

Original Article

# Lithium-based antioxidants: electrochemical properties and influence on immune cells

Evgenii Plotnikov<sup>1\*</sup>, Elena Korotkova<sup>1</sup>, Olesya Voronova<sup>1</sup>, Elena Dorozhko<sup>1</sup>, Nikolay Bohan<sup>2</sup>, Sergey Plotnikov<sup>1</sup>

1. Tomsk polytechnic university, 634050, Tomsk, Lenin av., 30, Russia
2. Mental Health Research Institute, Tomsk, Aleutskaya st. 4, Russia

## Abstract

**Introduction:** Lithium salts are known as effective psychotropic medicine for treatment bipolar disorder and may be used to treat alcoholism, schizoaffective disorders, and cluster headaches. The antioxidant activity and immunomodulatory effects of prospective lithium compounds have been investigated in this work.

**Materials and Methods:** The antioxidant properties were studied by the voltammetry method. Influence of the lithium compounds on the immune cells of human blood were determined by measuring phagocytic activity of leucocytes and by the rate of blastic transformation of lymphocytes.

**Results:** Relatively better antioxidant and immunotropic properties have been revealed for lithium ascorbate, compare to widely used drug-lithium carbonate. Lithium aspartate revealed antioxidant activity, as well.

**Conclusion:** Studied lithium salts can be considered as prospective psychotropic antioxidant with immunomodulatory effects. The combination of these properties significantly extends the potential medical applications of lithium salts.

## Keywords:

antioxidant activity;  
immunomodulatory effects;  
voltammetry;  
lithium ascorbate;  
lithium aspartate

## Received:

15 Apr 2015

## Accepted:

4 Aug 2015

## \*Correspondence to:

E. Plotnikov  
Tel: 0079528861086  
Fax: 007 (3822) 60-61-16  
Email: plotnikov.e@mail.ru

## Introduction

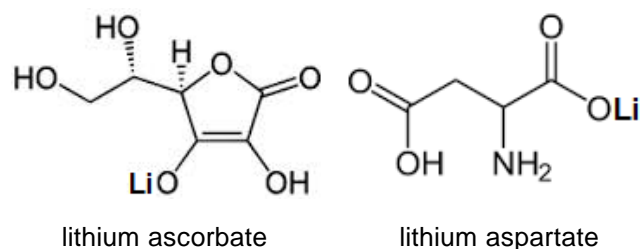
Lithium greatly affects neurochemical processes occurring in the human brain. It is well known; lithium is the primary choice for the treatment of bipolar disorder and used to treat alcoholism, cluster headaches and schizoaffective disorders (Ellenhorn et al., 1997). However, lithium has a relatively narrow therapeutic index and causes poisoning (Maj, 2003).

More severe consequences may occur in combination with additional medications, such as loop diuretics, thiazide, non-steroidal anti-inflammatory drugs (NSAIDs) and angiotensin-converting enzyme inhibitors (ACEIs) (Oruch et al., 2014). Proper doses of these drugs have to be chosen with care to avoid serious drug interactions (Lehmann, 1995). Therapeutic level of lithium in blood equals 0.6 mmol/L for psychoses treatment, but side effect can occur at levels equal 1.2 mmol/L and higher Lithium

levels must be carefully monitored to avoid toxic side effects. It is necessary to note that the brain has a comparatively high vulnerability to oxidative damage, and therefore, reactive oxygen species are an important link in the pathogenesis of mental disorders (Ng, Berk, 2008, Siwek et al., 2013). Work on antioxidant activity of the human blood cells confirm an exhaustion of antioxidant protective systems of a patients affected by the mental diseases (Korotkova et al., 2011). Investigations along these lines have revealed the necessity of antioxidant activity estimation of lithium salts for the oxidative stress correction. Nowadays, there are many methods of antioxidant activity determination. In this work, voltammetry was applied as an effective and convenient approach for the antioxidant activity detection (Korotkova et al., 2003). As a rule, mental diseases require long, often lifelong, medicinal /medical therapy. Therefore, it is very important to design new psychotropic remedies with low side effects, based on their antioxidant properties. The goal of present study was to investigate perspective lithium salts for revealing their respective antioxidant and immunomodulatory properties, as basis for designing new psychotropic drugs with expanded positive effect and low side effects.

