenerative diseases (De Vasconcellos et al. 2006; Camins et al. 2009). Despite all these beneficial effects of lithium, it has a very narrow therapeutic range with the risk of lithium intoxication (Haussmann et al. 2017). Although neuroprotective/neurotrophic effects of lithium have been demonstrated previously (Forlenza et al. 2014; Roux and Dosseto 2017), there have been reports of its neurotoxicity occurring even at therapeutic doses by altering oxidative stress status in the brain (Bhalla et al. 2007; Mezni et al. 2017). Various studies also suggested lithium may have adverse effects such as renal impairment, cardiotoxicity, cytotoxicity in the liver, and hypothyroidism (Eskandari et al. 2012; Nciri et al. 2012; Shine et al. 2015; Abdel Hamid et al. 2020; L'Abbate et al. 2023).

Scientists have been investigating lithium's properties for a long period of time. However, its mechanisms of action are not still fully understood. Furthermore, most of the studies about lithium were conducted in pathological conditions and only a few studies examined the role of lithium in non-pathological conditions. Therefore, herein we aimed to examine the effects of different doses of chronic lithium treatments to address the question of whether lithium has long-lasting effects on some oxidative stress parameters in the healthy rats' brain. We measured MDA as an oxidative stress indicator and the major antioxidant enzymes SOD and CAT.

Furthermore, lithium is used as a mood-stabilizing drug (Baldessarini 2013) and affects mood disorders *i.e.* suppressing mania and depression (Zhuo et al. 2022). However, significant neurocognitive and behavioral adverse effects often occur, due to its' narrow therapeutic index. It has been previously suggested that oxidative stress may contribute to anxious behavior (Fedoce et al. 2018) and lithium treatment may change oxidative status in the brain (Roux and Dosseto 2017; Popović et al. 2019; Gavrilović et al. 2022). In this study we aimed to evaluate different doses of long-term lithium treatment on anxiety-like behaviors and whether these possible changes are related to the alterations in oxidative stress status in the brain.

Material and Methods

Animals and grouping

A total of 32 adult Wistar albino male rats were randomized into control (C), low lithium (Li), moderate Li, and high Li treatment groups, each consisting of 8 animals. The control group was fed with a standard commercial rat pellet. Different doses of lithium bicarbonate ($Li_2 CO_3$) – 1.4 g/kg, 1.8 g/kg, and 2.2 g/kg, were added to diets of low Li, moderate Li, and high Li groups, respectively, for 30 days.

The animals were kept in polycarbonate cages in a group of 4/cage with wood chip bedding in standard lighting (12 h/12 h light/dark cycle) and temperature conditions (22 ± 3°C). Food and water were provided *ad libitum*. All experimental procedures were approved by the local ethics committee of Istanbul University (approval number: 201260).

Behavioral testing

Animals from each group were tested to measure the effects of different doses of lithium treatment on anxiety-like behaviors in an elevated plus maze (EPM) (Pellow et al. 1985; Eraslan et al. 2023) at the end of the experimental period during the light phase of the day (passive) between 8:00 a.m. – 12:00 p.m. The test apparatus was a plus-shaped open roofed construction with two open (50×10 cm) and two trilateral enclosed opposite arms (50×10×40 cm high).

The arms are joined to the central squire platform. The maze is placed 80 cm above the floor.

Animals were placed on the central squire platform facing one of the open arms and allowed to explore freely for 5 min. The behaviors of animals were videotaped. The test apparatus was cleaned using ethanol solution (5% v/v) and wiped dry between trials to prevent olfactory cues.

The video records were scored by a blinded researcher. The percentages of time spent in the open arms (100×time spent in the open arms/total time spent in the open and closed arms), the percentage frequency of entries in the open arms (100×number of entries into open arms/total entries into all arms), and total arm entries (total number of closed and open arm entries) were calculated as an index of anxiety-like behavior.

Sample collection and preparation

At the end of the experiments, all animals were anesthetized (xylazine/ketamine, 10/75 mg/kg) and blood samples were collected *via* cardiac puncture using EDTA tubes, and all rats were sacrificed by cervical dislocation. Brains were immediately removed and kept at –80°C until the analyses. Frozen brains were excised for PCR analyses. The rest of the brain tissue was homogenized in TNG-T Buffer using a rotor-stator homogenizer. The homogenate was centrifuged at 1000 × *g* for 10 min at 4°C and the supernatant was used for biochemical analyses.

RT-PCR

First of all, total RNA was isolated using a PureLink^{TM} RNA Mini Kit (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. The concentration of total RNA was measured fluorometrically (Qubit fluorimeter, Invitrogen, Carlsbad) using the Quant-iTTM RNA Assay Kit (Invitrogen, Eugene, OR). cDNA was synthesized from 1 μg total RNA using random primers (High-capacity RNA to cDNA Master Mix Kit, Applied Biosystems, Foster City, CA). The concentration of the resulting cDNA was measured using the Quant-iTTM DNA Br Assay Kit (Invitrogen, Eugene). Quantitative real-time PCR (qPCR) analyses were performed using 1 ng of cDNA with ABI7500 Real-Time PCR Systems (Applied Biosystems). Spesific oligonucleotide primers and probes for rat for CAT (assay ID: Rn01512560_m1) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (assay ID: Rn01775763_g1) were obtained as TaqMan Gene Expression Assays (ThermoFisher Scientific). The mRNA expression levels of the tested gene was normalized to those of GAPDH which is a housekeeping gene (Chan et al. 2006; Kolasa-Wołosiuk et al. 2019). Analyses of the data were performed using the ΔΔ Ct method (Livak and Schmittgen 2001). Fold changes of genes were calculated using the expression $2^{-\Delta\Delta Ct}$ with respect to the mean value of ΔCt in the control group.

