

MURINE STUDIES REGARDING THE VARIATION OF OXIDATIVE STATUS IN SERUM, HEPATIC AND BRAIN SAMPLES, AFTER ADMINISTRATION OF SOME CNS ACTIVE DRUGS

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Abstract

The imbalance between the production and accumulation of reactive oxygen species and the body's ability to manage them by exogenous and endogenous antioxidant defense systems leads to oxidative stress.

An important source to generate reactive oxygen species (ROS), as superoxide anions ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) is the mitochondrial and microsomal system.

The present study assessed the variation of oxidative status in mice, after the intraperitoneal administration of some CNS active drugs. Serum, hepatic and brain samples were used throughout the study. A chemiluminescent method was applied in order to evaluate the oxidative status.

Despite the fact that the brain is considered highly sensitive to oxidative damage, explained by the large amount of readily oxidizable fatty acids and poor antioxidant defenses, in our study, for cerebral and hepatic tissues we proved that the administration of tested drugs led to a decrease of the oxidative status, while in the case of serum it was developed an increase of ROS concentrations.

The oxidative stress attack limitation using therapeutic agents with additional antioxidant effect, could be a therapeutic option leading to reduced doses and adverse effects of some CNS active drugs.

Rezumat

Stresul oxidativ apare ca urmare a unui dezechilibru între producerea și acumularea speciilor reactive și capacitatea organismului de a le neutraliza prin folosirea sistemelor antioxidante exogene și endogene.

O sursă celulară importantă de specii oxigen reactive, cum sunt radicalul anion superoxid ($O_2^{\bullet-}$) și peroxidul de hidrogen (H_2O_2), o reprezintă lanțul respirator mitocondrial și microzomal.

Acest studiu a avut ca obiectiv evaluarea statusului oxidativ, la șoareci albi de laborator, după administrarea intraperitoneală a unor medicamente active la nivelul sistemului nervos central. Au fost recoltate probe de ser, țesut hepatic și țesut cerebral în vederea determinării concentrațiilor de specii oxigen reactive. S-a folosit o metodă chemiluminometrică de determinare a stresului oxidativ de la nivel seric, hepatic și cerebral. S-a observat că medicamente cercetate au determinat la nivel tisular o scădere a statusului oxidativ în comparație cu nivelul seric, unde s-a observat creșterea statusului oxidativ. Scăderea statusului oxidativ folosind agenți terapeutici cu efect antioxidant auxiliar, ar putea fi o opțiune terapeutică care ar conduce la reducerea frecvenței administrărilor și la efecte adverse diminuate ale unor medicamente active la nivel SNC.

Keywords: CNS active drugs, chemiluminescent method, reactive oxygen species (ROS)

Introduction

In the social context of modern life, it is necessary to define stress, as an overloading of the human body which includes both state of aggression and adaptation through defence reactions to the various factors from the internal and / or the external environment.

Stress can be differentiated into several types depending on the level at which it is carried out, chemical stress (caused by pollutants from air, water, food, radiation, polypharmacy), sensory stress (auditive and visual), decisional stress, stress related to the monotony of everyday life, mental stress, etc.

In medical terms, stress is the disruption of homeostasis through physical or psychological stimuli. Stressful stimuli can cause reactions such as mental, physiological, anatomical or physical. At the molecular level, oxidative stress is caused by an imbalance existent between the production of reactive oxygen and nitrogen species, and the ability of biological systems to detoxify these reactive intermediates or to repair oxidative damage results. The balance between pro-oxidant action of free radicals and antioxidant protective systems of an organism is essential to life and characterizes the resilience of an organism. [5-8]

This imbalance can affect all organs and tissues: brain, heart, blood vessels, intestines, lungs, muscles, parenchymal organs (liver, kidney, pancreas), eye, skin, joints, etc. The significant increase in the production of reactive oxygen species (ROS) in various biological systems has demonstrated the ability to alter oxidative biomolecules such as lipids, proteins and DNA [9-11].

Reactive oxygen and nitrogen species formed in the course of normal metabolism and under conditions of oxidative stress are involved in the etiology and development of a number of neurodegenerative diseases, as well as in normal aging. A significant number of studies suggest that in

particular, the brain is vulnerable to the effects of free radicals because of high levels of oxygen consumption, easily oxidizable substrates such as catecholamine and unsaturated lipids, and relative low levels of antioxidants [19]. Physical and psychological stress is known to be a factor that is affecting synaptic plasticity, dendritic morphology and causes neurotoxic damage in humans, through free radicals.