## Materials and methods

The following lithium compounds have been used in present study - lithium ascorbate, lithium aspartate (Figure 1), lithium carbonate (Sygma-Aldrich, Germany) as reference drug and ascorbic acid (Sygma-Aldrich, Germany) as a standard antioxidant for antioxidant activity testing. Lithium ascorbate and lithium aspartate were prepared in laboratory in the ion exchange reaction with the calculated amount of lithium carbonate and ascorbic or aspartic acids. To separate reaction products in the crystalline state we used a well-known purification method - salting out by proteolytic solvents (ethanol) from aqueous solutions. White crystalline powder were obtained after double recrystallization, used in further experiments. The concentration of lithium ions for all of the tested substances was confirmed by atomic emission spectrometry.



**Fig. 1.** Molecular structure of investigated lithium salts

Stock solutions of lithium salts were prepared in the distilled water. These solutions were diluted to the working concentration after mixing with phosphate buffer  $0.025 \text{ mol} \cdot \text{l}^{-1}$  (equimolar mixture of  $\text{Na}_2\text{HPO}_4$  and  $\text{KH}_2\text{PO}_4$ , pH 6.86), which was used as a supporting electrolyte for voltammetry analysis. The automated voltammetric analyzer "TA-2" ("Tomanalyt", Tomsk, Russia) with three-electrode open electrochemical cell was used for antioxidant activity detection.

## Detection of antioxidant activity by voltammetry

The electrochemical oxygen reduction ( $\text{ER O}_2$ ) on working mercury film electrode progresses at several stages with the formation of the reactive oxygen species (ROS), such as  $\text{O}_2^{\cdot-}$ . This process is similar to the oxygen reduction in the tissues of a living organism. The measurement includes the recording the current of oxygen at the cathode electrode with and without the investigated compounds under the following conditions: potential rate scan  $0.03 \text{ V s}^{-1}$ , potential range from 0.0V to -0.8V, amplitude 10 mV. All tested substances were added in the electrochemical cell in volume 0.1 ml. The following concentrations were chosen for more objective comparison in this test: equal-mass concentrations for all samples 0.5 mg/ml; equimolar concentrations  $1.36 \text{ } \mu\text{mol/ml}$ , this concentration for lithium ions may be considered as toxic level for medical application; the concentrations corresponding to therapeutic concentrations of lithium in the blood of patients ( $0.6 \text{ } \mu\text{mol/ml}$  of  $\text{Li}^+$  ions recalculated to substance weight). The last dosage is more important for comparison from medical point of view (Mashkovsky, 2002).

Antioxidants react with ROS and decrease of ROS concentration at the electrode. The current of  $\text{ER O}_2$

also decreases. Detailed scheme of these interactions has been previously described (Tur'yan et al., 2004). Coefficient of antioxidant activity of the substances,  $K$ , in  $\mu\text{mol l}^{-1}\text{min}^{-1}$ , reflects the amount of active oxygen radicals being scavenged by the antioxidants within certain time, according to the formula 1.

$$K = \left(1 - \frac{I}{I_0}\right) \times \left(\frac{C_0}{t}\right) \quad (1)$$

Where  $C_0$  ( $\mu\text{mol l}^{-1}$ ) is the oxygen concentration in solution,  $I$  is the ER  $\text{O}_2$  current with the investigated substance in the solution,  $I_0$  is the limiting ER  $\text{O}_2$  current without the substance in the solution,  $t$  (min) is time of the interaction between the reactive oxygen species and an antioxidant at the working electrode.

