Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2022 19(2): 811-818. **OPEN ACCESS**

Effects of Lithium treatment on oxidative stress markers in *Saccharomyces cerevisiae* wild type and superoxide dismutase, glutathione peroxidase, and glutathione reductase deficient strains

Ahmed Al-Mustafa

Department of Biological Sciences, Faculty of Science, Mutah University, Mutah, P.O. Box 7, Karak, 61710, **Jordan**

***Correspondence:** ahmedh65@gmail.com **Received 20-01-2022, Revised: 01-04-2022, Accepted: 10-04-2022 e-Published: 26-04-2022**

Lithium (Li) is commonly used as a mood stabilizer in the treatment of mental diseases due to its anti-oxidative capability, though other studies have demonstrated that Li possesses pro-oxidant effects in a variety of models. To elucidate some of the molecular mechanisms underlying lithium redox homeostasis, the activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR), and glutathione S-transferase (GST), as well as lipid peroxide malondialdehyde (MDA) levels were measured in wild type (WT) and SOD, GLR, and GPX deficient *Saccharomyces cerevisiae* strains cultured for 24 h in presence of lithium. A dose-dependent cell-growth inhibition was detected for Li at 1, 5, and 10 mM**.** Li doses that inhibited the growth of WT and SOD, GPX, and GLR deficient strains by 50% (IC_{50}) were 4.46 and 2.99, 3.15 and 3.32 mM, respectively. MDA levels in WT, GPX, and GLR deficient strains did not alter significantly following Li treatment ($P > 0.05$). Furthermore, when a SOD deficient strain is exposed to Li, the MDA level was increased significantly ($P < 0.05$). SOD activity did not change significantly ($P > 0.05$) in the WT or GPX deficient strain, however, its activity in the GLR deficient strain was significantly increased after Li treatment ($P > 0.05$). Li treatment decreased CAT activity in WT as well as in SOD, GPX, and GLR deficient strains. In addition, GPX activity was unaltered following Li treatment in WT and GLR deficient strain, compared to control, but GPX activity was significantly changed in SOD deficient strain. GST activity was reduced in the WT along with GPX and GLR deficient strains, whereas it increased significantly in SOD deficient strain ($P < 0.05$). The GR activity was increased in GPX deficient strain treated with Li, while the GR activity was significantly decreased in WT and SOD deficient strains compared to control. Overall, the current data imply that the Li concentration utilized in treatment is critical because it can either enhance the antioxidant system or has prooxidant activity due to its ability to generate ROS and be involved in the production of lipid peroxidation.

Keywords: Lithium; oxidative stress; yeast; antioxidant enzymes; Lipid peroxidation

INTRODUCTION

Reactive oxygen species (ROS) are naturally produced by cells because of a normal attribute of aerobic life and can be enhanced by stress. An increase in the concentrations of different ROS species might leads to molecular damage, assigned as oxidative distress (Sies, 2021). ROS such as superoxide and hydroxyl radicals, as well as nonradical hydrogen peroxide (H_2O_2), can initiate a variety of diseases by causing protein denaturation, enzyme inhibition, DNA damage, and lipid peroxidation (Mahadik et al. 2001; Wang et al. 2003; Frey et al. 2006; Machado-Vieira et al. 2007).

The lipid peroxide level (MDA) and the superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) enzyme activities are considered major markers of oxidative stress levels (Gsell et al. 1995; Hussain et al. 1995; Wang et al. 2004). SOD converts superoxide radicals to H2O2, while CAT converts H_2O_2 to O_2 and H_2O_2. Also, GPX is involved in the reduction of organic peroxides, whereas GR is involved in the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH). The enzyme GST detoxifies xenobiotics by conjugating them with GSH as an electron donor. Non-enzymatic antioxidant molecule GSH functions as a substrate for GPX and GST as well as a reductant for electrogenic toxicants (Hussain et al.1995; Halliwell and Gutteridge, 2015).

Moreover, ROS is known to play an important role in the pathogenesis of several diseases such as neurodegenerative and psychiatric disorders (Halliwell, 2001; Palmieri and Sblendorio, 2006; Frey et al. 2007; Liu et al., 2007; Machado-Vieira et al. 2007; Won et al. 2017). Therefore, tremendous progress has been made in understanding the role of these oxidants in physiology and

illness, particularly in the neurological, cardiovascular, and immunological systems (Williams and Harwood, 2000; Yan et al. 2019). Because of ROS's significance in redox signaling pathways, antioxidant compounds can be evaluated for their effectiveness in preventing disease initiation and progress. As a result, regulating certain ROS-signaling pathways is a promising future redox-medicine strategy. This includes the role of lithium (Hajikarimlou et al. 2020) as well as enzymatic defense systems such as SOD (Sies, 2021).

Lithium is known as a mood-stabilizing agent with documented capacity for DNA damage, free-radical formation, and lipid peroxidation prevention and/or reversion in diverse models (Shao et al. 2005; Andreazza et al. 2007, Machado-Vieira et al. 2009); lithium has neurotrophic and neuroprotective characteristics (Andreazza et al.2008). Worth noting, lithium's effects on oxidative stress biomarkers and antioxidant enzymes were investigated in animal as well as cell line models (Allagui et al. 2007;Frey et al. 2007; Paul et al. 2020). However, no previous research has examined the effects of lithium on antioxidant enzyme activities such as SOD, CAT, GPX, GLR, and GST in wild-type (WT) and in SOD, GPX, and GLR deficient yeasts.