In intact cells, the balance between the production of free radicals and their neutralization is maintained by complex mechanisms that include physical and spatial distribution of the sites that are generating free radicals, of the peroxide biomolecules and of antioxidant protective systems [3,12]. The biochemical state of the imbalance in the production of reactive oxygen species and nitrogen, is associated with systemic diseases, and diseases affecting the central nervous system. Increased neuronal oxidative stress induces deleterious effects on signal transduction, structural plasticity and cellular resilience, mostly by inducing lipid peroxidation in membranes, proteins and genes [4,7]. It has been hypothesized that these pathological processes occur in critical brain circuits that regulate affective functioning, emotions, motoric behaviour and other features involved in the bipolar disorder. Altered oxidative stress parameters have been described in the pathophysiology of bipolar disorder. The brain is particularly vulnerable to oxidative damage since it contains large amounts of polysaturated fatty acids and possesses low antioxidant capacity.

The study assessed the variation of oxidative status in mice, after the intraperitoneal administration of some CNS active drugs: lithium, valproic acid, fluoxetine, risperidone, and thioridazine.

Lithium is the gold standard mood stabilizing agent that has been found to prevent and/or reverse DNA damage, free radical formation and lipid peroxidation [11]. Lithium plays a neurotrophic and neuroprotective role and enhances total antioxidant activity in pathological models. In bipolar disorder, lithium treatment significantly reduces the levels of plasma lipid peroxides and improves the antioxidant status. Lithium has also been shown to decrease SOD levels in pre-clinical models compared to animals submitted to an animal model of mania in the prefrontal cortex.

Lithium exerts antioxidant-like properties in the brain, combining an excellent antioxidant activity, immune stimulating effect and low toxicity [1,13,14].

Valproic acid, anticonvulsant drug, is now commonly used in the management of bipolar disorder. It was been also used to treat agitation in patients with dementia, depression, and borderline personality disorder. This drug works through one of these mechanisms of action: potentiation of γ -

aminobutyric acid (GABA)-mediated neuronal inhibition, inhibition of glutamate-mediated excitatory postsynaptic receptors, or control of sodium and calcium voltage-gated ion channels. It is particularly important to note that anticonvulsant medications have proven to be a fruitful source of novel psychiatric compounds. **Fluoxetine**, antidepressant drug, SSRI (Selective serotonin reuptake inhibitor) is often used as first-line therapy because it has less effect on cardiac status and have a favorable overdose profile. It is indicated in the treatment of postpartum major depressive disorder, insomniac patients, generally nonsedating, and it is well tolerated. **Risperidone**, a serotonin-dopamine antagonist, is a potent antipsychotic drug which is mainly used to treat schizophrenia (including adolescent schizophrenia), schizoaffective disorder, mixed and manic states associated with bipolar disorder, and irritability in people with autism. **Thioridazine** is a typical antipsychotic drug belonging to the phenothiazine derivatives with indications in schizophrenia and psychosis [2,6,17,18].

Materials and Methods

The study was performed on Albino Swiss mice, having the approximate weight of 25-30 grams. The animals were brought from the “**Institute for Diagnosis and Animal Health (IDSA)**” Biobase, and located in the Biochemistry Laboratory of the Faculty of Pharmacy Bucharest. They were divided into eleven groups of 10 mice each, as follows:

- group 1 (CONTROL): animals that received saline solution (NaCl 0.9%) (0.1mL/10g bw, i.p.);
- group 2 (noted V): animals that received 10 mg/kgbw i.p. valproic acid;
- group 3 (R): animals that received 0.5 mg/kgbw i.p. risperidone
- group 4 (T): animals that received 10 mg/kgbw i.p. thioridazine
- group 5 (F): animals that received 10 mg/kgbw i.p. fluoxetine
- group 6 (Li): animals that received 70 mg/kgbw i.p. lithium carbonate
- group 7 (V+R): animals that received (simultaneously) 10mg/kgbw i.p. valproic acid and risperidone (0.5mg/kgbw).
- group 8 (V+T): animals that received (simultaneously) 10mg/kgbw i.p. valproic acid and thioridazine (10 mg/kgbw).
- group 9 (V+F): animals that received (simultaneously) 10 mg/kgbw i.p. valproic acid and fluoxetine (10 mg/kgbw).
- group 10 (V+Li): animals that received (simultaneously) 10mg/kgbw i.p. valproic acid and lithium carbonate (70 mg/kgbw).