## Influence of lithium compounds in the reaction of blastic transformation of lymphocytes

Measurement of proliferative responses of human lymphocytes allows estimating biological responses of immune cells before and after the addition of a stimulating agent. The method is based on phytohemagglutinin (PHA) - induced proliferative response of the lymphocytes. The blood samples obtained from healthy donors. Lymphocytes were isolated by gradient centrifugation and resuspended with RPMI 1640, containing 20% fetal bovine serum, L-glutamine, streptomycin. Aliquots of 0.1 ml ( $2 \times 10^6$  cells/ml) of the mixture were placed in a separate microculture plates. Substances were added to the plate in equal-molar concentrations, where all samples were in concentration 1.36  $\mu\text{mol/ml}$  and corresponding to the therapeutically acceptable levels.  $\mu\text{mol/ml}$  of  $\text{Li}^+$  ions recalculated to substance weight), with or without PHA. Control group contained no substances. Cultured plates were sealed and incubated for 72 hours at  $37^\circ\text{C}$ . Lymphocyte transformation was detected by method (Goldberg et al., 1992).

## Influence of lithium compounds on phagocytic activity of leucocytes

The phagocytic activity of the neutrophils was studied

using phagocytosis method (Novikov, Novikova, 1996). The Gram-positive bacteria, *Staphylococcus aureus* - H209, was used as the substrate. *Staphylococcus aureus* was added to the leukocyte suspension obtained from the human blood. The investigated substances were added to the microculture plates in different concentration (0.1-0.001 mg/ml). The phagocytic index was calculated as the number of neutrophils positive for *Staphylococcus aureus* (*S. aureus*) ingestion per 100 neutrophils. The avidity index was calculated as the total number of *S. aureus* cells engulfed per 100 positive neutrophils and divided by 100. The index of phagocytosis completeness was calculated as the number of *S. aureus* killed in phagocytes divided by the total number of the microbes engulfed by phagocytes per 100.

## Statistical Analysis

Statistical analysis was performed using Statistica 6.0 software. The mean changes in coefficients of antioxidant activity and cell influences of tested substances were calculated after running the experiment three time. Data are presented as mean  $\pm$  SD.

## Results

Mean coefficients of antioxidant activity for all substances are evaluated and shown in table 1. Coefficient  $K$  reflects the rate of interaction between oxygen radicals and antioxidant, otherwise known as velocity of radicals scavenging.

According to Table 1, the highest comparative antioxidant properties was expressed by lithium ascorbate for all concentrations. Lithium aspartate showed much lower activity, but for all substances, lowest antioxidant activity was detected at therapeutic concentration of lithium ions. Results of the influence of lithium compounds on immune blood cells "in vitro" (reaction of blastic transformation of lymphocytes) are presented in the table 2.

It is notable that there was a rising of spontaneous lymphocytes proliferation under influence of lithium aspartate, mean while ascorbate had no significant action and carbonate revealed suppressive activity.

**Table 1:** Antioxidant activity coefficients of the investigated lithium salts (n=10, mean±SD)

Substance name	Antioxidant activity coefficient, K ( $\mu\text{mol l}^{-1} \text{min}^{-1}$ )		
	C (equal-mass), 0.5 mg/ml	C (equal-molar), 1.36 $\mu\text{mol/ml}$	C (therapeutic) Li <sup>+</sup> 0.6 $\mu\text{mol/ml}$
Lithium ascorbate	1.55±0.23	0.83±0.11	0.34±0.08
Lithium aspartate	0.46±0.09	0.31±0.07	0.21±0.06
Lithium carbonate	0.11±0.09	0.09±0.08	0.09±0.06

**Table 2:** Lithium compounds influence on lymphocytes proliferation (asterisks indicate a significant difference from control, P <0.05)

