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The Effect of *Eleutherococcus Senticosus* (Rupr. et Maxim.) Maxim. Extract on Cadmium-Induced Toxicity in Mice

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ABSTRACT

The present study analyzed the effects of *Eleutherococcus senticosus* (Rupr. et Maxim.) Maxim. ethanol extract on long-term (6-week) intoxication induced by intraperitoneal injections of 0.05 LD_{50} of cadmium chloride solution. The experiments were performed with white laboratory mice. Two groups of mice were injected with ethanol extract of *E. senticosus*: 0.05 LD_{50} and 0.10 LD_{50}. Cadmium concentrations were investigated in blood, kidney, liver, spleen, heart, and skeletal muscle by atomic absorption spectroscopy. Long-term injections of *Eleutherococcus senticosus* (Rupr. et Maxim.) Maxim. ethanol extract combined with $CdCl_2$ led to significant increase of cadmium concentrations in the spleen, skeletal muscle, blood, kidney and liver of experimental mice. *E. senticosus* also decreased the mitotic activity of liver cells induced by cadmium.

Key words: cadmium, Eleutherococcus senticosus, mitotic index, atomic absorption spectroscopy

INTRODUCTION

Cadmium has a diversity of toxic effects including teratogenicity, carcinogenicity, nephrotoxicity, reproductive and endocrine toxicity[(2)]. Cadmium is a potent animal carcinogen[(3)] and has been recently upgraded to a human carcinogen by the International Agency for Research on Cancer[(4)]. Cadmium induces oxidative stress in tissues by increasing lipid peroxidation (LPO) and by altering the antioxidant status in tissues. Metabolic transformations of Cd^{2+} are limited to its binding to the protein and nonprotein sulfhydryl groups, and various macromolecules, such as metallothionein, which is especially important in the kidney and liver[(1,9)]. *Eleutherococcus senticosus* (Rupr. et Maxim.) Maxim. (ES) can modify cadmium effect and toxicity[(6)]. The beneficial effects of ES derive from the capability to exert protective and/or inhibitory action against free radicals[(7)].

Mechanisms of action for adaptogenic activity seem to fall into three categories: those that act to regulate the stress response via the neuro-endocrine HPA axis, those that act as antioxidants and those that either inhibit or enhance central nervous system (CNS) activity[(5,8)]. ES supports the body's stress response, immune system and endocrine system including the adrenal glands, spleen, liver and thymus gland[(11)]. Its action in the body is due to its stimulation of the hypothalamic-pituitary axis to secrete adrenal corticotropic hormone (ACTH)[(11,12,15)].

Our study investigated the effects of ES ethanol extract on the accumulation of Cd^{2+} in blood and in internal organs (liver, kidney, spleen , heart, and skeletal muscle) as well as on the mitotic activity of liver cells after the chronic intraperitoneal (*i.p.*) intoxication by Cd^{2+}.

MATERIALS AND METHODS

I. *Preparation of Extract from Root of ES*

The extract from roots of ES was prepared in the factory "Valentis" (Lithuania) for the investigations. The raw

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material of ES[8] was imported from Poland. One kilogram of the dried roots of ES was ground into 3 mm particles, and roots were evenly moistened with 1 L 40% aqueous ethanol (menstruum) and then placed in a closed vessel for 24 h. The moistened roots were introduced into a percolator. The percolator was placed in position, and 1 L 40% aqueous ethanol was poured in to make a 1 : 2 extract. Percolation was underway at the flow speed of extraction of 0.2 mL/min for 24 h. When the percolation was finished, the inert, fibrous part of ES was removed from the percolator and pressed and, the pressed liquid was then mixed with the percolate. The 1 : 1 liquid extract was made by concentration. The resulted percolate was then filtered. One milliliter extract contained 0.151 g of the dried root of ES.

II. *Sample Collection*

Experiments were carried out on 4 to 6-week-old outbred male white laboratory mice BALB/c weighing 20 to 25 g. Intraperitoneal injections (*i.p.*) of $CdCl_2$ and ES extract solutions were performed as follows. Experimental mice (10 mice in each group) were periodically injected *i.p.* for 6 weeks (three times a week) with metal salts and ES extract solutions of two different concentrations in deionized water:

(I) 0.05 LD_{50} Cd^{2+} (corresponding to Cd^{2+} 0.16 mg/kg body mass).