Therefore, this study was designed to evaluate the effects of lithium on lipid peroxide level (MDA: oxidative stress biomarker) and on SOD, CAT, GPX, GR, and GST activities (the major antioxidant enzymes) in *Saccharomyces cerevisiae* WT, and in SOD, GPX and GLR deficient strains models.

MATERIALS AND METHODS

Chemicals

Hydrogen peroxide 30% (H_2O_2) was purchased from GAINLAND Chemical company, U.K. 1-chloro-2,4-dinitrobenzene was purchased from (MERCK-Schuchardt, Germany). SOD Assay Kit, glutathione reduced form (min 98%), Thiobarbituric acid (TBA), and lithium chloride (LiCl) were purchased from Sigma- Aldrich company (Germany). L- Glutathione oxidized (Min 98%) and NADPH-Tetrasodium salt were purchased from Applichem (Germany).

Yeast Strains

Saccharomyces cerevisiae (*S. cerevisiae*) WT and GLR, GPX, and SOD deficient strains were used in this study. These strains were a kind gift from Dr. Antonios Makris and Dr. Odat Osama, International Center for Advanced Mediterranean Agronomic Studies, Chania, Greece.

Yeast culture media.

Yeast culture media (1% Yeast extract, 2% Peptone, casein1%, Proline 0.12%, 2% glucose) as a carbon source were used in this study. The medium was sterilized at 15 P.S.I for 20 min, at 121 ^oC, and adjusted to pH (5.8 ± 0.2).

Growth condition

yeast cells were grown to saturation for 24 h at 25 ^oC in liquid yeast media. Yeast strains were cultured in the absence or presence of lithium chloride (0,1 5,10 mM LiCl) for 24 h at pH 5.8, 25 ^oC, and 150 rpm agitation rate. After 24 h, yeast strains were collected and lysed by ultrasonication in 30 s intervals for 5 min in the ice, centrifuged at 15000 rpm for 30 min, and the supernatant was collected and stored at -70 ^oC for further studies.

Determination of lipid peroxidation

Lipid peroxidation was measured according to a procedure mentioned in Draper and Hadley (1990), it was represented by malondialdehyde (MDA) level as an indicator of ROS generation. The assay was based on the reaction of thiobarbituric reactive substances (TBARS) with malondialdehyde (MDA); MDA is one of the low-molecular-weight end products generated by the breakdown of lipid peroxidation products. 2 ml of 10% trichloroacetic acid solution was added to 1 ml of yeast strains homogenate with heating in a boiling water bath for 15 minutes. The tubes were chilled under tap water, centrifuged at 1000 rpm for 10 minutes, and 2 ml of the supernatants were added to 1 ml of 0.67% TBA solution in a test tube. The preparations were placed in a boiling water bath for 15 min. They were cooled under tap water and the absorbencies of the resulted reaction products were measured using a spectrophotometer at $\lambda_{532\ nm}$. In the control experiment, which followed the same experimental approach, distilled water was utilized instead of the TBA solution. MDA concentrations were determined by the measurement of pink-colored complex (MDA-TBA complex; absorbance coefficient $E = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$) formation at 532 nm. The MDA values were expressed in µmole/g protein.

Catalase activity

Catalase activity (CAT) was assayed according to the method of Aebi (1984). Briefly, 20 µl of yeast strains homogenates was added to 965 µl of phosphate buffer (pH 6.5, 0.2 M), then 50 µl of H_2O_2 (15 mM fresh solution) was added. Before the addition of H_2O_2, the absorbance value in the spectrophotometer was adjusted to auto-zero at 240 nm, then after the addition of H_2O_2, the decrease in absorbance value was monitored at 240 nm for 1 min and expressed as µmole H_2O_2 decomposed/mg protein/min (specific absorption coefficient is 43.6 $M^{-1}\ cm^{-1}$ H_2O_2 at 240 nm).

Superoxide dismutase activity

Superoxide dismutase (SOD) activity was determined using the SOD determination kit (Sigma- Aldrich, Germany), which was based on the method of (McCord and Fridovich, 1969). Xanthine and xanthine oxidase were used to generate superoxide anion radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride quantitatively to form a red formazan dye. SOD inhibits the reaction by converting the superoxide radical to

H_2O_2 and oxygen. A unit of SOD activity was expressed as unit/mg protein which was defined as the amount of enzyme that causes 50 % inhibition of cytochrome c reduction under standard conditions.

Glutathione peroxidase activity

The activity of glutathione peroxidase (GPX) in the supernatant of yeast strains homogenate was measured spectrophotometrically as described previously (Paglia and Valentine, 1967). The assay mixture contained 20 µl of 0.1 M GSH, 10 µl of the sample, 100 µl of 2 mM NADPH, 100 µl of 10 U/ml GR, 760 µl of 0.2 M Tris-HCl pH 8, and 10 µl of 7 mM t-butyl hydroperoxide. The rate of NADPH oxidation was followed at 340 nm. Controls without t-butyl hydroperoxide always accompanied each assay. The specific activity was expressed as µmol/mg protein/min estimating the decrease of NADPH for 1 min at 340 nm (specific absorption coefficient is 6.22 mM^{-1} cm^{-1} NADPH at 340 nm).