Substance	lymphocytes proliferation without PHA, percentage			PHA - induced proliferation of lymphocytes, percentage		
	Control	C (equal-molar) 1.36 $\mu\text{mol/ml}$	C (therapeutic) Li <sup>+</sup> 0.6 $\mu\text{mol/ml}$	Control	C (equal-molar) 1.36 $\mu\text{mol/ml}$	C (therapeutic) Li <sup>+</sup> 0.6 $\mu\text{mol/ml}$
Lithium ascorbate	6±0.5%	7±1.0%	5±1.5%	87±2.5%	*91±4.0%	89±4.5%
Lithium aspartate	6±0.5%	*9±2.0%	8±3.5%	87±2.5%	*95±4.5%	91±6.0%
Lithium carbonate	6±0.5%	*4±1.5%	6±1.0%	87±2.5%	85±7.0%	90±4.5%

Lithium aspartate also showed slight rising of PHA - induced lymphocytes proliferation, compared with that in the control, other salts had no significant influence on this parameter, as shown in Table 2. Ascorbate and aspartate lithium revealed no suppressive effect on stimulated and intact lymphocytes in all concentrations. The impact of the lithium compounds on Immune cells, as indicated by the phagocytic activity of neutrophilic leucocytes "in vitro" are presented in the tables 3, 4 and 5.

Data from Table 3 and Figure 2 shows activation of phagocytosis under the influence of lithium ascorbate, detected in a rising percentage of active phagocytes and percentage of phagocytosis completeness, but avidity index has insignificantly decreased to 9±4.

Similar influence was detected for lithium aspartate (Table 4) and carbonate (Table 5). Lithium aspartate enhanced the percentage of phagocytosis completeness even at therapeutic concentration of lithium salts. Figure 2 shows comparison of changes in phagocytosis activity under influence of different lithium salts at different concentrations.

Active phagocytosis percentage and avidity index changed slightly under action of aspartate, but lithium carbonate slightly decreased these parameters and increased phagocytosis completeness.

## Discussion

Antioxidants depending on the concentration can change their activity rate /intensity against free radicals. For comparison, lithium salts used three different doses, including therapeutic concentration of lithium ions. Study of antioxidant properties in equal-mass concentration revealed that lithium ascorbate had the highest effect, even compared with widely used antioxidant ascorbic acid, which revealed coefficient K of 1.15  $\mu\text{mol l}^{-1} \text{min}^{-1}$  in same concentration of 0.5 mg/ml. similar trend was revealed for equimolar concentration. It is well known ascorbic acid and its sodium salt detoxify the free radicals and turn them into harmless reduced substances in vitro and in vivo (Yourga et al., 1944), similar activity revealed for its lithium salts. Antioxidant and neuroprotective properties

**Table 3:** Influence of lithium ascorbate on neutrophils phagocytic activity (asterisks indicate a significant difference from control,  $P < 0.05$ )