Biochemical parameters

Lipid peroxidation

The MDA level was measured using the thiobarbituric acid reactive substance (TBARS) assay. The formation of TBARS was used as an indicator of lipoperoxidation. Plasma TBARS were estimated according to the method of Yoshioka et al. (1979). The assay was based on the reaction of two molecules of thiobarbituric acid with one molecule of MDA. This formed a colored complex with a maximum absorbance of 532 nm.

Catalase activity

CAT activity was determined by a modified method described by Yasmineh et al. (1995). The assay was based on the decomposition of H_2O_2 in the buffer by catalase enzyme in the tissue.

SOD activity

Tissue Cu-Zn superoxide dismutase SOD activity was determined according to the method of Sun et al. (1988) by inhibition of nitroblue tetrazolium (NBT) reduction with xanthine-xanthine oxidase used as a superoxide generator. One unit of SOD is defined as the amount of protein that inhibits the rate of NBT reduction by 50%.

A UV spectrophotometer (Chebios S.r.l. optimum-one, Rome, Italy) was used to measure absorbances.

Statistics

Statistical analysis was carried out on the SPSS software package (ver. 11.5.2.1, SPSS Inc., Chicago, IL, USA). Data were first tested for normality using the Shapiro-Wilk test. Normally distributed data were analyzed using one-way analysis of variance (ANOVA). Duncan's test was used for pairwise comparisons. Statistical significance was set at $p < 0.05$. If the normality assumption was violated, the non-parametric Kruskal-Wallis test was used. Afterward, the Mann-Whitney U test was applied for pairwise comparisons.

Results

We confirmed that serum lithium levels were significantly increased dose-dependently, (F (3, 30) = 65.77, $p < 0.05$). Serum levels of lithium-treated groups were significantly higher than those of control. In addition, lithium levels of

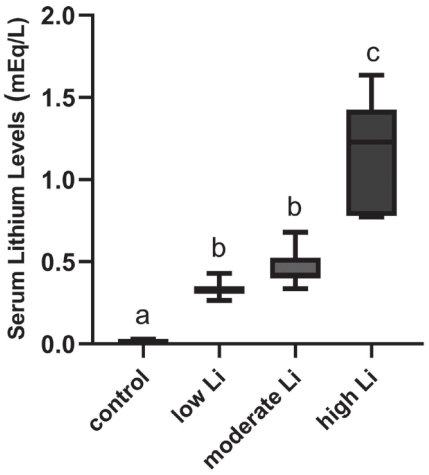

Figure 1. The serum lithium levels of the control and lithium treatment groups. Data are presented by box plots where the central lines represent the median, and the whiskers represent the minimum and maximum values. Different letters above the columns indicate a significant difference between the groups; serum levels of the control group (a) were significantly lower than those of the lithium treatment groups (b, c) ($p < 0.05$). In addition, lithium levels of the high Li group (c) were higher than the low and moderate Li groups (b) ($p < 0.05$).

the high Li group were higher than low and moderate Li groups (*post-hoc*, $p < 0.05$) (Fig. 1).

Considering oxidative stress parameters in the brain; the MDA level significantly increased in the high Li group compared to the control (*post-hoc*, $p < 0.05$ after ANOVA, F (3, 29) = 3.56, $p < 0.05$) (Fig. 2A). A Kruskal-Wallis test showed a tendency for a significant difference in CAT activity of different dose groups (χ^2 (3) = 7.301, $p = 0.06$). *Post-hoc* Man-Whitney U test showed that there was a significant difference between the high Li group and control ($z = -2.46$, $p < 0.05$), low Li ($z = -1.98$, $p < 0.05$), and moderate Li groups ($z = -1.98$, $p < 0.05$) (Fig. 2B). Similarly, SOD activity significantly differed between the groups (χ^2 (3) = 11.945, $p < 0.05$). It was lower in the high Li group than those of the control group ($z = -3.01$, $p < 0.05$), low Li ($z = -2.55$, $p < 0.05$), and moderate Li groups ($z = -2.62$, $p < 0.05$) (Fig. 2C).

The effect of lithium treatment was significant on CAT mRNA expression level (χ^2 (3) = 11.38, $p < 0.05$). It was significantly increased in the high Li group compared to control ($z = -2.32$, $p < 0.05$), low Li ($z = -2.55$, $p < 0.05$), and moderate Li ($z = -2.89$, $p < 0.05$) (Fig. 3).

