The Influence of *Sempervivum Tectorum* and Melatonin Administration on Erythrocyte Catalase in Rats Exposed to Aluminium Sulphate

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Abstract
The aim of the study was to highlight the effect of *Sempervivum tectorum* and melatonin administration in rats exposed to aluminium sulphate through drinking water on the erythrocyte catalase activity The researches were carried out on Wistar albinos rats, grouped in 8 lots: a control lot (C) and 7 experimental lots (E_1: 10% *Sempervivum tectorum* aqueous extract, 3 month; E_2: melatonin, 10 mg/100 ml water, 3 month; E_3: aluminium sulphate, 3 months; E_4: aluminium sulphate with 10% *Sempervivum tectorum* aqueous extract, 3 months; E_5: aluminium sulphate with melatonin 3 months; E_6: aluminium sulphate 3 months followed by 10% *Sempervivum tectorum* aqueous extract for a month; E_7: aluminium sulphate 3 months, followed by melatonin for a month). Al(3+) level in drinking water was 1000 ppb. It was registered decrease of catalase activity compared to C group in E_3, E_4 ($p<0.01$), E_5 group ($p>0.05$) and an insignificant increase in E_1, E_2, E_6, E_7 groups. *Sempervivum tectorum* and melatonin administration led to the increase of catalase activity comparing to the group exposed only to aluminium. The catalase activity increase was significantly higher in case of consecutive administration to aluminium intake. Melatonin effect was more well-marked as the one induced by *Sempervivum tectorum* ($p>0.05$).

Keywords: aluminium, catalase, melatonin, rat, *Sempervivum tectorum.*

1. Introduction

The biochemical and physiological role of aluminium is not fully explained [1,2]. The erythrocytes are extremely sensitive to endogenous or exogenous oxidising agents. Their role is to protect against reactive species of oxygen (ROS), and when their production is exaggerated the defensive systems are exceeded and the oxidative stress is installed. The erythrocytes have a protection role regarding the oxidative stress through the antioxidant systems, among which is also catalase (CAT) [3]. The carried out study had in view the effect of Sempervivum tectorum and melatonin administration on the erythrocyte catalase activity in rats exposed to aluminium sulphate. Sempervivum tectorum contains flavonoids, the main being kaempherol with anti-inflammatory, healing and antioxidant properties, and melatonin is a strong antioxidant able to destroy in a large proportion the free radicals responsible for some degenerative diseases [4,5,6].

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2. Materials and methods

The researches have been carried out on 40 white albinos Wistar adults rats, male, having a weight of 250±10 g grouped in 8 groups of 5 individuals: a control group (C) and 7 experimental groups (E_1, E_2, E_3, E_4, E_5, E_6, E_7).

E_1: administration of 10% *Sempervivum tectorum* infusion, 3 months; E_2: melatonin, administration 10 mg/100 ml water, 3 months; E_3: aluminium sulphate administration for 3 months; E_4 : administration of aluminium sulphate with 10% *Sempervivum tectorum* infusion, 3 months; E_5: administration of aluminium sulphate with melatonin (10 mg/100 ml water) 3 months; E_6: administration of aluminium sulphate 3 months, followed by administration of 10% *Sempervivum tectorum* infusion, a month; E_7: administration of aluminium sulphate 3 months, followed by administration of melatonin (10 mg/100 ml water) a month.

Aluminium was administered as aluminium sulphate ($Al_2(SO_4)_3$) in drinking water, at a level of 1000 ppb aluminium, level found in drinking water from wells situated round the primary aluminium industry and recorded as having the most harmful effect in the experiments carried out on rats. [7,8,9]

The blood samples were collected after euthanasia with ketamin 50 mg/kg b.w.

It was determined the catalase activity expressed as µmol of H_2O_2 consumed/min/ml. at 25^0C, using SINHA method (at T60 UV-VIS Spectrophotometer, λ=570 nm).

The data are presented as average values ±.SEM of the groups, including each 5 rats. The results have been statistically processed by ANOVA method. Differences were considered significant at $p<0.05$.

3. Results and discussion

The obtained results are presented in table 1 and figure 1.

Table 1. The average values of the catalase activity in the experimental and control groups

Group	C	E_1	E_2	E_3	E_4	E_5	E_6	E_7
$\bar{x}$ ± S.E.M.	79.63 ± 3.97	80.46 ± 3.90 ^{NS}	81.16 ± 1.32 ^{NS}	42.48 ±6.18**	69.81 ± 4.09*	72.81 ± 2.33 *	78.28 ± 3.71^{NS}	79.22 ± 3.81^{NS}

Unit: CAT (µmol H_2O_2 consumed/min/ml. at 25^0C)
Significance comparative to C group:
NS = Non significant
* p< 0,05
** p< 0,01