Glutathione reductase

Glutathione reductase (GR) activity in the supernatant of yeast strains homogenate was measured according to the method described by (Glatzle et al. 1974). The enzyme assay mixture contained 100 µl of 33.0 mM oxidized glutathione, 10 µl of the sample, 840 µl of 50 mM of sodium phosphate buffer, pH 7.5, and 50 µl of 2 mM NADPH. The rate of NADPH oxidation was followed photometrically at 340 nm. Controls without NADPH were used and the specific activity was expressed as µmol/min/mg protein.

Glutathione S-transferase

The activity of glutathione S-transferase (GST) was evaluated by an increase of absorbance for 1 min at 340 nm as a result of the GSH and CDNB (1-chloro-2,4-dinitrobenzene) conjugation as described by (Habig et al.1974). The test mixture consisted of 50 µl of 0.1 M reduced glutathione, 20 µl of the sample, 880 µl of sodium phosphate buffer, pH 6.8, and 50 µl of 100 mM 1-chloro2,4 dinitrobenzene to start the reaction. The enzyme's specific activity was measured in µmol/min/mg protein using controls that did not contain 1-chloro-2,4 dinitrobenzene. The specific absorption coefficient of the conjugate S-(2,4-dinitrophenyl)-glutathione is 9.6 mM^{-1} cm^{-1} at 340 nm.

Total protein levels

Total protein levels of the supernatants were measured at 750 nm after mixing with the Folin-phenol reagent according to the method of (Lowry et al.1951). Bovine serum albumin was used as standard, and the calculations were expressed as mg/mL.

Statistical methods

All enzymes, lipid peroxide, and growth of strains result,s in the presence and absence of lithium were performed 3 times. Data were expressed as Mean ± Standard Error and analyzed using a one-way analysis of variance (ANOVA) test with sigmaplot application. $P < 0.05$ was considered statistically significant

RESULTS AND DISCUSSION

The effect of lithium concentrations on the growth of yeast cells

Figure 1 shows that LiCl, after 24h of exposure, induced dose-dependent decreases of the cell number in culture. The lowest concentration that significantly inhibited WT and SOD, GPX, and GLR deficient strains growth was 1 mM. The concentrations that caused a 50% decrease in growth of WT, and SOD, GPX, and GLR deficient yeast strains (IC_{50}) were 4.46, 2.99, 3.15, and 3.32 mM LiCl, respectively; values dependent on the kind of the cells used. They were lower in SOD, GPX, and GLR deficient cells than in WT ones. Interestingly, IC_{50} values of LiCl against SOD, GPX, and GLR deficient strains were insignificantly different.

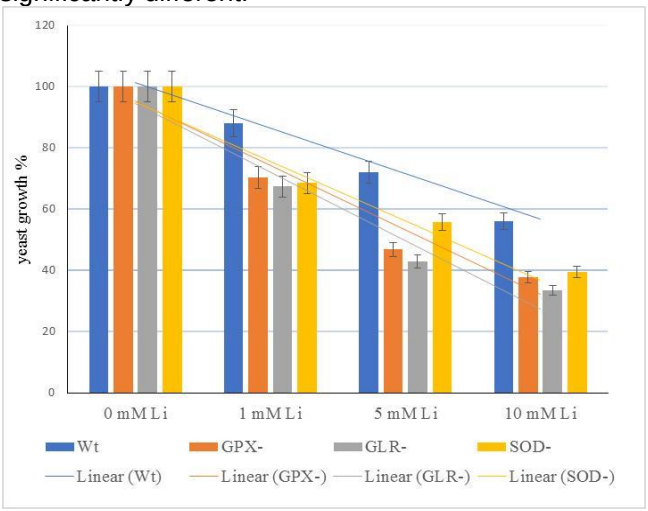

Figure 1: Dose effects of Li on the growth of *S. cerevisiae* strains cultured for 24 h in presence of LiCl. Values are the mean of 3 cultures/concentration ± standard error. Growth % is expressed relative to control which was regarded as 100%.

Table 1: The effects of LiCl on the growth of *S. cerevisiae* strains and expressed as IC_{50} value.

LiCl (IC_{50}) mM *S. cerevisiae* strain			
WT	SOD defcient	GPX defcient	GLR defcient
4.46 ± 0.14[a]	2.99 ± 0.09 [b]	3.15 ± 0.42 [b]	3.32 ± 0.21 [b]
y = -14.8x + 116 R^2 = 0.9956	y = -19.43x + 114.55 R^2 = 0.9586	y = -21.01x + 116.25 R^2 = 0.9523	y = -22.4x + 116.95 R^2 = 0.9482

IC_{50} values were determined from the dose growth curves. The results are presented as mean ± SD. IC50: the concentration that inhibits the growth of yeast by 50%. [a,b] Values were considered significant with $p < 0.05$.

The cytotoxic effects of Li in GPX, GLR, and SOD

deficient cells could be attributed to superoxide (O2˙-) accumulation which is account for the increased cell death. This result agrees with previous studies, which indicate that part of lithium toxicity is mediated by oxidative stress (Kielczykowska et al. 2004; Holley et al. 2014; Zhao et al. 2021).

