ORIGINAL ARTICLE

Annals of Agricultural and Environmental Medicine 2017, Vol 24, No 3, 423–427
www.aaem.pl

Lithium as a prooxidant? A possible protective role of selenium – *in vitro* study

Irena Musik[1], Małgorzata Kiełczykowska[1], Barbara Rajtar[2], Łukasz Świątek[2], Małgorzata Polz-Dacewicz[2], Joanna Kocot[1]

[1] *Chair and Department of Medical Chemistry, Medical University of Lublin, Lublin, Poland*
[2] *Department of Virology, Medical University of Lublin, Lublin, Poland*

Musik I, Kiełczykowska M, Rajtar B, Świątek Ł, Polz-Dacewicz M, Kocot J. Lithium as a prooxidant? A possible protective role of selenium – *in vitro* study. Ann Agric Environ Med. 2017; 24(3): 423–427. doi: 10.26444/aaem/74473

Abstract

Introduction and objective. Lithium is used in medicine but its application may cause diverse side effects. Selenium has been found to show protective properties against negative influence of different harmful factors. This study was aimed at evaluating the influence of non-toxic dose of lithium on antioxidant parameters in FaDu (ATCC HTB-43) and Vero (ECACC No. 84113001) cell lines as well as the possible protective effect of non-toxic concentration of sodium selenite.

Materials and method. The cells were subjected to 0.17 mmol/L of Li_2CO_3 and/or 2.9 µmol/L of $Na_2SeO_3 \cdot 5H_2O$ for Vero as well as 0.47 mmol/L of Li_2CO_3 and/or 3.0 µmol/L of $Na_2SeO_3 \cdot 5H_2O$ for FaDu cells. The incubation was continued for the subsequent 72 h. In the cells total antioxidant status (TAS) values, activities of antioxidant enzymes – superoxide dismutase (SOD) and glutathione peroxidase (GPx) as well as the reduced glutathione concentration (GSH) were determined.

Results and conclusion. In Vero cells lithium decreased all studied parameters, particularly GPx. Selenium co-treatment showed a distinct protective effect. In FaDu cells the similar effect was observed only in case of GSH. The results point to differences in action of lithium and selenium in physiological and pathological state. As long-term lithium therapy is applied in psychiatric patients the results regarding Vero line let suggest that selenium might be considered as an adjuvant alleviating side effects of Li-treatment.

Key words

lithium carbonate, sodium selenite, total antioxidant status, antioxidants, FaDu (ATCC HTB-43) cells, Vero (ECACC No. 84113001) cells

INTRODUCTION

Despite the negligible content in the human organism, selenium belongs to the most important essential microelements for its proper functioning [1]. Its presence in molecules of antioxidant enzymes: glutathione peroxidase and thioredoxin makes it one of the most important antioxidants [2–4]. The outcomes of research on its possible protective properties against prooxidative processes have seemed to be promising and encouraging. Inorganic selenium (sodium selenite) has been found to prevent oxidative stress caused by methamphetamine in neuronal cells [5] and by As_2O_3 in fish hepatoma cells [6], as well as *t*-butyl hydroperoxide-induced DNA damage in human mesenchymal stromal cells [7]. Other forms (organic compounds and nanoparticles) of selenium have also been studied and the research has revealed the dependence of the effects on the used form [3, 8–10].

Lithium salts have been used in medicine, first of all in psychiatry, for more than sixty years [11, 12]. Apart from its beneficial action lithium therapy may be accompanied by diverse side effects including disturbances of nervous and alimentary system as well as disorders of kidneys, eyes and glands [13, 14]. The main complications occurring in patients receiving lithium result from the fact that it displays positive effects only within a strongly determined range [12]. This is why its administration must be applied with caution. The studies have shown that lithium can cause oxidative stress as well as changes of antioxidant activity [12, 14, 15]. It has also been found to influence expression of genes encoding antioxidant enzymes in human A549 cell line [13]. These observations provoked research on the use of different antioxidants as protective agents against lithium toxicity [15, 16]. The consciousness of the growing contamination of the environment with lithium resulting from its growing industrial application [13] has made such investigations all the more worth undertaking.

OBJECTIVES

The aim of the current study was to evaluate the influence of non-toxic dose of lithium carbonate on parameters of oxidative stress in FaDu (ATCC HTB-43) and Vero (ECACC No. 84113001) cell lines as well as the possible protective effect of inorganic selenium (sodium selenite).