The effect of different doses of lithium treatment was significant on anxiety-like behavior when considering the percentage of time spent in the open arms (χ^2 (3) =10.27, $p < 0.05$) (Fig. 4A) and the ratio of open/total arm entries (χ^2 (3) = 12.40, $p < 0.05$) (Fig. 4B) in EPM. The high Li group animals significantly spent less time in the open arms of the maze compared to the control ($z = -2.92$, $p < 0.05$) and moderate Li group ($z = -2.03$, $p < 0.05$). Furthermore, open arm entries of high Li group animals were lower than those of control ($z = -3.31$, $p < 0.05$), low Li ($z = -2.82$, $p < 0.05$), and moderate Li groups ($z = -2.32$, $p < 0.05$) animals. Total arm entries of animals were also affected by lithium treatment (F (3, 27) = 3.41, $p < 0.05$). The high Li group animals

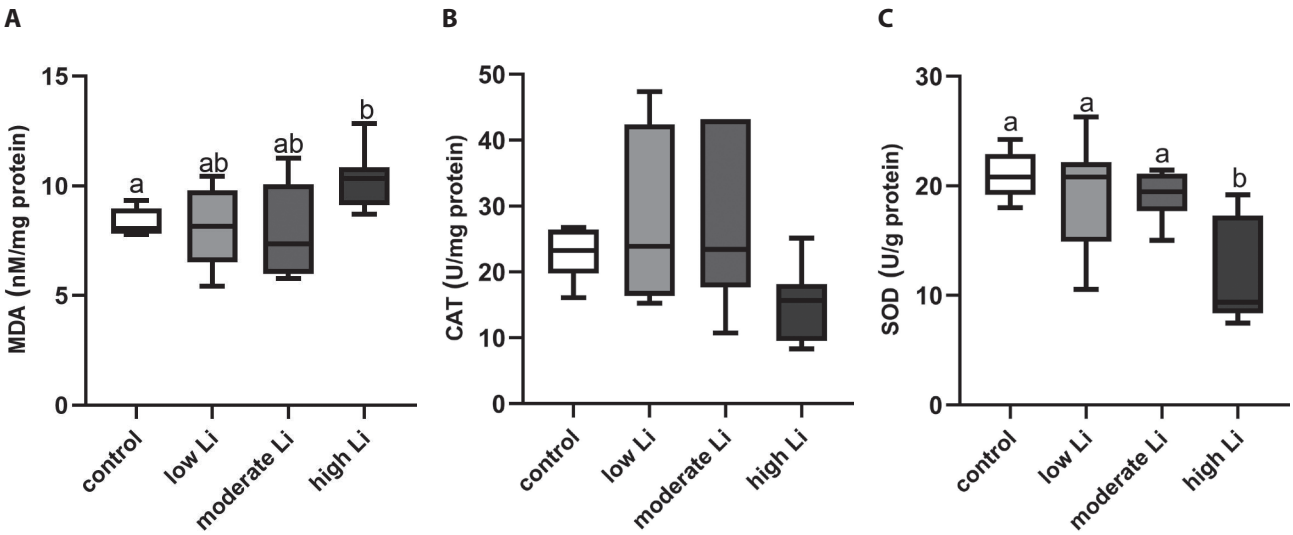

Figure 2. The effects of different doses of lithium administration on MDA (**A**), CAT (**B**), and SOD (**C**) activities in the brain. Data are presented by box plots where the central lines represent the median, and the whiskers represent the minimum and maximum values. Different letters above the columns indicate a significant difference between the groups; there was a significant difference between the control (a) and high Li group (b) considering MDA levels ($p < 0.05$). SOD levels of high Li group (b) were significantly lower than those of the control and other lithium treatment groups, ($p < 0.05$).

entered the arms of the maze less than the control and moderate Li group animals (*post-hoc*, $p < 0.05$) (Fig. 4C).

Discussion

In this study, we investigated the effects of different doses of chronic lithium treatment on some oxidative stress markers in the brain and anxiety-like behaviors in healthy rats. We found that while low Li and moderate Li did not alter these parameters, high Li, which is close to the upper therapeutic range, caused an alteration in brain oxidative stress status by increasing MDA, decreasing SOD, and CAT levels and also increasing anxiety-like behaviors.

In the present study, serum lithium levels of all groups were in the range of 0.3–1.2 mEq/l which is considered a therapeutic range (Hopkins and Gelenberg 2000). Serum levels of lithium in high Li group was significantly higher than the other groups and this result is in accordance with the results of the other parameters we investigated i.e., MDA, CAT, SOD levels and anxiety-like behaviors. Brain MDA levels were significantly increased in the highest dose lithium treatment group compared to the control which indicates increased lipid peroxidation (Tsikas 2017). It has been reported that lipid peroxidation increases after cell death or damage. In addition to this, cell membrane structure is deteriorated and cell integrity is damaged as a result of lipid peroxidation (Pisoschi and Pop 2015; Gaschler and Stockwell 2017; Iuchi et al. 2021). Therefore measurement of lipid peroxidation may be an excellent marker of tissue damage and cell deterioration (Gutteridge 1995; Garcia et al. 2013; Pisoschi and Pop 2015). Considering this, our results show that long-term lithium treatment with a 2.2 g/kg dose

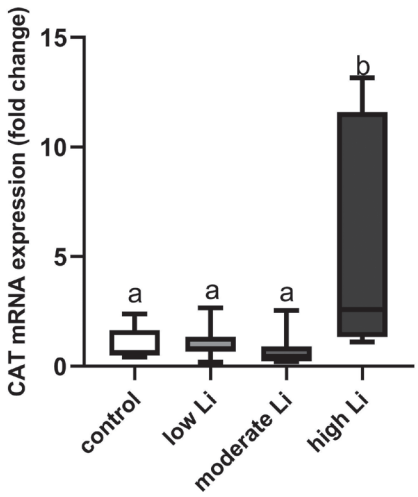

Figure 3. The effects of different doses of lithium administration on CAT mRNA expression in the brain. Data are presented by box plots where the central lines represent the median, and the whiskers represent the minimum and maximum values. Different letters above the columns indicate a significant difference between the groups; CAT mRNA levels of high Li group (b) were significantly higher than those of the control and the other lithium treatment groups (a), ($p < 0.05$).