Lipid peroxides (MDA)

The influence of Li on the production of MDA is depicted in Figure 2. After in vitro treatment of S. cerevisiae wild type and GLR and at lower concentrations in GPX deficient strains, with lithium, there was no significant change in MDA concentration, however, there was a significant rise in MDA after exposure of the GPX mutant to an increased concentration of Li ($P < 0.05$). In addition, MDA levels rise significantly after SOD is exposed to higher Li concentrations ($P < 0.05$). Moreover, there was a significant difference reported between SOD, wild type, GPX, and GLR deficient strains groups in response to Li.

These effects of Li on MDA levels in SOD, GR, and GPX deficient strains correspond to deficit effects of those enzymes in the detoxifications of ROS in cells and this is in accord with previous research (Tandon et al.1998; Ranjekar et al. 2003; Kielczykowska et al. 2004; Efrati et al. 2005; Allagui et al. 2007; Holley et al. 2014; Zhao et al. 2021). The nonsignificant change in MDA level in WT is attributable to CAT or GPX activity, both of which can detoxify hydroperoxides and other organic peroxides using GSH as a reducer (Grant et al.1998; Grant, 2001).

SOD activity

Figure 3 shows that after *in vitro* Li exposures, SOD activity did not change significantly ($P > 0.05$) in the WT or GPX deficient strains. Though, Li caused a significant change in WT when compared to the control group at 10 mM. SOD activity in the GLR deficient strain was increased

significantly after Li treatment ($P < 0.05$). There was also a significant change in SOD activity in WT, GPX, and GLR deficient strains after exposure to concentrations ≥ 5 mM of Li *in vitro*. The changes in SOD activities were found to be

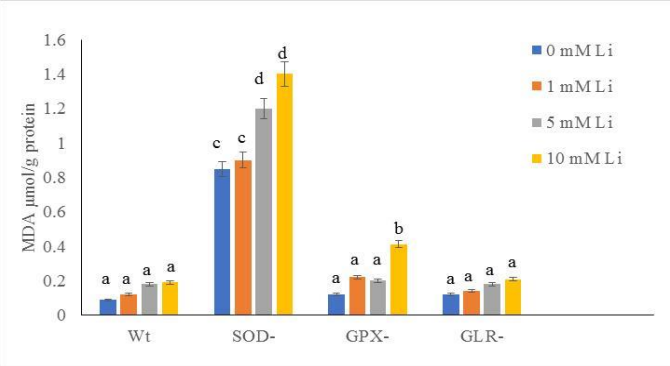

Figure 2: The effect of Li on lipid peroxide adducts (MDA) levels in *S. cerevisiae* WT and SOD, GR, and GPX deficient strains. Different letters indicate the significant differences between the control and exposed groups and among the exposure concentrations, respectively ($P < 0.05$). Data represents the mean± standard error.

increased in WT and GLR deficient strains confirming the hypothesis that lithium-induced the production of O2˙- by the SOD to O2 and H_2O_2 (Allagui et al. 2007). SOD gene in A549 cells was found to be down-regulated and its activity is closely related to lithium concentrations and levels of ROS production ((Allagui et al. 2007).

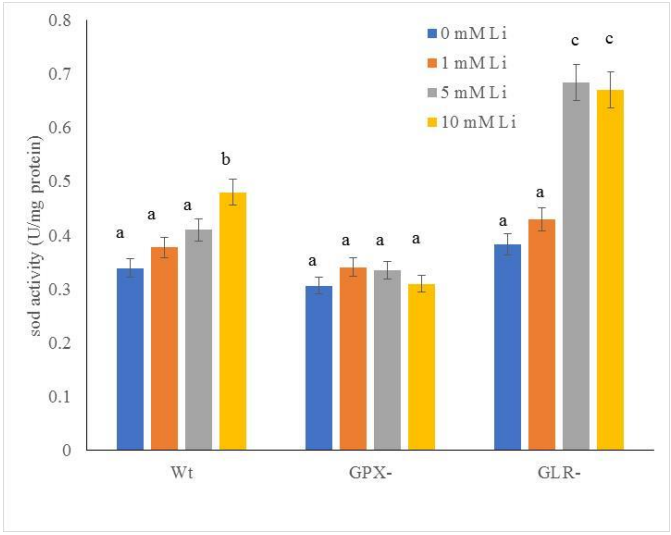

Figure 3: The effect of Li on the SOD activity in *S. cerevisiae* WT and GPX and GLR deficient strains. Different letters indicate the significant differences between the control and exposed groups and among the exposure concentrations, respectively ($P < 0.05$). Data represents the mean± standard error.

CAT activity

Lithium treatment decreased CAT activity in wild type, GPX, SOD, and GLR deficient strains in a dose-dependent manner (Figure 4). Furthermore, there was a significant difference in CAT activity between WT, GPX, SOD, and GLR deficient strains. Worth noting, there were significant differences in CAT activities within each yeast group at different concentrations of Li ($P < 0.05$). These results coincided with the reported effect of lithium on the reduction of CAT activities and SOD via lowering the generation of H_2O_2 and hydroxyl radicals or by directly binding to those enzymes (Pinto et al.2003). Moreover, an increase in GPX activity is correlated with a decrease in CAT activity due to differences in distinct subcellular localisations and their affinities to H_2O_2 (Barata et al.2005).