MATERIAL AND METHODS

Cytotoxicity assays. The cytotoxicity of the studied compounds ($Na_2SeO_3 \cdot 5H_2O$ and Li_2CO_3) was performed against cell lines: FaDu (ATCC HTB-43) and Vero (ECACC No. 84113001). The stock solutions of the compounds were prepared in water and then diluted in incubating medium. After 24-hour-incubation in growth medium with addition of fetal bovine serum medium was replaced with the new one containing 0–1900 µmol/L or 0–6.757 mmol/L of Na_2SeO_3

Adress for correspondence: Joanna Kocot, Chair and Department of Medical Chemistry, Medical University of Lublin, 20-093 Lublin, Chodźki 4a, Poland
E-mail: joanna.kocot@umlub.pl

Received: 12 December 2016; accepted: 10 April 2017; *first published: June 2017*

· $5H_2O$ or Li_2CO_3, respectively. The cells were incubated at 37°C in the presence of 5% CO_2 for 72 h.

The cytotoxicity was determined using MTT formazan test. The MTT method is a quantitative colorimetric toxicity test, based on the transformation of yellow, soluble tetrazolium salts (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) to purple-blue insoluble formazane, by cellular dehydrogenases.

After 72 h incubation with compounds cell cultures were supplemented with 10 µL per well of 5 mg/mL MTT (Sigma-Aldrich, Saint Louis, MO, USA) stock in PBS (BIOMED, Lublin, Poland), and the incubation was continued for 4 h at 37˚C. Then, 100 µL of aqueous solution containing 50% dimethylformamide (POCH, Gliwice, Poland) and 20% SDS (sodium dodecyl sulphate, 99% pure) from AppliChem (Darmstadt, Germany) was added to solubilise the formazane precipitates produced by MTT. After the all-night incubation the absorbance was measured by the Epoch plate reader (BioTek, Winooski, Vermont, USA) at two wavelengths – 540 and 620 nm. On the basis of the obtained results the IC_{50} value, which is the amount of tested substance that is required to reduce the number of viable cells by 50% compared to the control culture, was determined and was calculated using the Gen 5 2.01.14 software (BioTek, Winooski, Vermont, USA). The investigation was carried out in triplicate.

Preparation of the material for determination of antioxidant parameters. After 24-hour-incubation the cells were subjected to non-toxic concentrations of the studied compound, having been determined in the previous part of the experiment (0.17 mmol/L of Li_2CO_3 and/or 2.9 µmol/L of $Na_2SeO_3 \cdot 5H_2O$ for Vero cell line and 0.47 mmol/L of Li_2CO_3 and/or 3.0 µmol/L of $Na_2SeO_3 \cdot 5H_2O$ for FaDu cell line). The incubation was continued for the subsequent 72 h. After removing the medium the cells were washed with PBS and treated with trypsin. After centrifugation (300 × g, 3 min.) the cells were washed with PBS, centrifuged again (300 × g, 3 min.) and the supernatant was removed. The obtained cells were suspended in PBS and stored at -20 °C for further assays.

Determination of antioxidant parameters. The following oxidant parameters were determined in the cells prepared as described above: total antioxidant status (TAS) values, activities of antioxidant enzymes – superoxide dismutase (SOD) and glutathione peroxidase (GPx) as well as the concentration of reduced glutathione (GSH).

TAS values in plasma were assayed using diagnostic kit by RANDOX (Randox Laboratories Limited, Crumlin, County Antrim, United Kingdom) and expressed in mmol of TAS/g of protein. SOD and GPx activities were determined using diagnostic kits RANSOD and RANSEL produced by RANDOX and expressed in U of SOD/mg of protein and U of GPx/g of protein, respectively. GSH concentration was determined using BIOXYTECH® GSH-400™ kit produced by OxisResearch™ (OXIS Health Products, Inc., Portland, Oregon, USA) and expressed in µmol of GSH/g of protein. Protein was measured using method of Bradford (Bradford 1976). The assays were performed with use of spectrophotometer SPECORD M40 (Zeiss Jena).

Statistical analysis. All statistical analyses were performed using STATISTICA program (version 10.0). The normality of data distribution was verified using Shapiro-Wilk test. The differences among the studied groups were analysed using a one-way analysis of variance (ANOVA), followed by Tukey test. Values were considered significant with $p < 0.05$.