- group 11 (R+T): animals that received (simultaneously) 0.5mg/kgbw i.p. risperidone and thioridazine (10 mg/kgbw).

The mice were housed in a room and maintained at 25 ± 2 °C and 45-55% relative humidity, with an alternating 12h light-dark cycle. They had free access to food and water until the morning of the experiment. All animals used in this study were maintained in facilities fully accredited and the experiments described here were performed in compliance with the European Communities Council Directive 1986 (86/609/EEC) and Ordinance No. 37 of the Romanian Government from 2nd February 2002.

Substances

In this study, there were used: valproic acid, risperidone, thioridazine and lithium carbonate, of high purity purchased from SIGMA, USA. Fluoxetine was a kind gift from the Department of Toxicology of the Faculty of Pharmacy, Bucharest. Other routine reagents were of the highest purity commercially available.

Samples

In order to assess the oxidative status, the animals were sacrificed and blood, liver and brain were taken.

The organs, maintained on ice (liver and brain) were washed with 0.9% NaCl solution, minced, and then homogenized in 10% (w / v) phosphate buffer, pH 7.4 using a Potter type homogenizer. The homogenates were deproteinized with 10% trichloroacetic acid solution and centrifuged at 3000 rpm for 10 minutes. Subsequent determinations were carried out on the supernatant. All operations of homogenization and centrifugation were carried out at a temperature of 0-4°C.

The blood was centrifuged at 3000 rpm for 10 minutes, to separate the serum in maximum 3 hours after collection, serum was separated immediately, brought into Eppendorf tubes and stored at -20°C until determinations.

In this study we used a modern chemiluminescent method to evaluate the oxidative stress.

The determination is based on a method which involves oxidation reaction of luminol (5-amino-2,3-dihydro-1,4-ftalazindione) by sodium hypochlorite. Hence is obtained diazoquinone that in presence of the reactive oxygen species (ROS): $^1\text{O}_2$, H_2O_2 , O_2^- , $\cdot\text{OH}$, $\text{ROO}\cdot$, $\text{ONOO}\cdot$, is converted into an aminophthalate molecule (a dicarboxylated dianion). Following the evolution of the aminophthalate from an excited status into a basal status a photon signal is emitted that will be registered as a

chemiluminescent signal. The intensity of the light signal is proportional to the concentration of reactive oxygen species which are participating in the reaction [15,16].

Determinations were carried out as follows: in reading cells of the chemiluminometer there were placed: 10^{-4} M luminol solution, the biological sample and 10^{-4} M NaOCl solution. Results are expressed in relative chemiluminescent units (r.l.u) and directly reflect the concentration of reactive oxygen species in the samples.

The study was conducted within the Biochemistry Department, Faculty of Pharmacy from Bucharest, using a Perkin Elmer LS 50B chemiluminometer equipped with an external thermostat sample system and magnetic stirring in sample medium.

Statistical analysis

The data were analyzed using Student t test. p values < 0.05 were considered statistically significant. Pearson correlation test was used to examine correlations.

We quantified the percentage variation of ROS concentration (expressed as % effect) using the following equation:

$$(\%) \text{ effect} = [(r.l.u \text{ sample} - r.l.u \text{ control}) / r.l.u \text{ control}] \times 100$$

where: r.l.u sample (in relative luminescence units) is the luminescence intensity in the presence of tested compound, r.l.u control is the luminescence intensity of the control group (animals that received saline solution).

Results and Discussion

The chemiluminescent intensities which directly reflect the concentration of reactive oxygen species in serum, liver and brain, for the eleven studied groups, are depicted in figure 1.

The highest chemiluminescent intensities were quantified in serum for V + R and V + T groups ($p < 0.01$) vs. control.

For the hepatic tissues, the registered data were significantly lower ($p < 0.01$) for the groups which received lithium only, and also for those treated with V + F, V + Li.

Regarding the brain tissue, the levels of luminescent signals were significantly lower ($p < 0.001$) for most groups, compared with the control. A particular case is observed for the mice treated in combination with V + Li and R+T, where the differences are not statistically significant (Figure 1).