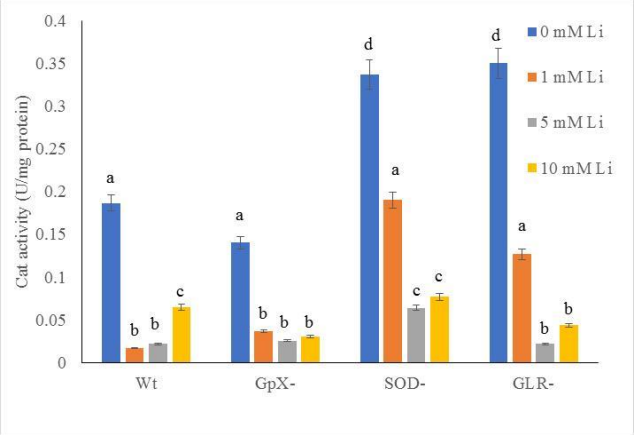

Figure 4: The effect of Li on CAT activity in *S. cerevisiae* WT and GPX, SOD, and GLR deficient strains. Different letters indicate the significant differences between the control and exposed groups and among the exposure concentrations, respectively (P < 0.05). Data represents the mean± standard error.

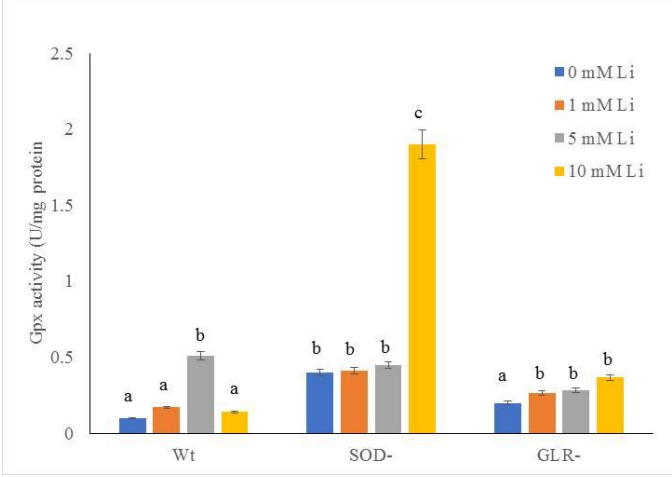

Figure 5: The effect of Li on GPX activity in *S. cerevisiae* WT, SOD, and GLR deficient strains. Different letters indicate the significant differences between the control and exposed groups and among the exposure concentrations, respectively (P < 0.05). Data represents the mean± standard error.

GPX activity

Higher Li treatments significantly increased GPX activity of SOD deficient strain relative to controls, but at lower Li concentrations had no significant effect (Figure 5). In addition, in both WT and GLR deficient strains, GPX activity was slightly altered following Li treatments compared to control. When GPX activities in SOD and GLR deficient strains are evaluated, it is found to be significantly higher than that of the WT (P <0.05).

In this regard, the unchanged GPX activity at low levels of Li can be related to the protection afforded by lithium and might be due, at least in part, to its inhibition of $O_2^{\bullet-}$ from mitochondrial complexes I and III as previously shown (Maurer et al.2009). However, inhibition of GPX activity in WT by higher Li concentration might be due to $O_2^{\bullet-}$ (Eraković et al. 2000; Oktem et al. 2005). The GPX activity was increased in SOD and GLR deficient strains at high Li concentrations as a consequence of a deficiency in SOD and CAT production (Halliwell and Gutteridge, 2015).

GST activity

After Li exposure, GST activity was reduced in the WT, GPX, and GLR deficient strains, with lower GST values in the GLR deficient strains at high Li concentrations compared to lower concentrations, whereas its activity was increased significantly in SOD deficient strain (P < 0.05) (Figure 6). Furthermore, no significant differences in GST activities were detected between strains, except that they were increased in SOD deficient and decreased in GLR deficient strains at greater concentrations of Li.

The increase of GST activity in SOD deficient strains is related to its role in detoxifying organic hydroperoxide due to ROS production. Meanwhile, its lower activity in GLR and GPX deficient strains is attributed to a decrease in the GSH level under oxidation stress (Downs et al. 2001; Engin et al. 2005; Eroglu et al. 2015) and/or inhibition of antioxidant enzyme activities (Oktem et al. 2005; Allagui et al. 2007;Shao et al. 2008)

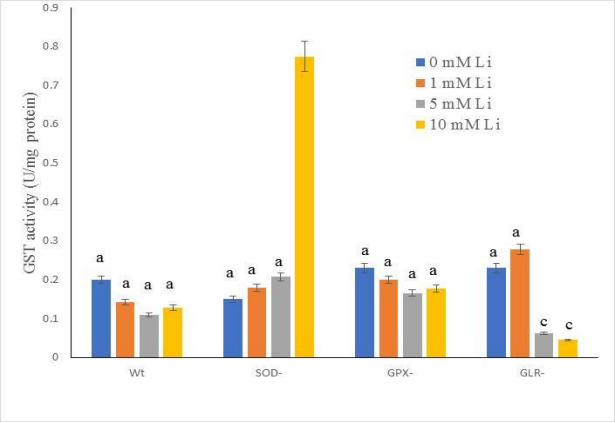

Figure 6: The effect of Li on GST activity in *S. cerevisiae* WT, GPX, GLR, and SOD deficient strains. Different letters indicate the significant differences between the control and exposed groups and among the exposure concentrations, respectively (P < 0.05). Data represents the mean± standard error.