RESULTS

Cytotoxicity assays. As shown in Fig. 1 and Fig. 2, the viability of FaDu and Vero cell lines was significantly affected by Li_2CO_3 and $Na_2SeO_3 \cdot 5H_2O$ in a concentration-dependent manner.

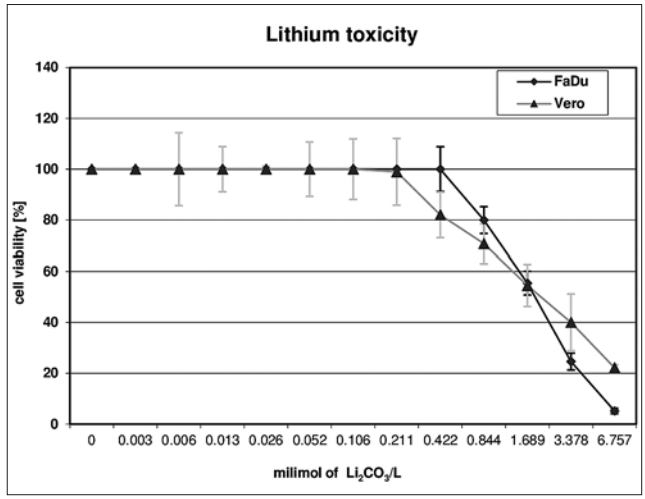

Figure 1. The effects of 72-hour incubation of FaDu and Vero cell lines with Li_2CO_3. Cell viability is expressed in 100% vs. control where the value obtained for control was regarded as 100%

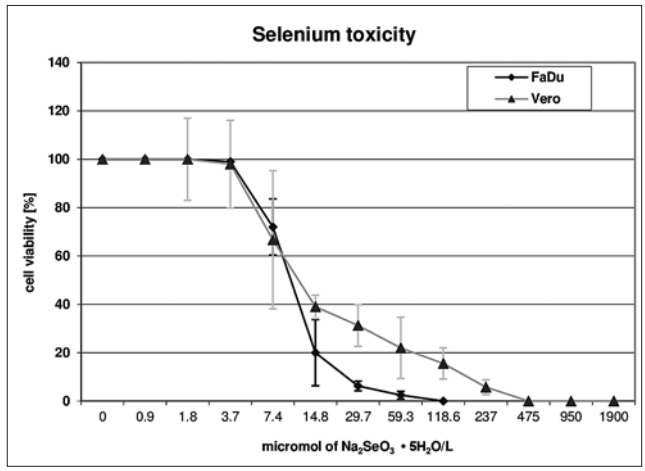

Figure 2. The effects of 72-hour incubation of FaDu and Vero cell lines with $Na_2SeO_3 \cdot 5H_2O$. Cell viability is expressed in 100% vs. control where the value obtained for control was regarded as 100%

The non-toxic values for the studied compounds were dependent on the kind of the used cells. They were lower in Vero cells than in FaDu ones in case of both Li_2CO_3 and $Na_2SeO_3 \cdot 5H_2O$. In contrast, IC_{50} values showed no differences between FaDu and Vero cell lines. Non-toxic and IC_{50} values for the studied compounds are presented in Tab. 1.

Oxidant parameters in Vero cells. In cells incubated in the presence of lithium SOD activity was slightly decreased vs. control, whereas in Li+Se-treated no difference in comparison with control was observed. Se alone enhanced

Table 1. Non-toxic and IC_{50} values for the studied compounds in μmol/L for $Na_2SeO_3 \cdot 5H_2O$ and mmol/L for Li_2CO_3

compound	cell line FaDu (ATCC HTB-43)		cell line Vero (ECACC No. 84113001)	
	NT	IC_{50}	NT	IC_{50}
$Na_2SeO_3 \cdot 5H_2O$	3.00±1.10	11.18±1.44	2.85±0.84	11.41±3.76
Li_2CO_3	0.47±0.14	1.61±0.13	0.17±0.05	1.64±0.07

Non-toxic and IC_{50} values were determined from the dose response curves. The results are presented as mean ± SD

SOD vs. all the other groups, but the significance was found only vs. Li-treatment. Lithium caused significant decrease in GPx activity vs. Se-alone and control groups. This negative effect was considerably alleviated by additional selenium. Selenium alone significantly increased GPx compared to Li+Se-treatment. TAS value was diminished in Li group, significantly compared to Li+Se-treatment and slightly vs. control. In Li+Se-treated cells in turn TAS value was increased in comparison to control. No statistical significance was observed in case of GSH concentration values.