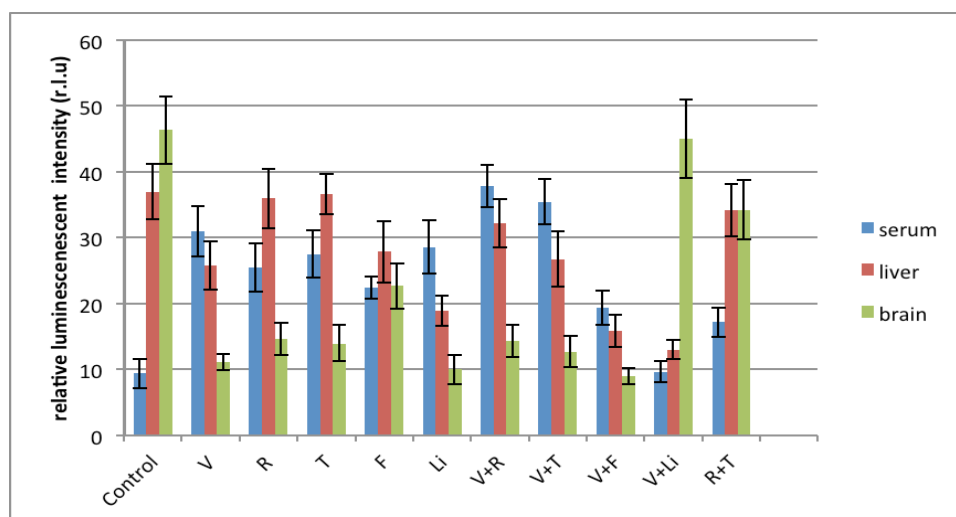


Figure 1

The relative luminescence intensity (r.l.u) for serum, liver and brain, for the eleven studied animal groups

For the evaluation of our results, we calculated the percentage variation (effect %) of the reactive oxygen species concentrations found in serum and tissue, for all groups of studied animals, compared to the control.

The simultaneous administration of drugs led to an increase of serum ROS concentrations for group V+R as follows: an increase of 302.66% vs. control, 21.88% vs. V group and 48.62 % vs. R group (table I and figure 2) In all groups we registered a significant increase in the chemiluminescent intensities ($p < 0.001$), except the case of V+Li group, which exhibited a similar oxidative status to the control group.

Table I

The percentual variation of reactive oxygen species (% effect) on serum level compared to the control group and reference drugs administrated singularly.

Animals groups	% Effect vs. control group (percentage)	% Effect vs. reference drugs administrated singularly (percentage)				
		V	F	T	R	Li
V	230.35	-	-	-	-	-
R	170.92	-	-	-	-	-
T	193.07	-	-	-	-	-
F	139.08	-	-	-	-	-
Li	203.94	-	-	-	-	-
V+R	302.66	21.88	-	-	48.62	-
V+T	277.74	14.34	-	28.89	-	-
V+F	105.85	-37.68	-13.9	-	-	-
V+Li	2.55	-68.95	-	-	-	-71.53
R+T	82.74	-	-	-37.64	-32.54	-