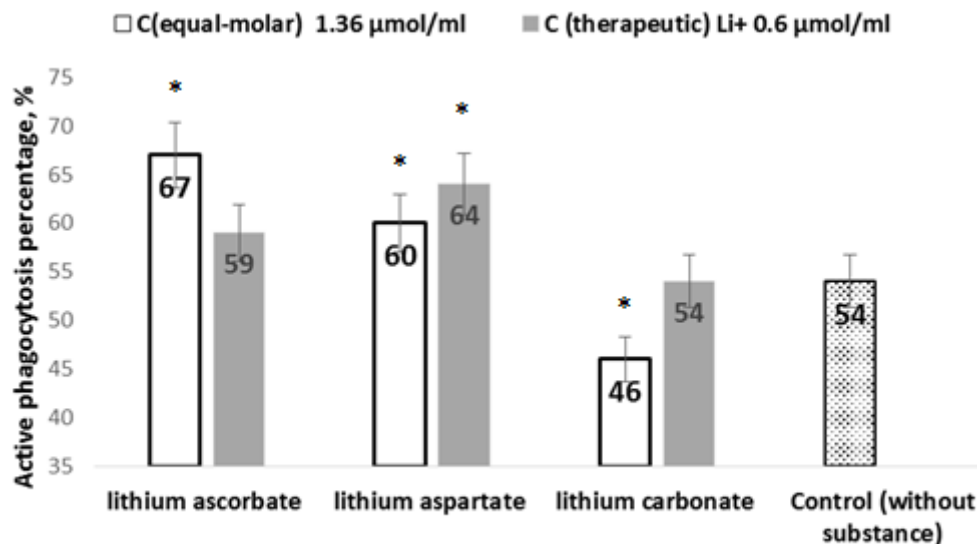
Indicator	Control (without substance)	C (equal-molar) 1.36 $\mu\text{mol/ml}$	C (therapeutic) $\text{Li}^+$ 0.6 $\mu\text{mol/ml}$
Avidity index (bacteria/phagocyte)	11 $\pm$ 3	11 $\pm$ 3	9 $\pm$ 4
Percentage of phagocytosis completeness	63 $\pm$ 7%	*71 $\pm$ 7%	*69 $\pm$ 8%

**Table 4:** Influence of lithium aspartate on neutrophils phagocytic activity (asterisks indicate a significant difference from control,  $P < 0.05$ )

Indicator	Control (without substance)	C(equal-molar) 1.36 $\mu\text{mol/ml}$	C (therapeutic) $\text{Li}^+$ 0.6 $\mu\text{mol/ml}$
Avidity index (bacteria/phagocyte)	11 $\pm$ 3	10 $\pm$ 3	11 $\pm$ 3
Percentage of phagocytosis completeness	63 $\pm$ 7%	*87 $\pm$ 9%	*82 $\pm$ 7%

**Table 5:** Influence of lithium carbonate on neutrophils phagocytic activity (asterisks indicate a significant difference from control,  $P < 0.05$ )

Indicator	Control (without substance)	C(equal-molar) 1.36 $\mu\text{mol/ml}$	C (therapeutic) $\text{Li}^+$ 0.6 $\mu\text{mol/ml}$
Avidity index (bacteria/phagocyte)	11 $\pm$ 3	*8 $\pm$ 3	*9 $\pm$ 3
Percentage of phagocytosis completeness	63 $\pm$ 7%	65 $\pm$ 7%	*75 $\pm$ 7%

**Fig. 2.** Influence of lithium salts on phagocytosis activity (asterisks indicate a significant difference from control,  $P < 0.05$ )

of lithium salts are also confirmed by recent works (Thuy et al., 2015). Lithium ions are possibly enhancing antioxidant activity of salts in vitro and provide psychotropic properties. Thus, lithium-based antioxidant could be considered as prospective mood

stabilizer (normothymics) for bipolar psychosis and alcoholism treatment. It should be noted, that lithium could provide indirect antioxidant activity, as it was shown in work (Vasconcellos et al., 2006) and concluded that lithium presented antioxidant properties,

but is not able to prevent oxidative damage induced by chronic stress. Lithium can recover/revive behavioral and neurochemical impairments induced by stress (Parveen et al., 2013). From this point of view, it was extremely important to compare properties of lithium compounds in equal therapeutic concentration of lithium ions in blood of patients with bipolar psychosis. Lithium ascorbate shows antioxidant activity, higher than other lithium compounds in all concentration ranges (Table 1). Excellent antioxidant activity of lithium ascorbate was revealed even at level of therapeutic lithium concentration 0.6  $\mu\text{mol/ml}$  (0.11 mg/ml of substance weight), that provides an important advantage for medical application, taken into account depletion of antioxidant systems/reservoirs of the body (Korotkova et al., 2013). According to this result, lithium aspartate shows rather moderate antioxidant properties. The “gold standard” mood stabilizer - lithium carbonate revealed low antioxidant properties in all concentration ranges by voltammetry method.