GR activity

The GR activity was increased in GPX deficient strain treated with Li (Figure 7), while its activity was significantly decreased in WT and SOD deficient strains compared to control (without Li). Moreover, the higher concentration of Li (10 mM) caused an increase in the wild-type GR activity when compared to the lower concentration of Li (5 mM).

The decrease of GR activity in WT and SOD deficient strains is related to the oxidation of GSH as the substrate used by GPX and GST to detoxify ROS (Sharma,2004). Meanwhile, GR activity was increased in GPX deficient strain and this related to the inability of GPX deficient strain to consume the GSH.

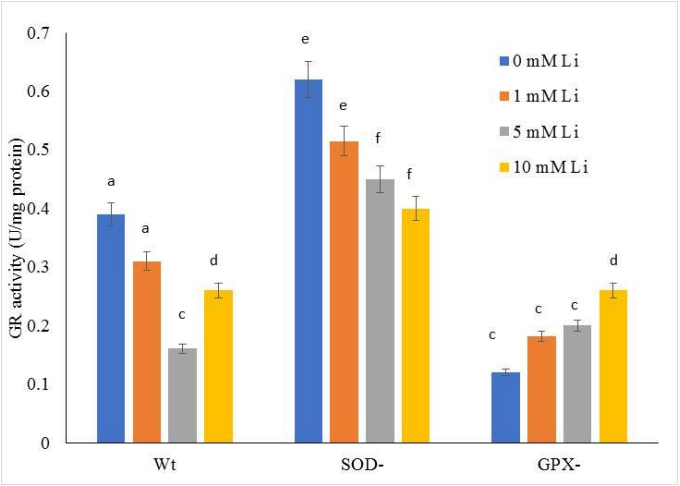

Figure 7: The effect of Li on GR activity in *S. cerevisiae* WT, GPX, and SOD deficient strains. Different letters indicate the significant differences between the control and exposed groups and among the exposure concentrations, respectively ($P < 0.05$). Data represents the mean± standard error.

CONCLUSION

Under lithium treatment, SOD, CAT, GPX, and GST activities as well as lipid peroxides levels in WT and SOD, GPX, and GR deficient strains were changed. Likewise, the lithium effects in ROS production are related to oxidative stress regulation by antioxidant enzymes. ROS generation is involved in lipid peroxides production. Interesting Lithium at very low concentrations protects WT cells against oxidative stress and verifies that lithium enhances the antioxidant defense system against oxidative stress. Moreover, a cytotoxic effect of Li in GR, SOD, and GPX deficient strains was found at higher doses.

CONFLICT OF INTEREST

The authors declared that the present study was performed in absence of any conflict of interest.

ACKNOWLEDGEMENT

The author would like to thank Bilal Al- Hwamdih for his help in the laboratory work. This work was carried out at the Department of Biology, Mutah University, Karak, Jordan.

AUTHOR CONTRIBUTIONS

AA has designed the study, wrote the manuscript, and performed bench experiments as well as results analysis.

REFERENCES

Aebi H (1984). Catalase in vitro. Methods Enzymol. 105:121-6. doi: 10.1016/s0076-6879(84)05016-3.

Allagui MS, Vincent C, El feki A, Gaubin Y, Croute F (2007). Lithium toxicity and expression of stress-related genes or proteins in A549 cells. Biochim Biophys Acta. 2007 Jul;1773(7):1107-15.doi:10.1016/j.bbamcr.2007.04 .007.

Andreazza AC, Cassini C, Rosa AR, Leite MC, de Almeida LM, Nardin P, Cunha AB, Ceresér KM, Santin A, Gottfried C, Salvador M, Kapczinski F, Gonçalves CA (2007). Serum S100B and antioxidant enzymes in bipolar patients. J Psychiatr Res. 41(6):523-9. doi: 10.1016/j.jpsychires.2006.07.013.

Andreazza AC, Kauer-Sant'Anna M, Frey BN, Stertz L, Zanotto C, Ribeiro L, Giasson K, Valvassori SS, Réus GZ, Salvador M, Quevedo J, Gonçalves CA, Kapczinski F(2008). Effects of mood stabilizers on DNA damage in an animal model of mania. J Psychiatry Neurosci. 33(6):516-24. PMID: 18982174.

Barata C, Varo I, Navarro JC, Arun S, Porte C (2005). Antioxidant enzyme activities and lipid peroxidation in the freshwater cladoceran Daphnia magna exposed to redox cycling compounds. Comp Biochem Physiol C Toxicol Pharmacol. 140(2):175-186. doi: 10.1016/j.cca.2005.01.013. Epub 2005 Feb 24. PMID: 15907763.doi: 10.2165/00002512-200118090-00004.

Downs CA, Dillon RT, Fauth JE, Woodley CM (2001). A molecular biomarker system for assessing the health of gastropods (Ilyanassa obsoleta) exposed to natural and anthropogenic stressors. J Exp Mar Biol Ecol. 259(2):189-214. doi: 10.1016/s0022-0981(01)00233-7. PMID: 11343712.

Draper HH, Hadley M (1990) . Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol. 1990;186:421-31. doi: 10.1016/0076-6879(90)86135-i. PMID: 2233309.

Efrati S, Averbukh M, Berman S, Feldman L, Dishy V, Kachko L, Weissgarten J, Golik A, Averbukh Z (2005). N-Acetylcysteine ameliorates lithium-induced renal failure in rats. Nephrol Dial Transplant. 2005 20(1):65-70. doi: 10.1093/ndt/gfh573. Epub 2004 Nov 16.