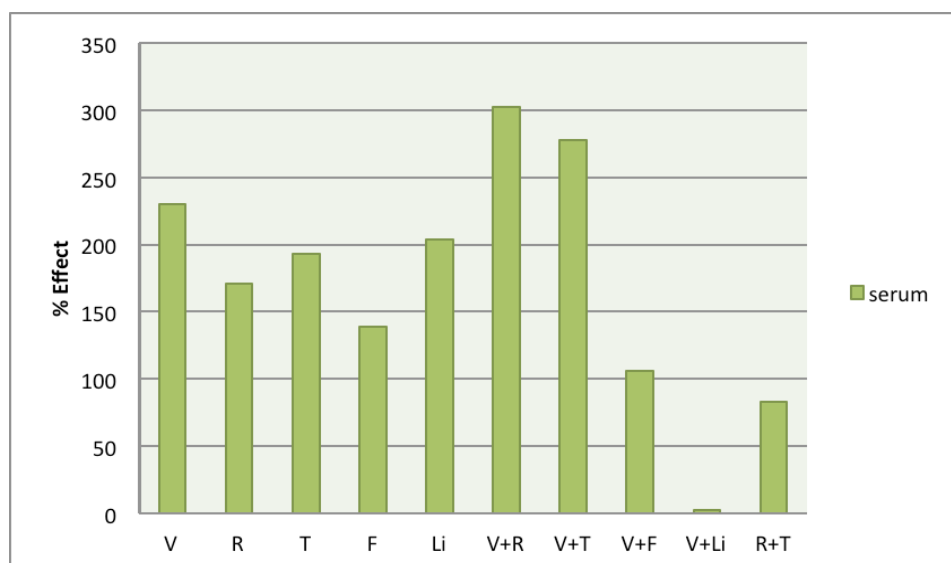


Figure 2

The percentual variation of ROS (% effect) on serum level vs. control group

In the case of hepatic and cerebral tissues the administration of the studied drugs led to a decrease of oxidative status in comparison with the control group. On the contrary, the results obtained for serum exhibited an increase of the ROS levels (Figure 3).

In the case of cerebral tissue we registered an inhibition of ROS development which suggests an antioxidant potential of our studied drugs. This aspect is especially relevant for valproic acid (-76.16% decrease) and lithium (-78.49% decrease) (Table II). The same pattern was observed for the hepatic tissue (Table III).

Nevertheless a particular aspect should be discussed. In association, valproic acid+lithium (V+Li) showed a contradictory behaviour. In co-administration they behave as pro-oxidants with the highest values of brain ROS levels: 307.05 % increase vs. V group, 351.15% vs. Li group (Figure 3).

Our results agree once more with other published data that sustain that lithium is a very actual psychotropic antioxidant, and may increase efficacy of addictive disorders and bipolar psychosis therapy [2,13].

Furthermore our results demonstrated cerebral antioxidant like behavior for valproic acid.

Table II

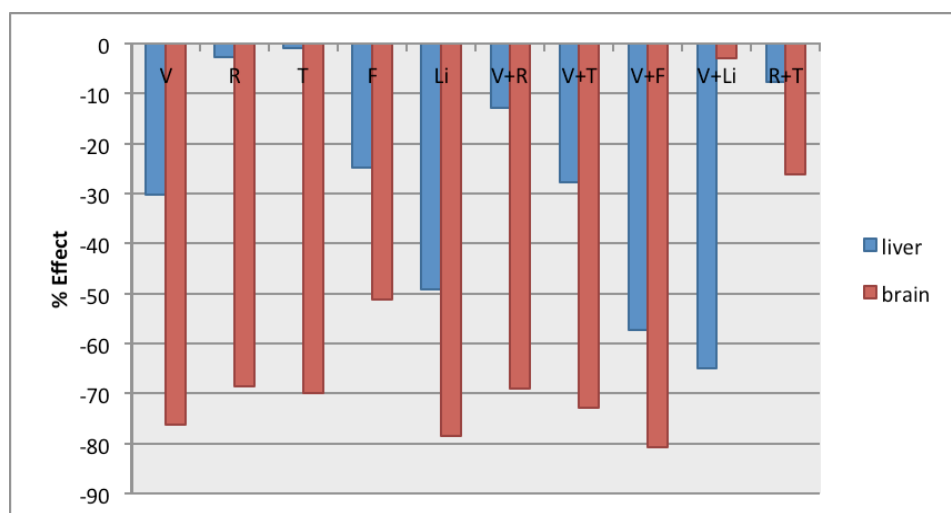
The percentual variation of reactive oxygen species (% effect) for brain level compared to the control group and reference drugs administrated singularly

Animals groups	% Effect vs. control group (percentage)	% Effect vs. reference drugs administrated singularly (percentage)				
		V	F	T	R	Li
V	-76.16	-	-	-	-	-
R	-68.59	-	-	-	-	-
T	-69.99	-	-	-	-	-
F	-51.16	-	-	-	-	-
Li	-78.49	-	-	-	-	-
V+R	-69.11	29.59	-	-	-1.64	-
V+T	-72.75	14.29	-	-9.20	-	-
V+F	-80.69	-19.00	-60.46	-	-	-
V+Li	-2.97	307.05	-	-	-	351.15
R+T	-26.16	-	-	146.08	135.09	-