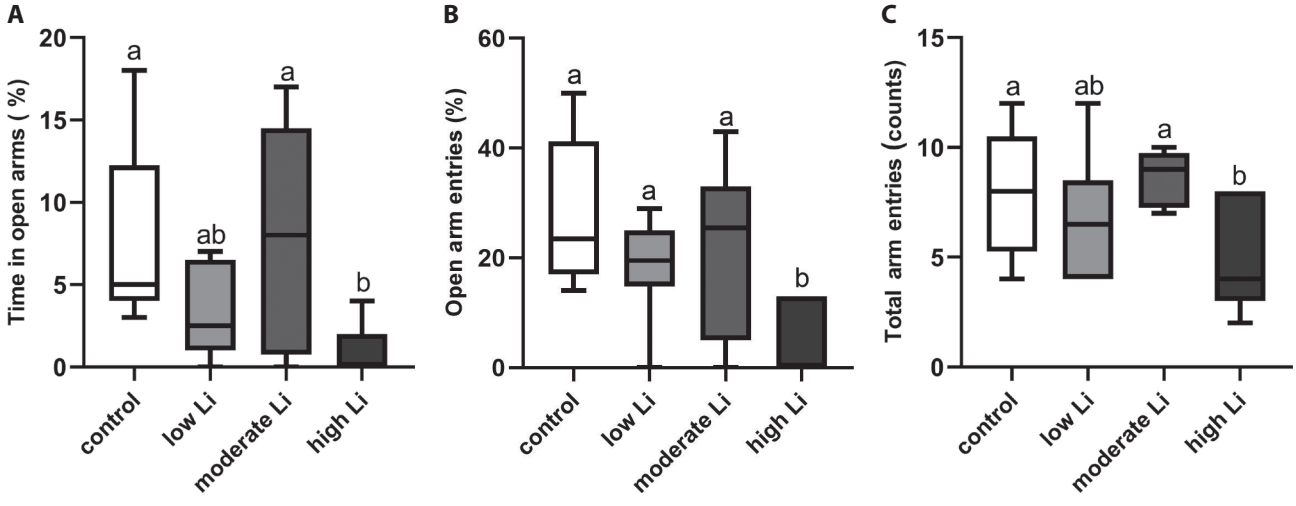

Figure 4. Behavioral data obtained in the elevated plus maze (EPM) Data are presented by box plots where the central lines represent the median, and the whiskers represent the minimum and maximum values. Different letters above the columns indicate a significant difference between the groups, ($p < 0.05$). **A.** The percentage of time spent in the open arms. The high Li group animals (b) significantly spent less time in the open arms of the maze compared to the control and moderate Li groups (a), ($p < 0.05$). **B.** The percentage of open arm entries. The open arm entries of high Li group animals (b) were significantly lower than those of control and the other lithium treatment groups (a), ($p < 0.05$). **C.** Total arm entries of animals. The high Li group animals (b) entered the arms of the maze significantly less than the control and moderate Li group animals (a), ($p < 0.05$).

may cause cellular deterioration by way of increased lipid peroxidation. Although the mechanism of lithium's adverse effects has not been solved yet, it is considered to be related to oxidative stress as it was previously shown that lithium carbonate increased lipid peroxidation in the kidney and liver in rats (Nciri et al. 2012; Ossani et al. 2019).

Furthermore, SOD activity was decreased in this high Li treatment group, while CAT activity tended to decrease in the same group. SOD and CAT play an important role in the free radical detoxification of the brain. During physiological states, SOD metabolizes superoxide anion (O_2^-), producing hydrogen peroxide (H_2O_2), which can react with iron to generate highly reactant hydroxyl radicals and CAT is the most important peroxidase in detoxifying excess hydrogen peroxide to prevent hydroxyl production (Ighodaro and Akinloye 2018). The decreased enzyme activity indicates a high degree of oxidative stress (Ranjekar et al. 2003). Therefore, decreased SOD and CAT activities in the high Li group in our study may suggest the overconsumption of these enzymes due to oxidative stress. Hence, we can conclude that there is an increase in free radical generation and impairment in antioxidant defense after a 2.2 g/kg dose of lithium treatment suggesting toxication in the relevant group. Supporting our findings, it was reported that 4 weeks of lithium treatment, even by the lower dose, which was 1.1 g/kg diet, than we used, caused lipid peroxidation and decreased CAT activity in the cerebrum and cerebellum of rats after 4 weeks treatment (Bhalla et al. 2007). In the same direction, it was reported that SOD levels in the serum of healthy volunteers significantly decreased after lithium treatment compared to baseline levels while CAT and MDA levels were not changed following lithium treatment in this study (Khairova et al. 2012). On the other hand, it was stated that the similar doses of chronic lithium treatment that we used, did not induce significant changes in MDA level, SOD, and CAT activities in mice brains suggesting these doses of treatment hadn't created toxicity in the brains of treated mice (Riadh et al. 2011). The different lengths of lithium treatment, the type of lithium salts used, the way of lithium administration, and sample handling might have led to discrepancies in the results of the mentioned studies. We also found that CAT mRNA expression levels were higher in the high Li group than control, low Li, and moderate Li groups. In line with this, we found that CAT activity in the high Li group tended to be lower than those of the control group. This may show that expression of the CAT gene is up-regulated in response to the oxidative stress that was observed in this high Li group. Although it has been previously suggested that long-term exposure to only low lithium concentrations could confer some protection against oxidative stress (Camins et al. 2009), our results show that long-term administration of the doses close to the highest therapeutic range may cause toxicity in the brain by the way of increasing oxidative stress.