PMID: 15546888.

Engin A, Altan N, Isik E (2005). Erythrocyte glutathione levels in lithium-induced hypothyroidism. Drugs R D. 2005;6(1):35-40. doi:10.2165/00126839-200506010-00004

Eraković V, Zupan G, Varljen J, Laginja J, Simonić A (2000). Lithium plus pilocarpine induced status epilepticus--biochemical changes. Neurosci Res. 36(2):157-66. doi: 10.1016/s0168-0102(99)00120-0.

Eroglu A, Dogan Z, Kanak EG, Atli G, Canli M (2015). Effects of heavy metals (Cd, Cu, Cr, Pb, Zn) on fish glutathione metabolism. Environ Sci Pollut Res Int. 22(5):3229-37. doi: 10.1007/s11356-014-2972-y.

Frey BN, Andreazza AC, Kunz M, Gomes FA, Quevedo J, Salvador M, Gonçalves CA, Kapczinski F (2007). Increased oxidative stress and DNA damage in bipolar disorder: a twin-case report. Prog Neuropsychopharmacol Biol Psychiatry. 30;31(1):283-5. doi: 10.1016/j.pnpbp.2006.06.011.

Frey BN, Valvassori SS, Réus GZ, Martins MR, Petronilho FC, Bardini K, Dal-Pizzol F, Kapczinski F, Quevedo J (2006). Effects of lithium and valproate on amphetamine-induced oxidative stress generation in an animal model of mania. J Psychiatry Neurosci. 31(5):326-32.PMID:16951735;PMCID: PMC1557682.

Glatzle D, Vuilleumier JP, Weber F, Decker K (1974). Glutathione reductase test with whole blood, a convenient procedure for the assessment of the riboflavin status in humans. Experientia. 30(6):665-7. doi: 10.1007/BF01921531. PMID: 4151937.

Grant CM, Perrone G, Dawes IW (1998). Glutathione and catalase provide overlapping defenses for protection against hydrogen peroxide in the yeast Saccharomyces cerevisiae. Biochem Biophys Res Commun.253(3):893-898.doi:10.1006/bbrc.1998. 9864.

Grant CM. (2001). Role of the glutathione/glutaredoxin and thioredoxin systems in yeast growth and response to stress conditions. Molecular Microbiology, 39: 533-541. https://doi.org/10.1046/j.13652958.2001.02283. x.

Gsell W, Conrad R, Hickethier M, Sofic E, Frölich L, Wichart I, Jellinger K, Moll G, Ransmayr G, Beckmann H, Riederer P (1995). Decreased catalase activity but unchanged superoxide dismutase activity in brains of patients with dementia of Alzheimer type. J Neurochem. J Neurochem. 64(3):1216-1223. doi:10.1046/j.1471-4159.1995.64031216.x.

Habig WH, Pabst MJ, Jakoby WB (1974). Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem. 249(22):7130-9. PMID: 4436300.

Hajikarimlou M, Hunt K, Kirby G, Takallou S, Jagadeesan SK, Omidi K, Hooshyar M, Burnside D, Moteshareie H, Babu M, Smith M, Holcik M, Samanfar B, Golshani A (2020). Lithium Chloride Sensitivity in Yeast and Regulation of Translation. Int J Mol Sci.21(16):5730. doi: 10.3390/ijms21165730.

Halliwell B (2001). Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. Drugs Aging ; 18(9):685-716.

Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. 5th edition ed. Oxford University Press; 2015.DOI:10.1093/acprof:oso/9780198717478.001.0001.

Holley AK, Miao L, St Clair DK, St Clair WH (2014). Redox-modulated phenomena and radiation therapy: the central role of superoxide dismutases. Antioxid Redox Signal.20(10):1567-1589.doi:10.1089/ars.2012.5000.

Hussain S, Slikker W Jr, Ali SF(1995). Age-related changes in antioxidant enzymes, superoxide dismutase, catalase, glutathione peroxidase and glutathione in different regions of mouse brain. Int J Dev Neurosci. 13(8):811-817. doi:10.1016/0736-5748(95)00071-2.

Kielczykowska M,Pastermak K, Musik I, Wroniska J (2004). The effect of lithium administration in a diet on the chosen parameters of the antioxidant barrier in rats, Ann. Univ. Mariae Curie-Sklodowsk a Sectio D: Medicina. 59(2) :140–145.

Liu Q, Xie F, Rolston R, Moreira PI, Nunomura A, Zhu X, Smith MA, Perry G (2007). Prevention and treatment of Alzheimer disease and aging: antioxidants. Mini Rev.Med.Chem.2007;7(2):171-180.doi:10.2174/ 138955707779802552.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin phenol reagent. J Biol Chem. 193(1):265-75. PMID: 14907713.

Machado-Vieira R, Andreazza AC, Viale CI, Zanatto V, Cereser V Jr, da Silva Vargas R, Kapczinski F, Portela LV, Souza DO, Salvador M, Gentil V(2007). Oxidative stress parameters in unmedicated and treated bipolar subjects during initial manic episode: a possible role for lithium antioxidant effects. Neurosci Lett. 421(1):33-6. doi: 10.1016/j.neulet.2007.05.016.