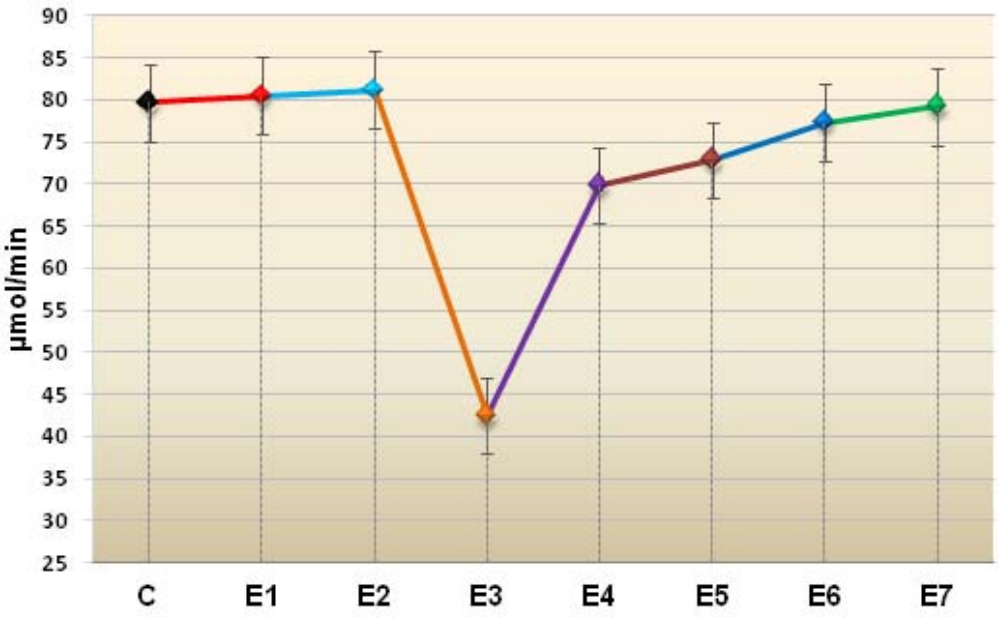

Figure 1. The catalase activity in the experimental and control groups

The *Sempervivum tectorum* extract (E_1) and melatonin (E_2) administration (Table 1) led to an insignificant increase of the erythrocyte catalase activity in comparison with the control group. When aluminium sulphate was administered (E_3) it was recorded a high significant decrease ($p<0.01$) of the erythrocyte catalase activity (E_3/C: -46.65%).

The simultaneous administration of aluminium and *Sempervivum tectorum* (E_4), respectively aluminium and melatonin (E_5), had as consequence the significant decrease ($p<0.05$) of the catalase activity compared to the C group for groups E_4 and E_5 (E_4/C: -2.33%; E_5/C:-8.56%) but an increase in comparison with lot E_3 ($p< 0,01$), as much for group E_4 (E_4/E_3: +64.33%) as for group E_5 (E_5/E_3:+71.39%), proving the beneficial effect as well of the *Sempervivum tectorum* extract as of melatonin.

The administration of *Sempervivum tectorum* extract (E_6) and melatonin (E_7) consecutively to the aluminium intake led to the insignificant decrease ($p>0.05$) of the erythrocyte catalase activity compared to the control group (C) (E_6/C: -1.69%; E_7/C: -0.51%) but highly significant ($p<0.01$) compared to the group exposed only to aluminium E_3 (E_6/E_3: +85.15%; E_7/E_3:+86.48 %).

The erythrocyte catalase activity in case of *Sempervivum tectorum* administration, respectively melatonin, consecutively to aluminium intake (E_6, E_7) was significantly higher ($p<0,01$), than in case of their simultaneous administration with aluminium sulphate (E_4, E_5).

The differences between the beneficial effects of *Sempervivum tectorum* and melatonin were insignificant ($p>0.05$).

The aluminium is involved in the pathogeneses of some sever diseases. The toxic effects of aluminium on the sanguine elements suggest that these would lead to ROS generation and to induction of the oxidative stress [10]. Aluminium stimulates NADPH oxidation and takes part in the process of the free radicals formation [1]. The main extracellular antioxidant defence mechanism is represented by the plasmatic protein (haptaglobin, ceruloplasmin and erythrocyte) [3]. Erythrocytes are efficacious in the inactivation of hydrogen peroxide. The inhibition of catalase from erythrocytes reduces the protection effect [3]. Oral administration of aluminium induces free radicals and causes the decrease of superoxide dismutase (SOD) and CAT [3,10,11]. Studies regarding the effect on sanguine antioxidants in rats, induced by melatonin administration showed the increase of their level suggesting the protector effect of melatonin in terms of reducing the oxidative stress [4,12,13]. It was proved that melatonin stimulates either the activity of the genes expression of antioxidant enzymes or the enzymatic activity (SOD, CAT, GSPx, GSH) [6]. Melatonin is scavenger for the hydroxyl radicals, performing an antioxidant protection role [14].

There have been also carried out studies regarding the counteracting metabolic alterations produced by aluminium through the administration of *Sempervivum tectorum* that contains the flavonoid kaempherol with antioxidant properties [5]. It was remarked the favourable action of the extract on the protection of rats' with hyperlipidemic livers and the detoxification properties with the elimination of Al, Ba, Ni and Ti from the liver [5,15].

Due to the carried out study it was observed the beneficial effect on the erythrocyte catalase induced by melatonin and by *Sempervivum tectorum* infusion, the catalase being an antioxidant enzyme with a protection role against the toxic effects generated by xenobiotics, among which is also aluminium.