Considering the anxiety-like behaviors of rats in the EPM test, we found that the percentage time in open arms, open arm entries, and total arm entries of high Li group animals were lower than the other groups suggesting anxiety type profile in this group. Supporting our results, chronic lithium overdose treatment was found to alter rat and mice behavior by inducing anxiety in the EPM test (Hanak et al. 2017; Smagin et al. 2021). Similarly, therapeutic doses of lithium treatment during preadolescence in rats increased measures of innate anxiety in the open field and on the EPM (Youngs et al. 2006). On the other hand, it was previously reported that therapeutic concentrations of lithium modulated antioxidant status and reduced oxidative stress, furthermore stabilizing behavior in animals with anxiety-like behavior after chronic stress exposure (Popović et al. 2019). However, in our study, oxidative stress parameters increased in the brain in high Li group animals which also shows anxiety-like behaviors. Oxidative stress state in the brain may alter neurotransmission, neuronal function, and overall brain activity, even neuronal demise may result (Andersen 2004; Jomova et al. 2023). As anxiety is controlled by the nervous system, abnormalities in these regulatory systems can result in anxious behavior (Martin et al. 2009; Duval et al. 2015). It is also possible that oxidative stress may affect corticolimbic regions involved in mediating and regulating anxiety (Masood et al. 2008; Mathew et al. 2008). It has been previously suggested that there is a direct involvement of oxidative stress in anxiety-like behaviors in rodents; oxidative stress has been implicated in high anxiety levels (Bouayed et al. 2009; Hassan et al. 2014). Masood et al. (2008), Bouayed et al. (2009) and Salim et al. (2010) reported that oxidative stress in the brain induced anxiety-like behaviors in mice and rats. Social defeat stress led to oxidative stress induced anxiety-like behaviors in rats (Patki et al. 2013). Furthermore, it was shown that overexpression of genes glyoxalase 1 and glutathione reductase 1, which are involved in oxidative stress metabolism, in mice brains resulted in increased anxiety-like behavior (Hovatta et al. 2005). In addition, it was found that hydrogen peroxide, a component of oxidative stress, treated mice showed increased anxiety in a battery of behavioral tests (Bouayed and Soulimani 2019). Considering all these reports, our finding about increased anxiety-like behaviors in the high Li group may be explained by increased oxidative stress parameters in this group. Therefore, we can conclude that oxidative stress in the brain caused by a 2.2 g/kg dose of long-term lithium treatment may be the underlying mechanism of increased anxiety-like behaviors of rats in our study.

The study has a limitation. As lithium is used for the treatment of different neurodegenerative diseases, the effect of lithium on different aspects of behavior such as depression

and also on cognitive functions could have been examined. Due to our laboratory conditions at the time of experiments, we could only conduct EPM. A more detailed study investigating the effect of lithium on other behaviors and cognition may be conducted in the future.

Our data show that normal rats might be unfavorably affected by long-term lithium treatment with a dose close to the upper therapeutic range considering alterations in brain MDA, SOD, CAT levels, and anxiety-like behaviors. It's important to note that the effects of lithium on these parameters are not entirely consistent across all studies. Factors such as the specific experimental conditions, the brain regions examined, and the dosage of lithium administered can lead to variable results. Overall, while there is evidence to suggest that lithium can influence oxidative stress markers like CAT, SOD, and MDA in the brain, more research is needed to fully understand the mechanisms and implications of these effects.

Conflict of interest. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Abdel Hamid OI, Ibrahim EM, Hussien MHS, ElKhateeb SA (2020): The molecular mechanisms of lithium-induced cardiotoxicity in male rats and its amelioration by N-acetyl cysteine. Hum. Exp. Toxicol. **39,** 696-711 https://doi.org/10.1177/0960327119897759

Andersen JK (2004): Oxidative stress in neurodegeneration: Cause or consequence? Nat. Rev. Neurosci. **10,** S18 https://doi.org/10.1038/nrn1434

Baldessarini RJ (2013): Mood-Stabilizing Agents, in: Chemotherapy in Psychiatry. Springer, New York https://doi.org/10.1007/978-1-4614-3710-9

Betteridge DJ (2000): What is oxidative stress? Metabolism **49,** 3-8 https://doi.org/10.1016/S0026-0495(00)80077-3