The obtained results are presented in Fig. 3.

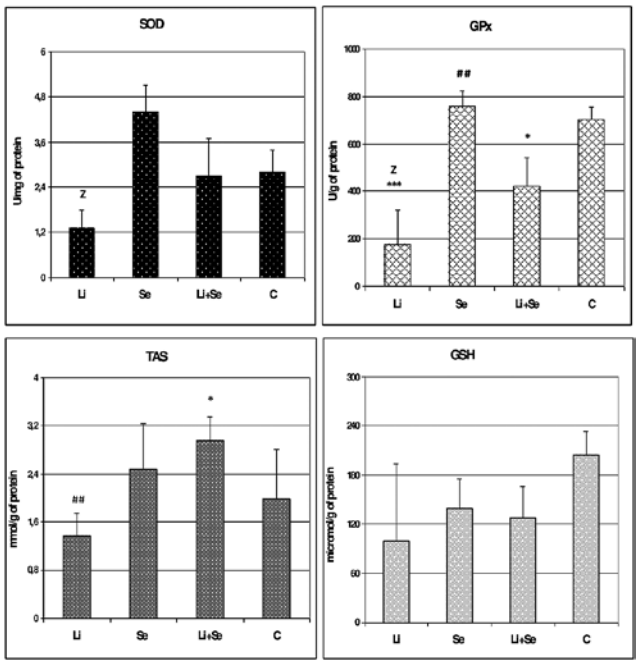

Figure 3. Antioxidant parameters in Vero cells incubated in the presence of lithium and/or selenium. C – control cells incubated in the medium with no lithium or selenium compounds.
The results are presented as mean ± SD.
The differences among the studied groups were analysed using a one-way analysis of variance (ANOVA), followed by Tukey test. Values were considered significant with $p < 0.05$.
*$p < 0.05$ vs. control; *** $p < 0.001$ vs. control; ##$p < 0.01$ vs. Li+Se group; ᶻ$p < 0.001$ vs. Se-group

Oxidant parameters in FaDu cells. SOD activity values showed no statistically significant differences among the studied treatments. GPx activity values were similar in the studied groups except for cells treated with selenium alone. In this case statistically significant increase vs. all the other groups was observed. Li significantly increased TAS in FaDu cells vs. all the other groups. Se-treatment showed no effect compared to control. In Li+Se-treated cells TAS value was markedly enhanced in comparison with the control. Both Li and Se alone decreased GSH concentration in comparison with two other groups although this effect was significant only in case of Se alone treatment.

The obtained results are presented in Fig. 4.