Table III

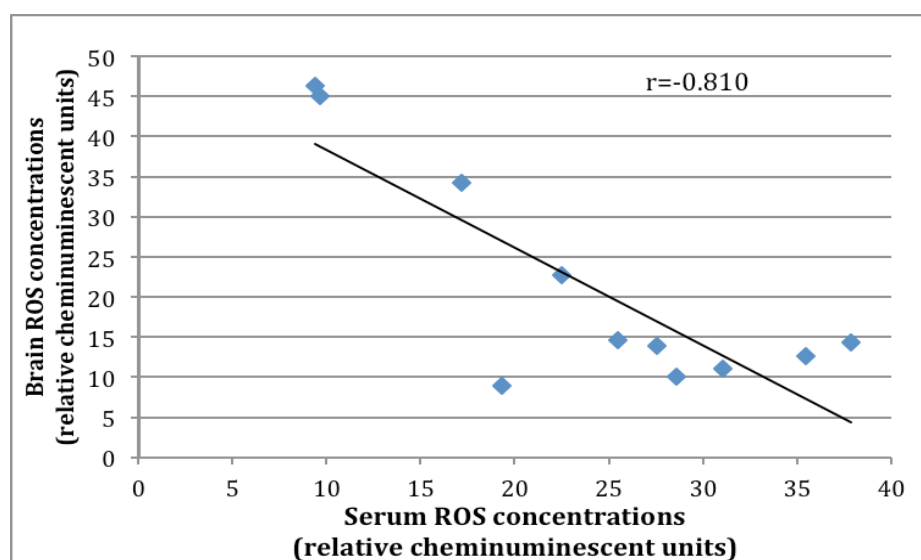
The percentual variation of reactive oxygen species (% effect) for liver level compared to the control group and reference drugs administrated singularly

Animals groups	% Effect vs. control group (percentage)	% Effect vs. reference drugs administrated singularly (percentage)				
		V	F	T	R	Li
V	-30.27	-	-	-	-	-
R	-2.72	-	-	-	-	-
T	-0.97	-	-	-	-	-
F	-24.72	-	-	-	-	-
Li	-49.10	-	-	-	-	-
V+R	-12.89	24.92	-	-	- 10.44	-
V+T	-27.70	3.68	-	-26.99	-	-
V+F	-57.37	-38.87	-43.37	-	-	-
V+Li	-64.91	-49.68	-	-	-	-31.06
R+T	-7.56	-	-	-24.01	- 22.64	-

**Figure 3**

Percentual variation of ROS at the liver and brain level vs. control group

Our results showed that ROS concentrations reach a statistical correlation at the brain level (Pearson coefficient $r=-0.810$) with ROS levels in serum samples (figure 4).

**Figure 4**

Pearson correlation between seric and brain ROS concentrations

Conclusions

In recent years, the role of oxidative stress in central nervous system impairments has been attracting increased attention. In such diseases, an imbalance exists in the antioxidant defense system, in different degrees, mainly due to mitochondrial DNA mutation, decreased mitochondrial respiration, mitochondrial calcium dysregulation, lipid oxidation, protein modification, mitochondrial permeability transition, among other factors. The decrease in oxidative stress using therapeutic agents with additional antioxidant effect, could be a therapeutic option leading to the reduced drug doses and adverse effects of some CNS active drugs.

The benefit of protection against oxidative damage for attenuation of neuronal degeneration is proposed, in consequence, as a therapeutic approach in the treatment of CNS diseases. Further assessment of the physiopathological, biochemical, cellular and molecular mechanisms involved in this drug-resistant pathology is required. Given the few alternatives for patients with neurological pathologies, the use of CNS active drugs with additional antioxidant effect should certainly be considered as a therapeutic alternative.