Bhalla P, Dhar R, Dhawan DK, Dam Chadha V (2007): Neuroprotective effects of zinc on antioxidant defense system in lithium treated rat brain. Indian J. Exp. Biol. **45,** 954-958

Bouayed J, Rammal H, Soulimani R (2009): Oxidative stress and anxiety. Relationship and cellular pathways. Oxid. Med. Cell. Longev. **2,** 63-67 https://doi.org/10.4161/oxim.2.2.7944

Bouayed J, Soulimani R (2019): Evidence that hydrogen peroxide, a component of oxidative stress, induces high-anxiety-related behaviour in mice. Behav. Brain Res. **359,** 292-297 https://doi.org/10.1016/j.bbr.2018.11.009

Camins A, Verdaguer E, Junyent F, Yeste-Velasco M, Pelegrí C, Vilaplana J, Pallás M (2009): Potential mechanisms involved in the prevention of neurodegenerative diseases by lithium. CNS Neurosci. Ther. **15,** 333-344 https://doi.org/10.1111/j.1755-5949.2009.00086.x

Cecerska-Heryć E, Polikowska A, Serwin N, Roszak M, Grygorcewicz B, Heryć R, Michalczyk A, Dołęgowska B (2022): Importance of oxidative stress in the pathogenesis, diagnosis, and monitoring of patients with neuropsychiatric disorders, a review. Neurochem. Int. **153,** 1-16 https://doi.org/10.1016/j.neuint.2021.105269

Chan SHH, Tai MH, Li CY, Chan JYH (2006): Reduction in molecular synthesis or enzyme activity of superoxide dismutases and catalase contributes to oxidative stress and neurogenic hypertension in spontaneously hypertensive rats. Free Radic. Biol. Med. **40,** 2028-2039 https://doi.org/10.1016/j.freeradbiomed.2006.01.032

Chiu C, Chuang D (2011): Molecular actions and therapeutic potential of lithium in preclinical and clinical studies of CNS disorders. Mol. Neurobiol. **128,** 281-304 https://doi.org/10.1016/j.pharmthera.2010.07.006

Cordiano R, Di Gioacchino M, Mangifesta R, Panzera C, Gangemi S, Minciullo PL (2023): Malondialdehyde as a potential oxidative stress marker for allergy-oriented diseases: An update. Molecules **28,** 1-22 https://doi.org/10.3390/molecules28165979

De Vasconcellos APS, Nieto FB, Crema LM, Diehl LA, De Almeida LM, Prediger ME, Da Rocha ER, Dalmaz C (2006): Chronic lithium treatment has antioxidant properties but does not prevent oxidative damage induced by chronic variate stress. Neurochem. Res. **31,** 1141-1151 https://doi.org/10.1007/s11064-006-9139-2

Duval ER, Javanbakht A, Liberzon I (2015): Neural circuits in anxiety and stress disorders: A focused review. Ther. Clin. Risk Manag. **11,** 115-126 https://doi.org/10.2147/TCRM.S48528

Eraslan E, Castelhano-Carlos M, Amorim L, Soares-Cunha C, Rodrigues A, Sousa N (2023): Home-cage behavior is impacted by stress exposure in rats. Front. Behav. Neurosci. **17,** 1-9 https://doi.org/10.3389/fnbeh.2023.1195011

Eskandari MR, Fard JK, Hosseini MJ, Pourahmad J (2012): Glutathione mediated reductive activation and mitochondrial dysfunction play key roles in lithium induced oxidative stress and cytotoxicity in liver. BioMetals **25,** 863-873 https://doi.org/10.1007/s10534-012-9552-8

Fedoce A das G, Ferreira F, Bota RG, Bonet-Costa V, Sun PY, Davies KJA (2018): The role of oxidative stress in anxiety disorder: cause or consequence? Free Radic. Res. **52,** 737-750 https://doi.org/10.1080/10715762.2018.1475733

Forlenza OV, De-Paula VJR, Diniz BSO (2014): Neuroprotective effects of lithium: Implications for the treatment of Alzheimer's disease and related neurodegenerative disorders. ACS Chem. Neurosci. **5,** 443-450 https://doi.org/10.1021/cn5000309

Garcia SC, Grotto D, Bulcão RP, Moro AM, Roehrs M, Valentini J, De Freitas FA, Paniz C, Bubols GB, Charão MF (2013): Evaluation of lipid damage related to pathological and physiological conditions. Drug Chem. Toxicol. **36,** 306-312 https://doi.org/10.3109/01480545.2012.720989

Gaschler MM, Stockwell BR (2017): Lipid peroxidation in cell death. Biochem. Biophys. Res. Commun. **482,** 419-425 https://doi.org/10.1016/j.bbrc.2016.10.086

Gavrilović L, Popović N, Stojiljković V, Pejić S, Todorović A, Vujović P, Pajović SB (2022): Antioxidant defense system in the prefrontal cortex of chronically stressed rats treated with lithium. PeerJ. **10,** 1-12 https://doi.org/10.7717/peerj.13020

Ghanaatfar F, Ghanaatfar A, Isapour P, Farokhi N, Bozorgniahosseini S, Javadi M, Gholami M, Ulloa L, Coleman-Fuller N, Motaghinejad M (2023): Is lithium neuroprotective? An updated mechanistic illustrated review. Fundemental Clin. Pharmocology **37,** 4-30 https://doi.org/10.1111/fcp.12826