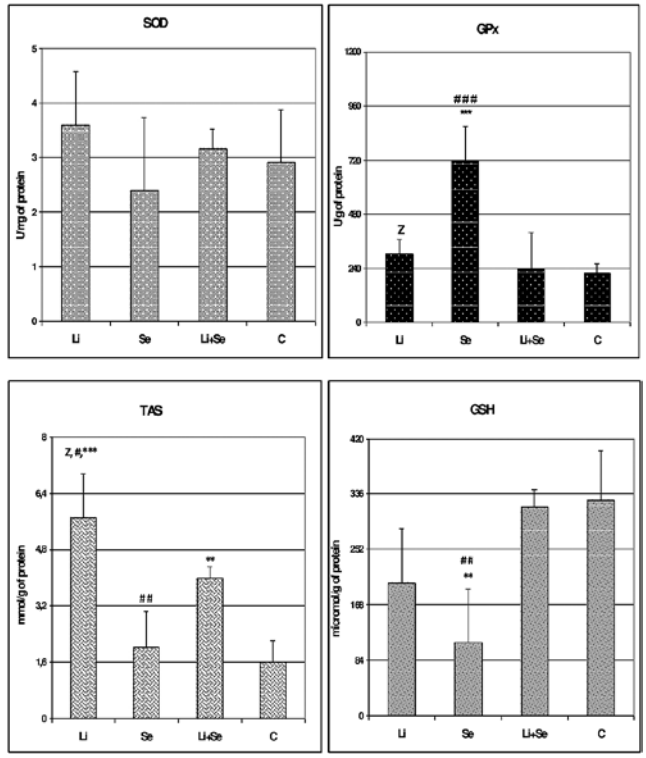

Figure 4. Antioxidant parameters in FaDu cells incubated in the presence of lithium and/or selenium. C – control cells incubated in the medium with no lithium or selenium compounds.
The results are presented as mean ± SD.
The differences among the studied groups were analysed using a one-way analysis of variance (ANOVA), followed by Tukey test. Values were considered significant with $p < 0.05$.
**$p < 0.01$ vs. control;
***$p < 0.001$ vs. control; #$p < 0.05$ vs. Li+Se group; ##$p < 0.01$ vs. Li+Se group;
###$p < 0.001$ vs. Li+Se group; ᶻ$p < 0.001$ vs. Se-group

DISCUSSION

As expected, in the current study inorganic selenium markedly increased GPx activity in both studied cell lines. Such results are consistent with other authors' findings. As selenium is a constituent of GPx, the issue of its effect on GPx activity was the subject of numerous studies performed on diverse typed of cell lines.

Sodium selenite (1μM in the culture media, 11 days) significantly enhanced GPx in human hepatoma Hep G2 cells [17]. Sodium selenite (100 nM) supplementation caused significant increase in GPx activity in bone marrow stromal cells as well as in telomerase-immortalized human mesenchymal stem cell line [18]. According to Barayuga et al. sodium selenite (10 or 100 nM for a week) was found to increase GPx1 and GPx4 isoforms in SH-SY5Y neuronal cells in dose-dependent way [5]. Rusolo et al. observed the similar effect in human hepatoma cell lines HepG2 and Huh7, treated for 24 h with 0.25, 0.5 or 1 μM of sodium selenite. The expression of GPx1 was found to be increased in dose-dependent way [19].

Research revealed that other forms of selenium showed the same effect. Khera et al. reported that both organic selenium (selenomethionine) and sodium selenite (500 nM and 100 nM for 24 h, respectively) markedly enhanced GPx activity in trophoblast BeWo cells [20]. Erkekoğlu et al., in turn, found that sodium selenite (30 nM) and selenomethionine (10 μM) markedly enhanced GPx1 activity in LNCaP human prostatic cancer cell line [1]. In one of the recently published studies the comparison of three different forms of selenium (methylseleninic acid, methylselenocysteine and Se yeast) was undertaken. The authors carried out the experiment on ER-positive MCF-7 and triple-negative MDA-MB 231 human breast cancer cell lines. GPx was increased in cells incubated in the presence of all types of selenium but methylseleninic acid was decidedly the most effective, particularly in case of MDA-MB 231 cells [21].

Selenium nanoparticles (0.5–5 μg/mL, 48 h) increased GPx activity in intestinal epithelial cells of crucian carp. The results were dose and size-dependent as the larger particles showed the more distinct effect when the concentration was higher. Interestingly, the increase was observed up to a specific concentration pointing to a kind of "saturation "and this effect was the most distinct in case of the smallest particles [22]. Such an effect was also reported by other scientists. Incubation in the presence of sodium selenite (0–500 nM for at least 7 days) resulted in enhancement of the GPx isoforms GPx1 and GPx4 in human lung cancer cell lines in dose-dependent way up to a certain point, but further increase in Se concentration did not change the observed activity. Interestingly, the concentration which caused that "saturation" was dependent on the kind of the cells. In case of GPx1 for H1944 cells it was 20 nM, for HPL1D 10 nM whereas for H460 increase was observed in the whole range of Se doses. In case of GPx4 the Se "saturation" doses were 100 nM, 250 nM, 100 nM and 40 nM for HPL1D, H460, H1944, and H1703 cells, respectively [23]. The next experiment, performed on human trophoblast cells, revealed that both sodium selenite (25 nM – 800 nM) and selenomethionine (250 nM – 1500 nM) caused GPx activity increase up to 100 nM (selenite) and 500 nM (selenomethionine) but the further enhancement of Se dose resulted in a distinct decrease [24].