4. Conclusions

The researches regarding the influence of *Sempervivum tectorum* aqueous extract and melatonin administration on the erythrocyte catalase in rats exposed to aluminium sulphate in drinking water pointed out:

The highly significant decrease of erythrocyte catalase activity under the circumstances of exposing them to aluminium sulphate.

The significant decrease of erythrocyte catalase activity in comparison with the one of the control group, consecutively to the aluminium association with *Sempervivum tectorum* or melatonin.

Insignificant differences between the beneficial effect of *Sempervivum tectorum* aqueous extract and melatonin, regardless of their administration way, simultaneously or consecutively with the aluminium sulphate administration.

Insignificant increase of erythrocyte catalase activity in comparison with the one of the control

group in case of *Sempervivum tectorum* aqueous extract and melatonin administration consecutively to the aluminium sulphate administration.

The highly significant increase of erythrocyte catalase activity in comparison with the group exposed to aluminium sulphate as well *Sempervivum tectorum* aqueous extract and melatonin are administered simultaneous or consecutively to aluminium sulphate.

The highly significant more increased activity of erythrocyte catalase in the groups where *Sempervivum tectorum* extract and melatonin was administered consecutively to the aluminium sulphate intake, in comparison with those to whom it was administered simultaneously.

References

1. Zaman, K., Zaman, A., Batcabe, J., Hematological effects of aluminum on living organisms, Comparative Biochemistry and Physiology-Part C: Toxicology & Pharmacology, 1993, 106 (2), 285-293

2. Mahieu, S., Contini, M., Gonzales, M., Millen, N., Elias, M., Aluminium toxicity, Hematological effects, Toxicology Letters, 2000, 111, 235-242

3. Dejica, D., Stresul oxidativ in bolile interne, Ed.Casa Cărţii de ştiinţă, Cluj-Napoca, 2000, pp.403-493

4. Rishi, P., Bharrhan, S., Bhalla, M.P.S., Koul, A., Chopra, K., Inhibition of endotoxin-induced hepatotoxicity by melatonin in rats, International Journal of Biomedical Science, 2008, 4(2), 103-112

5. Szentmihályi, K., Fehér, E., Vinkler, P., Kéry, Á., Blázovics, A., Metabolic alterations of toxic and nonessential elements by the treatment of sempervivum tectorum extract in a hyperlipidemic rat model, Toxicologic Pathology, 2004, 32, 50-57

6. Gupta, Y. K**.** Gupta, M., Kohli, K., Neuroprotective role of melatonin in oxidative stress vulnerable brain, Indian journal of physiology and pharmacology, 2003, 47 (4), 373–386

7. Trif, A., Drugă, M., Brudiu, I., Muselin, F., Dynamics of some serum enzymes in rats during chronic aluminium sulphate intake, Veterinary Clinical Pathology, 2005, 34, Suppl., 312-313

8. Drugă, M., Trif, A., Drugă, M., Brudiu, I., Ştef D., The consequences of dietary aluminium oxide on some biochemical parameters in broilers, Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Veterinary Medicine, Ed. Academic Press, Cluj Napoca, 2005, 62, 404-408

9. Trif, A., Druga, M., Stana, L., Druga, M., Muselin, F., Brudiu I., Aluminium oxide dietary intake consequences on some hematological parameters in broilers, Scientifical Papers:Veterinary Medicine, 2005, Vol. XXXVIII, 671-676

10. Al-Hashem, F., Camel's milk protects against aluminium chloride-induced normocytic normocromic anemia, lipid peroxidation and oxidative stress in erytrocytes of White albinos rats, American Journal of Biochemistry and Biotechnology, 2009, 5(3), 126-136

11. Chainy, G.B., Samanta, L., Rout, N.B., Effect of aluminium on superoxid dismutase, catalase and lipid peroxidation of rat liver, Research Communications in Molecular Pathology & Pharmacology, 1996, 94 (2), 217-220

12. Mirunalini, S., Karthishwaran, K., Dhamodharm, G., Mohan, S., Melatonin attenuates lipid peroxidation and enhances circulatory antioxidants durring mammary carcinogenesis in rats, Journal of Biochemical Technology, 2010, 2(3), 171-174

13. Çöl, C., Dinler, K., Hasdemir, O., Büyükaşik O., Buğdayci G., Oxidative stress and lipid peroxidation products: effect of pinealectomy or exogenous melatonin injections on biomarkers of tissue damage during acute pancreatitis, Hepatobiliary & pancreatic diseases international, 2010, 9(1), 78-82

14. Pandi-Perumal, S. R., Srinivasan, V., Maestroni, G. J. M., Cardinali, D. P., Poeggeler, B., Hardeland, R., Melatonin nature's most versatile biological signal? FEBS Journal, 2006, 273, 2813–2838

15. Chmielnicka, J., Nasiadek, M., Lewandowska-Zyndul, E., and Pinkovski, R., Effect of aluminium on hematopoiesis after intraperitoneal exposure in rats, Ecotoxicology and Environmental Safety, 1996, 33(3), 201-206