Gutteridge JMC (1995): Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin. Chem. **41,** 1819-1828 https://doi.org/10.1093/clinchem/41.12.1819

Hanak A, Chevillard L, Lebeau R, Risède P (2017): Neurobehavioral effects of lithium in the rat : Investigation of the effect / concentration relationships and the contribution of the poisoning pattern. Prog. Neuropsychopharmacol. Biol. Psychiatry **76,** 124-133 https://doi.org/10.1016/j.pnpbp.2017.03.005

Hassan W, Barroso Silva C, Mohammadzai IU, Teixeira da Rocha J, Landeira-Fernandez J (2014): Association of oxidative stress to the genesis of anxiety: Implications for possible therapeutic ınterventions. Curr. Neuropharmacol. **12,** 120-139 https://doi.org/10.2174/1570159X11666131120232135

Haupt M, Bähr M, Doeppner T (2021): Lithium beyond psychiatric indications: The reincarnation of a new old drug. Neural Regen. Res. **16,** 2383-2387 https://doi.org/10.4103/1673-5374.313015

Haussmann R, Lewitzka U, Severus E, Bauer M (2017): Correct treatment of mood disorders with lithium. Nervenarzt. **88,** 1323-1334 https://doi.org/10.1007/s00115-017-0421-0

Haussmann R, Noppes F, Brandt MD, Bauer M, Donix M (2021): Lithium: A therapeutic option in Alzheimer‘s disease and its prodromal stages? Neurosci. Lett. **760,** 136044 https://doi.org/10.1016/j.neulet.2021.136044

Hopkins HS, Gelenberg A (2000): Serum lithium levels and the outcome of maintenance therapy of bipolar disorder. Bipolar Disord. **2,** 174-179 https://doi.org/10.1034/j.1399-5618.2000.020304.x

Hovatta I, Tennant RS, Helton R, Marr RA, Singer O, Redwine JM, Ellison JA, Schadt EE, Verma IM, Lockhart DJ, Barlow C (2005): Glyoxalase 1 and glutathione reductase 1 regulate anxiety in mice. Nature **438,** 662-666 https://doi.org/10.1038/nature04250

Ighodaro OM, Akinloye OA (2018): First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria J. Med. **54,** 287-293 https://doi.org/10.1016/j.ajme.2017.09.001

Iuchi K, Takai T, Hisatomi H (2021): Cell death via lipid peroxidation and protein aggregation diseases. Biology (Basel) **10,** 1-19 https://doi.org/10.3390/biology10050399

Jomova K, Raptova R, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, Valko M (2023): Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. Arch. Toxicol. **97,** 2499- 2574 https://doi.org/10.1007/s00204-023-03562-9

Khairova R, Pawar R, Salvadore G, Juruena MF, De Sousa RT, Soeiro-De-Souza MG, Salvador M, Zarate CA, Gattaz WF, Machado-Vieira R. (2012): Effects of lithium on oxidative stress parameters in healthy subjects. Mol. Med. Rep. **5,** 680-682

Kolasa-Wołosiuk A, Tarnowski M, Baranowska-Bosiacka I, Chlubek D, Wiszniewska B (2019): Antioxidant enzyme expression of mRNA and protein in the epididymis of finasteride-treated male rat offspring during postnatal development. Arch. Med. Sci. **15,** 797-810 https://doi.org/10.5114/aoms.2017.68528

L'Abbate S, Nicolini G, Marchetti S, Forte G, Lepore E, Unfer V, Kusmic C (2023): Lithium treatment ınduces cardiac dysfunction in mice. Int. J. Mol. Sci. **24,** 1-15 https://doi.org/10.3390/ijms242115872

Livak KJ, Schmittgen TD (2001): Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods **25,** 402-408 https://doi.org/10.1006/meth.2001.1262

Martin EI, Ressler KJ, Binder E, Nemeroff CB (2009): The neurobiology of anxiety disorders: Brain ımaging, genetics, and psychoneuroendocrinology. Psychiatr. Clin. North Am. **32,** 549-575 https://doi.org/10.1016/j.psc.2009.05.004

Masood A, Nadeem A, Mustafa SJ, O'Donnell JM (2008): Reversal of oxidative stress-induced anxiety by inhibition of phosphodiesterase-2 in mice. J. Pharmacol. Exp. Ther. **326,** 369-379 https://doi.org/10.1124/jpet.108.137208

Mathew SJ, Price RB, Charney DS (2008): Recent advances in the neurobiology of anxiety disorders: Implications for novel therapeutics. Am. J. Med. Genet. Part C Semin. Med. Genet. **148,** 89-98 https://doi.org/10.1002/ajmg.c.30172

Mezni A, Aoua H, Limam F, Aouani E (2017): Neuroprotective effect of grape seed and skin extract on lithium-induced oxidative stress in healthy rat brain. Neurochem. J. **11,** 85-94 https://doi.org/10.1134/S1819712417010093

Nciri R, Allagui MS, Bourogaa E, Saoudi M, Murat JC, Croute F, Elfeki A (2012): Lipid peroxidation, antioxidant activities and stress protein (HSP72/73, GRP94) expression in kidney and liver of rats under lithium treatment. J. Physiol. Biochem. **68,** 11-18 https://doi.org/10.1007/s13105-011-0113-3