In the present experiment selenium alone did not change SOD vs. control in a significant way. In this case the available reports showed divergent results. Similarly as in our study, 48-hour-exposure to selenium nanoparticles showed no significant influence on SOD in intestinal epithelial cells of crucian carp [22]. On the other hand, treatment with 100 nM sodium selenite caused increase in SOD activity in telomerase-immortalized human mesenchymal stem cell line [18]. In another interesting study the comparison of three different forms of selenium (methylseleninic acid, methylselenocysteine and Se yeast) was performed on ER-positive MCF-7 and triple-negative MDA-MB 231 human breast cancer cell lines. Selenium generally caused a slight increase in SOD activity, but methylseleninic acid was the most effective form [21]. Fu et al. also compared three Se-form (sodium selenite, selenomethionine and nanoparticles at a dose of 100 nM of Se) and showed that expression of superoxide dismutase (Cu-Zn isoform) in Caco-2 cells was decreased, particularly by the two latter forms [9].

The lack of significant effect of selenium alone on GSH concentration in Vero cells and decrease in FaDu ones, observed in the current study, is partially consistent with results obtained by other authors. Organic (selenomethionine) and inorganic (selenite) selenium did not affect GSH concentration in LNCaP human prostatic cancer cell line, but in those exposed to phthalates used as additives in plastics a significant increase was observed [1]. Wang et al. reported no influence of 48-hour-incubation in the presence of selenium nanoparticles on GSH concentration in intestinal epithelial cells of crucian carp, irrespective of Se concentration and size of particles [22]. In SH-SY5Y neuronal cells, differentiated in media containing sodium selenite (10 nM, 2 weeks), total intracellular GSH was not changed by the subsequent exposure to selenite at a dose of 10 nM for a week but decreased by a higher dose (100 nM for a week). In contrast, both Se doses caused well-marked decrease in total extracellular GSH [5]. The comparison of three Se-form (sodium selenite, selenomethionine and nanoparticles 100 nM of Se) revealed an increase in expression of glutathione synthase in Caco-2 cells, but two latter forms exerted a significantly greater effect [9]. In A549 human lung cancer cell line sodium selenite (0.5 μM) did not alter GSH content, while organic selenium (ethaselen 3 μM) and combination of these two forms caused GSH decrease, being intensified along the time of experiment [25].

In the present experiment, the impairment of antioxidant barrier resulting from Li-exposure was observed. Other studies also revealed the lithium's effect on oxidative processes but the outcomes are quite divergent. Eskandari et al. reported the increased generation of reactive oxygen species in hepatocytes incubated in the presence of Li_2CO_3 (EC50 dose of 2 mM was used) [12]. Allagui et al. found that rather high, toxic concentrations (5 or 10 mM) of lithium carbonate caused down regulation of genes of enzymes involved into antioxidant defence in lung alveolar human cells (A549) [13]. On the other hand, some authors suggested that lithium neuroprotective action could be connected with its antioxidative properties. However, such effect of lithium was proved to be dependent on the presence of other drugs as its combination with haloperidol was suggested to enhance oxidative stress of SH-SY5Y neuronal cells [26]. Arraf et al. reported that lithium pretreatment (LiCl 2 or 4 mM, 7 days) could prevent the toxicity of prooxidative hydrogen peroxide in cell culture (human neuroblastoma SH-SY5Y cells). However, using *in vitro* model of lipid peroxidation, those authors did not observe any antioxidant effect of lithium [11]. In the current experiment non-toxic lithium dose slightly decreased intracellular GSH vs. control in both studied cell lines. These outcomes are consistent with those obtained by Eskandari et al. who reported significant intracellular GSH depletion in hepatocytes incubated in the presence of EC50 dose Li_2CO_3 (2 mM) accompanied with well-marked extracellular GSH increase [12].

The deterioration of antioxidant defence, observed in Vero cells, was alleviated by coadministration of selenium. The possible protective properties of selenium were also the topic of other studies performed on cell lines. Both organic and inorganic selenium was proved to be effective in restoring a GPx1 decrease, caused by exposure to phthalates used as additives in plastics, in LNCaP human prostatic cancer cell line [1]. According to Zhou et al., cadmium-induced increase in ROS production in LLC-PK_1 cells (20 μM Cd for 12 h) was fully reversed by pretreatment with selenium (5, 10, or 20 μM for 0.5 h as sodium selenite) [4]. The similar protective effect of pretreatment with sodium selenite was observed by

Selvaraj et al. in fish hepatoma cell line PLHC-1 exposed to arsenic trioxide. In cells incubated in the presence of As_2O_3 (100 μM – IC_{50} concentration for 10, 20 or 40 h) decreased GPx activity was observed. After 10 and 20 h, in cells pre-incubated with selenium (1, 5 or 10 μM) for 2 h this effect was alleviated by all doses, whereas after 40 h the higher doses proved to be entirely ineffective [6].