References

1. Ana Paula Santana de Vasconcellos, Fabiane Battistela Nieto, Leonardo Machado Crema, Luisa Amália Diehl, Lúcia Maria de Almeida, Martha Elisa Prediger, Elizabete Rocha da Rocha, Carla Dalmaz. Chronic Lithium Treatment has Antioxidant Properties but does not Prevent Oxidative Damage Induced by Chronic Variate Stress. *Neurochemical Research*. 2006, 31, 9, 1141-1151.
2. Aycicek A., Iscan A. The Effects of Carbamazepine, Valproic Acid and Phenobarbital on the Oxidative and Antioxidative Balance in Epileptic Children. *Eur Neurol* 2007, 57, 65-69.
3. Bae Y.S., Oh H., Rhee S.G., Yoo Y.D. Regulation of reactive oxygen species generation in cell signaling. *Mol. Cells*. 2011, 32, 491-509.
4. Benedusi V., Martorana F., Brambilla L., Maggi A., Rossi D. The Peroxisome Proliferator-Activated Receptor γ (PPAR γ) controls natural protective mechanisms against lipid peroxidation in Amyotrophic Lateral Sclerosis. *J. Biol. Chem.* 2012. *in press*.
5. Burke K.E., Wei H. Synergistic damage by UVA radiation and pollutants. *Toxicol. Ind. Health* 2009, 25, 219-224.
6. Collet J.F., Messens J. Structure, function and mechanism of thioredoxin proteins. *Antioxid. Redox Signal.* 2010, 13, 1205-1216.
7. Cova E., Bongioanni P., Cereda C., Metelli M.R., Salvaneschi L., Bernuzzi S., Guareschi S., Rossi B., Ceroni M. Time course of oxidant markers and antioxidant defenses in subgroups of amyotrophic lateral sclerosis patients. *Neurochem. Int.* 2010, 56, 687-693.
8. Da Costa L.A., Badawi. A., El-Sohemy. A. Nutrigenetics and modulation of oxidative stress. *Ann. Nutr. Metab.* 2012, 60, 27-36.
9. Daniela Miricescu, Maria Greabu, Alexandra Totan, Maria Mohora, Andreea Didilescu, Niculina Mitrea, Andreea Arsene, Tudor Spinu, Cosmin Totan, Radu Rădulescu. Oxidative stress a possible link between systemic and oral diseases. *Farmacia*, 2011, 59, 3, 329-337.

10. Daniela-Saveta Popa, Bela Kiss, Laurian Vlase, Anca Pop, Rita Iepure, Ramona Păltinean, Felicia Loghin. Study of oxidative stress induction after exposure to bisphenol a and methylparaben in rats. *Farmacia*, 2011, 59, 4, 359-549.
11. Gencer M., Dasdemir S., Cakmakoglu B., Cetinkaya Y., Varlibas F., Tireli H., Kucukali C.I., Ozkok E., Aydin M. DNA repair genes in Parkinson's disease. *Genet. Test Mol. Biomark.* 2012, 16, 504-507.
12. Livia Gratiela Mihai, Niculina Mitrea, Raluca Ioana Papacoea, Anca Ioana Badarau. Dynamic activity of some antioxidant enzymes in primary cultures of neurons under hypoxia and ischemia. *Farmacia*, 2012, 60,1, 49-57.
13. Luciano K. Jornada, Samira S. Valvassori, Amanda V. Steckert, Morgana Moretti, Francielle Mina, Camila L. Ferreira, Camila O. Arent, Felipe Dal-Pizzol, João Quevedo. Lithium and valproate modulate antioxidant enzymes and prevent ouabain-induced oxidative damage in an animal model of mania. *Journal of Psychiatric Research*. 2011, 45, 2, 162-168.
14. M. Kielczykowska, K. Pasternak, I. Musik, J. Wrońska-Tyra, A. Hordyjewska. The Influence of Different Doses of Lithium Administered in Drinking Water on Lipid Peroxidation and the Activity of Antioxidant Enzymes in Rats. *Polish J. of Environ. Stud.* 2006, 15, 5, 747-751.
15. Mueller S, Arnhold J. Fast and sensitive chemiluminescence determination of H₂O₂ concentration in stimulated human neutrophils. *J Biolumin Chemilumin.* 1995, 10, 4, 229-237.
16. Mueller S, Millonig G, Waite GN. The GOX/CAT system: a novel enzymatic method to independently control hydrogen peroxide and hypoxia in cell culture. *Adv Med Sci.* 2009, 54, 2, 121-135.
17. Naseer M.I., Ullah I., Ullah N., Lee H.Y., Cheon E.W., Chung J., Kim M.O. Neuroprotective effect of vitamin C against PTZ induced apoptotic neurodegeneration in adult rat brain. *Pak. J. Pharm. Sci.* 2011, 24, 263-268.
18. Sanyal J., Bandyopadhyay S.K., Banerjee T.K., Mukherjee S.C., Chakraborty D.P., Ray B.C., Rao V.R. Plasma levels of lipid peroxides in patients with Parkinson's disease. *Eur. Rev. Med. Pharmacol. Sci.* 2009, 13, 129-132.
19. Van der Veen B.S., de Winther M.P., Heeringa P. Myeloperoxidase: Molecular mechanisms of action and their relevance to human health and disease. *Antioxid. Redox Signal.* 2009, 11, 2899-2937.

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