Ossani GP, Uceda AM, Acosta JM, Lago NR, Repetto MG, Martino DJ, Toblli JE (2019): Role of oxidative stress in lithium-ınduced nephropathy. Biol. Trace Elem. Res. **191,** 412-418 https://doi.org/10.1007/s12011-018-1617-2

Patki G, Solanki N, Atrooz F, Allam F, Salim S (2013): Depression, anxiety-like behavior and memory impairment are associated with increased oxidative stress and inflammation in a rat model of social stress Gaurav. Brain Res. **1539,** 73-86 https://doi.org/10.1016/j.brainres.2013.09.033

Pellow S, Chopin P, File SE, Briley M (1985): Validation of open : closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods **14,** 149-167

https://doi.org/10.1016/0165-0270(85)90031-7
Pisoschi AM, Pop A (2015): The role of antioxidants in the chemistry of oxidative stress: A review. Eur. J. Med. Chem. **97,** 55-74 https://doi.org/10.1016/j.ejmech.2015.04.040
Popović N, Stojiljković V, Pejić S, Todorović A, Pavlović I, Gavrilović L, Pajović SB (2019): Modulation of hippocampal antioxidant defense system in chronically stressed rats by lithium. Oxid. Med. Cell. Longev. **2019,** 1-11 https://doi.org/10.1155/2019/8745376
Ranjekar PK, Hinge A, Hegde MV, Ghate M, Kale A, Sitasawad S, Wagh UV, Debsikdar VB, Mahadik SP (2003): Decreased antioxidant enzymes and membrane essential polyunsaturated fatty acids in schizophrenic and bipolar mood disorder patients. Psychiatry Res. **121,** 109-122 https://doi.org/10.1016/S0165-1781(03)00220-8
Riadh N, Allagui MS, Bourogaa E, Vincent C, Croute F, Elfeki A (2011): Neuroprotective and neurotrophic effects of long term lithium treatment in mouse brain. BioMetals **24,** 747-757 https://doi.org/10.1007/s10534-011-9433-6
Roux M, Dosseto A (2017): From direct to indirect lithium targets: A comprehensive review of omics data. Metallomics **9,** 1326-1351 https://doi.org/10.1039/C7MT00203C
Salim S, Asghar M, Chugh G, Taneja M, Xia Z, Saha K (2010): Oxidative stress: A potential recipe for anxiety, hypertension and ınsulin resistance. Brain Res. **1359,** 178-185 https://doi.org/10.1016/j.brainres.2010.08.093
Shine B, Mcknight RF, Leaver L, Geddes JR (2015): Long-term effects of lithium on renal, thyroid, and parathyroid function: A retrospective analysis of laboratory data. Lancet **386,** 461-468 https://doi.org/10.1016/S0140-6736(14)61842-0
Shukla V, Mishra SK, Pant HC (2011): Oxidative stress in neurodegeneration. Adv. Pharmacol. Sci. **2011,** 1-13 https://doi.org/10.1155/2011/572634
Sies H (1997): Oxidative stress : Oxidants and antioxidants. Exp. Physiol. **82,** 291-295 https://doi.org/10.1113/expphysiol.1997.sp004024
Smagin DA, Kovalenko IL, Galyamina AG, Belozertseva IV, Tamkovich NV, Baranov KO, Kudryavtseva NN (2021): Chronic lithium treatment affects anxious behaviors and the expression of serotonergic genes in midbrain raphe nuclei of defeated male mice. Biomedicines **9,** 1-16 https://doi.org/10.3390/biomedicines9101293
Sun Y, Oberley L, Li Y (1988): A simple method for clinical assay of superoxide dismutase. Clin. Chem. **34,** 497-500 https://doi.org/10.1093/clinchem/34.3.497
Tang V, Wang J (2012): Oxidative stress in bipolar disorder. Biochem. Anal. Biochem. **S2,** 1-8
Tsikas D (2017): Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. Anal. Biochem. **524,** 13-30 https://doi.org/10.1016/j.ab.2016.10.021
Yasmineh W, Kaur T, Blazar B, Theologides A (1995): Serum catalase as marker of graft-vs-host disease in allogeneic bone marrow transplant recipients: pilot study. Comp. Study **41,** 1574-1580 https://doi.org/10.1093/clinchem/41.11.1574
Yoshioka T, Kawada K, Shimada T, Mori M (1979): Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. Am. J. Obs. Gynecol. **135,** 372-376 https://doi.org/10.1016/0002-9378(79)90708-7
Youngs RM, Chu MS, Meloni EG, Naydenov A, Carlezon WA, Konradi C (2006): Lithium administration to preadolescent rats causes long-lasting increases in anxiety-like behavior and has molecular consequences. J. Neurosci. **26,** 6031-6039 https://doi.org/10.1523/JNEUROSCI.0580-06.2006
Zhuo C, Chen G, Chen J, Tian H, Ma X, Li Q, Yang L, Zhang Q, Li R, Song X, Huang C (2022): Lithium bidirectionally regulates depression- and mania-related brain functional alterations without worsening cognitive function in patients with bipolar disorder. Front. Psychiatry **13,** 1-13 https://doi.org/10.3389/fpsyt.2022.963005

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