CONCLUSIONS

The obtained results concerning Vero cells allow to suggest that selenium could play a protective role against lithium toxicity resulting from prooxidative effect. In FaDu line the outcomes were different, pointing to differences in action of lithium and selenium in physiological and pathological state. However, regarding the fact that lithium therapy is applied in psychiatric patients the results regarding Vero line seem to confirm the assumption that selenium might be taken into account as an adjuvant alleviating side effects of lithium therapy.

Acknowledgements
This research did not receive any specific grant from funding agencies in the public, commercial, or non-for-profit sectors and was funded in full by Medical University of Lublin.

REFERENCES

1. Erkekoğlu P, Rachidi W, De Rosa V, Giray B, Favier A, Hincal F. Protective effect of selenium supplementation on the genotoxicity of di(2-ethylhexyl)phthalate and mono(2-ethylhexyl)phthalate treatment in LNCaP cells. Free Radic Biol Med 2010; 49(4): 559–566. doi: 10.1016/j.freeradbiomed.2010.04.038
2. Al-Rasheed NM, Attia HA, Mohamed RA, Al-Rasheed NM, Al-Amin MA. Preventive effects of selenium yeast, chromium picolinate, zinc sulfate and their combination on oxidative stress, inflammation, impaired angiogenesis and atherogenesis in myocardial infarction in rats. J Pharm Pharm Sci. 2013; 16(5): 848–867.
3. Cuello S, Ramos S, Mateos R, Martín MA, Madrid Y, Cámara C, et al. Selenium methylselenocysteine protects human hepatoma HepG2 cells against oxidative stress induced by tert-butyl hydroperoxide. Anal Bioanal Chem. 2007; 389(7–8): 2167–2178. doi: 10.1007/s00216-007-1626-z.
4. Zhou YJ, Zhang SP, Liu CW, Cai YQ. The protection of selenium on ROS mediated-apoptosis by mitochondria dysfunction in cadmium-induced LLC-PK(1) cells. Toxicol in Vitro. 2009; 23(2): 288–294. doi: 10.1016/j.tiv.2008.12.009
5. Barayuga SM, Pang X, Andres MA, Panee J, Bellinger FP. Methamphetamine decreases levels of glutathione peroxidases 1 and 4 in SH-SY5Y neuronal cells: protective effects of selenium. Neurotoxicology. 2013; 37: 240–246. doi: 10.1016/j.neuro.2013.05.009
6. Selvaraj V, Yeager-Armstead M, Murray E. Protective and antioxidant role of selenium on arsenic trioxide-induced oxidative stress and genotoxicity in the fish hepatoma cell line PLHC-1. Environ Toxicol Chem. 2012; 31(12): 2861–2869. doi: 10.1002/etc.2022
7. Alves H, Mentink A, Le B, van Blitterswijk CA, de Boer J. Effect of antioxidant supplementation on the total yield, oxidative stress levels, and multipotency of bone marrow-derived human mesenchymal stromal cells. Tissue Eng Part A. 2013; 19(7–8): 928–937. doi: 10.1089/ten.TEA.2011.0700
8. Wang Y, Fu L. Forms of selenium affect its transport, uptake and glutathione peroxidase activity in the Caco-2 cell model. Biol Trace Elem Res. 2012; 149(1): 110–116. doi: 10.1007/s12011-012-9395-8
9. Fu L, Yan X, Ruan X, Lin J, Wang Y. Differential protein expression of Caco-2 cells treated with selenium nanoparticles compared with sodium selenite and selenomethionine. Nanoscale Res Lett. 2014; 9(1): 589. doi: 10.1186/1556-276X-9-589. eCollection 2014
10. Misra S, Boylan M, Selvam A, Spallholz JE, Björnstedt M. Redox-active selenium compounds--from toxicity and cell death to cancer treatment. Nutrients. 2015; 7(5): 3536–3556. doi: 10.3390/nu7053536
11. Arraf Z, Amit T, Youdim MB, Farah R. Lithium and oxidative stress lessons from the MPTP model of Parkinson's disease. Neurosci Lett. 2012; 516(1): 57–61. doi: 10.1016/j.neulet.2012.03.055