Machado-Vieira R, Manji HK, Zarate CA Jr (2009). The role of lithium in the treatment of bipolar disorder: convergent evidence for neurotrophic effects as a unifying hypothesis. Bipolar Disord. 2009 Jun;11 Suppl 2(Suppl 2):92-109. doi: 10.1111/j.1399-5618.2009.00714.x.

Mahadik SP, Evans D, Lal H (2001) Oxidative stress and role of antioxidant and omega-3 essential fatty acid supplementation in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry. 25(3):463-493. doi:10.1016/s0278-5846(00)00181-0.

Maurer IC, Schippel P, Volz HP (2009). Lithium-induced enhancement of mitochondrial oxidative phosphorylation in human brain tissue. Bipolar Disord. 11(5):515-22.doi: 10.1111/j.1399-5618.2009.00729.x.

McCord JM, Fridovich I (1969). Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem. 244(22):6049-55. PMID: 5389100.

Oktem F, Ozguner F, Sulak O, Olgar S, Akturk O, Yilmaz

HR, Altuntas I (2005). Lithium-induced renal toxicity in rats: protection by a novel antioxidant caffeic acid phenethyl ester. Mol Cell Biochem. 277(1-2):109-15. doi: 10.1007/s11010-005-5426-5. PMID: 16132721.

Paglia DE, Valentine WN (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 70(1):158-69. PMID: 6066618.

Palmieri B, Sblendorio V(2006)Oxidative stress detection: what for? Part I. Eur Rev Med Pharmacol Sci. 10(6):291-317. PMID: 17274534.

Paul P, Iyer S, Nadella RK, Nayak R, Chellappa AS, Ambardar S, Sud R, Sukumaran SK, Purushottam M, Jain S; ADBS Consortium (ADBS: The Accelerator program for Discovery in Brain disorders using Stem cells), Viswanath B (2020). Lithium response in bipolar disorder correlates with improved cell viability of patient derived cell lines. Sci Rep. 4;10(1):7428. doi: 10.1038/s41598-020-64202-1.

Pinto E, Sigaud-Kutner TCS, Leitao MAS, Okamoto OK, Morse D, Colepicolo P(2003) Heavy metal-induced oxidative stress in algae, J. Phycol. 39;1008–1018. https://doi.org/10.1111/j.0022-3646.2003.02-193.x.

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(2):109-22. doi: 10.1016/s0165-1781(03)00220-8.

Shao L, Cui J, Young LT, Wang JF (2008). The effect of mood stabilizer lithium on expression and activity of glutathione s-transferase isoenzymes. Neuroscience. 151(2):51824.doi:10.1016/j.neuroscience.2007.10.041.

Shao L, Young LT, Wang JF(2005). Chronic treatment with mood stabilizers lithium and valproate prevents excitotoxicity by inhibiting oxidative stress in rat cerebral cortical cells. Biol Psychiatry. 58(11):879-84. doi: 10.1016/j.biopsych.2005.04.052.

Sharma R, Yang Y, Sharma A, Awasthi S, Awasthi YC (2004). Antioxidant role of glutathione S-transferases: protection against oxidant toxicity and regulation of stress-mediated apoptosis. Antioxid Redox Signal. 6(2):289-300. doi: 10.1089/152308604322899350.

Sies H (2021).Oxidative eustress: On constant alert for redox homeostasis. Redox Biol. 41:101867. doi:10.1016/j.redox.2021.101867.

Tandon A, Dhawan DK, Nagpaul JP (1998). Effect of lithium on hepatic lipid peroxidation and antioxidative enzymes under different dietary protein regimens. J Appl Toxicol. 18(3):187-190. doi: 10.1002/(sici)1099-1263(199805/06)18:3<187::aid-jat495>3.0.co;2-y.

Wang JF, Azzam JE, Young LT (2003). Valproate inhibits oxidative damage to lipid and protein in primary cultured rat cerebrocortical cells. Neuroscience. 116(2):485-489.doi:10.1016/s0306-4522(02)00655-3.

Wang JF, Shao L, Sun X, Young LT (2004). Glutathione S-transferase is a novel target for mood stabilizing drugs in primary cultured neurons. J Neurochem. 88(6):1477-84.doi:10.1046/j.1471-4159.2003.02276.x.

Williams RS, Harwood A (2000). Lithium therapy and signal transduction. Trends Pharmacol. Sci. 21:61–64. doi: 10.1016/S0165-6147(99)01428-5.

Won E, Kim YK (2017). An Oldie but Goodie: Lithium in the Treatment of Bipolar Disorder through Neuroprotective and Neurotrophic Mechanisms. Int J Mol Sci. 18(12):2679. doi: 10.3390/ijms18122679. PMID: 29232923; PMCID: PMC5751281.

Yan P, Xu D, Ji Y, Yin F, Cui J, Su R, Wang Y, Zhu Y, Wei S, Lai J (2019). LiCl Pretreatment Ameliorates Adolescent Methamphetamine Exposure-Induced Long-Term Alterations in Behavior and Hippocampal Ultrastructure in Adulthood in Mice. Int J Neuropsychopharmacol.22(4):303-316. doi: 10.1093/ijnp/pyz001. PMID: 30649326; PMCID: PMC6441133.

Zhao H, Zhang R, Yan X, Fan K (2021). Superoxide dismutase nanozymes: an emerging star for anti-oxidation. J Mater Chem B. 9(35):6939-6957. Published 2021 Sep 15. doi:10.1039/d1tb00720c.