For assessment immunomodulatory properties *in vitro*, lithium ascorbate (best relative antioxidant, as established before), lithium aspartate and lithium carbonate (reference drug) were tested in therapeutic concentration range of lithium ions (for bipolar psychosis treatment 0.005-0.008 mg/ml), recalculated to substance weight. Lithium ascorbate did not affect spontaneous lymphocytes proliferation in all investigated doses. PHA-stimulated blastogenic transformation of lymphocytes revealed no significant differences between the reaction with and without lithium salts, with the exclusion of aspartate which showed a slight stimulatory effect. This can be interpreted as the absence of suppressive or toxic influence on immune cells. Lithium ascorbate does not appreciably influence on the phagocytic activity (table 3). As can be seen in table 4, similar data were obtained for lithium aspartate in all dosage, but index of phagocytosis completeness in all investigated concentrations significantly increases. This reflects improving functional activity of the neutrophils and correlates to increasing of proliferative ability *in vitro* and natural killer cell toxicity up to 2.5 times, as it was shown for lithium chloride (Kubera et al., 1994), but immunostimulatory effect lasted only for prolonged administration of lithium chloride. Thus,

immunomodulatory effect of lithium depends on the type of cell and administration and is manifested mainly indirectly *in vivo*.

Findings indicate slightly stimulating influence of lithium salts on the phagocytic-related immunity and the absence of any toxic effects on immune blood cells. A noticeable stimulation effect on phagocytosis was revealed for lithium aspartate in therapeutic dosage. Lithium ascorbate has low toxicity on mice of a BALB/c line with intraperitoneal introduction. As it shown in earlier studies (Balashov et al., 2008), lithium ascorbate revealed absence of toxic action on the experimental animals ( $\text{LD}_{50}$ =4840 mg/kg), while for carbonate lithium  $\text{LD}_{50}$  is 531 mg/kg (Badmaev, Gudkovsky, 1988.). that was not the case.

Besides, lithium ascorbate (in comparison with lithium carbonate) had no damaging influence on brain cells of the experimental animals. Because of the known possible risk of kidney failure by long-term lithium treatment and other side effects (Timmer, Sands, 1999), we encourage further research on this topic, with the focus on lithium compounds that possess excellent antioxidant properties, as a possible way to minimize their undesirable effects.

## Conclusions

Lithium ascorbate shows the best antioxidant activity, higher than other investigated lithium compounds. Lithium ascorbate and lithium aspartate reveal lack of immune cells suppressive effects and the presence of phagocytic stimulating activity in the human blood “*in vitro*”. Therefore, lithium ascorbate could be considered as a new perspective normothymic agent with possible stimulating activity on, especially complex therapy of addictive disorders and alcoholism associated with depletion of antioxidant and defective immune system (Korotkova et al., 2013) and even for healthy people (Khairova et al., 2011), due to excellent antioxidant properties and low toxicity.

## Acknowledgments

The reported study was supported by Russian Fund Basic Research, project No. 15-04-01110, by the Ministry of Education and Science of Russian Federation (project “Science”).

## Author Contributions

EP—experiment conception, experimental part, EK, NB—RBTL experiment, SP – phagocytosis experiment, OV, ED— experimental part for antioxidant activity.

## Conflict of interest

The authors declare no conflict of interest.