12. Eskandari MR, Fard JK, Hosseini MJ, Pourahmad J. Glutathione mediated reductive activation and mitochondrial dysfunction play key roles in lithium induced oxidative stress and cytotoxicity in liver. Biometals. 2012; 25(5): 863–873. doi: 10.1007/s10534-012-9552-8
13. Allagui MS, Vincent C, Elfeki A, Gaubin Y, Croute F. Lithium toxicity and expression of stress-related genes or proteins in A549 cells. Biochim Biophys Acta. 2007; 1773(7): 1107–1115.
14. Nciri R, Allagui MS, Bourogaa E, Saoudi M, Murat JC, Croute F, et al. Lipid peroxidation, antioxidant activities and stress protein (HSP72/73, GRP94) expression in kidney and liver of rats under lithium treatment. J Physiol Biochem. 2012; 68(1): 11–18. doi: 10.1007/s13105-011-0113-3
15. Malhotra A, Dhawan DK. Zinc improves antioxidative enzymes in red blood cells and hematology in lithium-treated rats. Nutr Res. 2008; 28(1): 43–50. doi: 10.1016/j.nutres.2007.11.002
16. Vijaimohan K, Mallika J, Shyamala DC. Chemoprotective Effect of Sobatum against Lithium-Induced Oxidative Damage in Rats. J Young Pharm. 2010; 2(1): 68–73. doi: 10.4103/0975-1483.62217
17. Helmy MH, Ismail SS, Fayed H, El-Bassiouni EA. Effect of selenium supplementation on the activities of glutathione metabolizing enzymes in human hepatoma Hep G2 cell line. Toxicology. 2000; 144(1–3): 57–61.
18. Ebert R, Ulmer M, Zeck S, Meissner-Weigl J, Schneider D, Stopper H, et al. Selenium supplementation restores the antioxidative capacity and prevents cell damage in bone marrow stromal cells in vitro. Stem Cells. 2006; 24(5): 1226–1235.
19. Rusolo F, Pucci B, Colonna G, Capone F, Guerriero E, Milone MR, et al. Evaluation of selenite effects on selenoproteins and cytokinome in human hepatoma cell lines. Molecules. 2013; 18(3): 2549–2562. doi: 10.3390/molecules18032549
20. Khera A, Vanderlelie JJ, Perkins AV. Selenium supplementation protects trophoblast cells from mitochondrial oxidative stress. Placenta. 2013; 34(7): 594–598. doi: 10.1016/j.placenta.2013.04.010
21. Guo CH, Hsia S, Shih MY, Hsieh FC, Chen PC. Effects of Selenium Yeast on Oxidative Stress, Growth Inhibition, and Apoptosis in Human Breast Cancer Cells. Int J Med Sci. 2015(9); 12: 748–758. doi: 10.7150/ijms.12177. eCollection 2015
22. Wang Y, Yan X, Fu L. Effect of selenium nanoparticles with different sizes in primary cultured intestinal epithelial cells of crucian carp, *Carassius auratus gibelio.* Int J Nanomedicine. 2013; 8: 4007–4013. doi: 10.2147/IJN.S43691
23. Romanowska M, Kikawa KD, Fields JR, Maciag A, North SL, Shiao YH, et al. Effects of selenium supplementation on expression of glutathione peroxidase isoforms in cultured human lung adenocarcinoma cell lines Lung Cancer. 2007; 55(1): 35–42. doi: 10.1016/j.lungcan.2006.09.007
24. Watson M, van Leer L, Vanderlelie JJ, Perkins AV. Selenium supplementation protects trophoblast cells from oxidative stress. Placenta. 2012; 33(12): 1012–1019. doi: 10.1016/j.placenta.2012.09.014
25. Xu W, Ma WW, Zeng HH. Synergistic effect of ethaselen and selenite treatment against A549 human non-small cell lung cancer cells. Asian Pac J Cancer Prev. 2014; 15(17): 7129–7135. doi: http://dx.doi.org/10.7314/APJCP.2014.15.17.7129
26. Gawlik-Kotelnicka O, Mielicki W, Rabe-Jabłońska J, Lazarek J, Strzelecki D. Impact of lithium alone or in combination with haloperidol on oxidative stress parameters and cell viability in SH-SY5Y cell culture. Acta Neuropsychiatr. 2016; 28(1): 38–44. doi: 10.1017/neu.2015.47