## References

- Badmaev AL, Gudzovsky GA. Harmful chemical substances. Reference book. Leningrad: Chemistry, 1988.
- Balashov PP, Anikina EYu, Plotnikov EV, Potapov AV, Chuchalin VS. Comparative research of neurotoxic action of lithium salts. *Siberian J. psych. and narcology*. 2008; 4:83-4.
- Ellenhorn MJ, Schonwald S, Ordog G, Wasserberger J. Lithium. In: *Medical Toxicology: Diagnosis and Treatment of Human Poisoning*. Baltimore: Williams and Wilkins, 1997.
- Goldberg ED, Dygai AM, Shahov VP. Tissue culture methods in hematology. Tomsk: Publishing House of the Tomsk State University, 1992.
- Khairova R, Pawar R, Salvadore G, Juruena M, Sousa J, Soera-deSouza M. Effects of lithium on oxidative stress parameters in healthy subjects. *Molecular Medicine Reports*. 2011; 5(3): 680-2.
- Korotkova EI, Freinbichler W, Linert W, Dorozhko EV, Bukkel MV, Plotnikov EV, Voronova OA. Study of total antioxidant activity of human serum blood in the pathology of alcoholism. *Molecules*. 2013; 18(2):1811-8.
- Korotkova EI, Karbainov YA, Avramchik OA. Investigation of antioxidant and catalytic properties of some biologically active substances by voltammetry. *Anal. Bioanal. Chem*. 2003; 37:465-8.
- Korotkova EI, Misini B, Dorozhko EV, Bukkel MV, Plotnikov EV, Linert W. Study of OH radicals in human serum blood of healthy individuals and those with pathological schizophrenia. *Int. J. Mol. Sci*. 2011; 12:401-9.
- Kubera M, Bubak-Satora M, Holanb V, Krolc W, Basta-Kaim A, Roman A, Skowron-Cendrzak K, Shani J. Modulation of Cell-Mediated Immunity by Lithium Chloride. *Zeitschrift für Naturforschung*. 1994; 49:679-83.
- Lehmann K, Ritz E. Angiotensin-converting enzyme inhibitors may cause renal dysfunction in patients on long-term lithium treatment. *Am J Kidney Dis*. 1995; 25:82-87.
- Maj M. The effect of lithium in bipolar disorder: a review of recent research evidence. *Bipolar Disord*. 2003; 5:180-8.
- Mashkovsky MD. (2002) *The Medicinal Preparations*, 14th ed.; Moscow.
- Ng F, Berk M, Dean O, Bush AI. Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. *Int J Neuropsychopharmacol*. 2008; 11:851-76.
- Novikov DK, Novikova VA. *Immune Status Assessment*. Moscow: Medicine, 1996.
- Oruch R, Elderbi MA, Khatlab HA, Pryme IF, Lund A. Lithium: a review of pharmacology, clinical uses, and toxicity. *Eur J Pharmacol*. 2014; 5:464-73.
- Parveen T, Haider S, Mumtaz W, Razi F, Tabassum S, Haleem DJ. Attenuation of stress-induced behavioral deficits by lithium administration via serotonin metabolism. *Pharmacological Reports*. 2013; 65:336–42.
- Siwek M, Sowa-Kućma M, Dudek D, Styczeń K, Szewczyk B, Kotarska K, Misztak P, Pilc A, Wolak M, Nowak G. Oxidative stress markers in affective disorders. *Pharmacological Reports*. 2013; 65:1558–71.
- Thuy M, Perry P, Ellerby M, Bohnert K. Is lithium a neuroprotective agent? *Annals of Clinical Psychiatry*. 2015; 27(1):49-54.
- Timmer RT, Sands JM. Lithium Intoxication. *JASN*. 1999; 10:666-74.
- Tur'yan YI, Gorenbein P, Kohen R. Theory of the oxygen voltammetric electroreduction process in the presence of an antioxidant for estimation of antioxidant activity. *J. Electroanal. Chem*. 2004; 2:183-8.
- Vasconcellos AP, Nieto FB, Crema LM, Diehl LA, de Almeida LM, Prediger ME, da Rocha ER, Dalmaz C. Chronic lithium treatment has antioxidant properties but does not prevent oxidative damage induced by chronic variate stress. *Neurochem Res*. 2006; 31:1141-51.
- Yourga FJ, Esselen WB, Fellers CR. Some antioxidant properties of d-iso ascorbic acid and its sodium salt. *Journal of Food Science*. 1944; 9(3):188–96.