(II) 0.05 LD_{50} ES (corresponding to ES ethanol extract (1 : 1) 3.65 mL/kg and dry extract - 0.55 g/kg body mass).

(III) 0.10 LD_{50} ES (corresponding to ES ethanol extract 7.3 mL/kg and dry extract - 1.07 g/kg body mass).

(IV) 0.05 LD_{50} Cd^{2+} and 0.05 LD_{50} ES.

(V) 0.05 LD_{50} Cd^{2+} and 0.10 LD_{50} ES.

For an ethanol extract of ES the intraperitoneal LD_{50} in mice was 14.5 mL/kg[13].

For the fluid of $CdCl_2$ the intraperitoneal LD_{50} in mice was 3.2 mg Cd/kg body mass. To determine the median lethal dose LD_{50} was calculated using the following formula: $lgLD_{50} = lgD_N - \delta(\sum L_i$ y. where D_N is the highest dose of the study substance administered to mice, δ is the logarithm of the ratio between the doses of the substance administered, and L_i is the ratio between the number of dead mice and the number of mice used to determine the dose effect[10].

Control group mice were periodically injected *i.p.* with the same volume of saline solution. The mice were weighed weekly and decapitated after 6 weeks according to the rules defined by European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (License N. 0028).

III. *Detection of Metals in Organs*

The concentration of Cd^{2+} in whole blood and tissue specimens from liver and kidney were determined on electrothermal graphite furnace atomic absorption spectrophotometer-(Perkin-Elmer Zeeman/3030, Perkin-Elmer Life and Analytical Sciences, Inc., Wellesley, MA, USA). The venous blood was obtained using a single-use syringes with anticoagulant. Tissue specimens were dissolved with 0.125 M NaOH at 90°C, and the digests were diluted till the appropriate volume with twice distilled water. The modified method, as decribed by Schlemmer, was employed for the analysis of heavy metals in biological samples[22].

IV. *Histology*

The samples from liver tissue were fixed in 10%

Table 1. Cd^{2+} concentration in blood and internal organs of mice

In organ / groups	Blood, μg/dL	Liver, μg/g	Kidney, μg/g	Spleen, μg/g	Heart, μg/g	Muscle, μg/g
			Cd conc.			
			CI			
Control group	0.0034	0.0203	0.2063	0.0041	0.0024	0.0008
	0.0008 - 0.0154	0.0128 - 0.0319	0.1462 - 0.2909	0.0029 - 0.0057	0.0008 - 0.0065	0.0002 - 0.0031
ES 0.05	0.0015	0.0357	0.0791*	0.0045	0.0005	0.0008
	0.0001 - 0.0144	0.0145 - 0.0876	0.0571 - 0.1093	0.0027 - 0.0073	0.0001 - 0.0020	0.00006 - 0.0113
ES 0.1	0.0089	0.0158	0.0968*	0.0045	0.0017	0.0052*
	0.0053 - 0.01512	0.0060 - 0.0415	0.0747 - 0.1253	0.0025 - 0.0079	0.0008 - 0.0036	0.0016 - 0.0165
Cd+ES 0.05	4.1117	21.0553	38.5963	3.3781°	0.9380	0.2990
	3.5431 - 4.7715	15.9478 - 27.7986	27.9029 - 53.3877	2.5056 - 4.5543	0.4827 - 1.8227	0.1281 - 0.6981
Cd+ES 0.1	2.8908	18.7878	42.4446	3.1325°	0.6674°	0.4531°
	0.3639 - 3.6147	15.9262 - 22.1636	36.7341 - 49.0427	2.4192 - 4.0559	0.5205 - 0.8555	0.2720 - 0.7548
Cd	1.6788	13.1112	22.3965	1.9027	0.9809	0.2454
	0.2812 - 10.0228	6.5151 - 26.3856	10.8839 - 46.0866	1.6063 - 2.2538	0.7298 - 1.3184	0.1957 - 0.3076

* indicates significant difference at $p < 0.05$ – compared to control group.

° indicates significant difference at $p < 0.05$ – compared to Cd group.

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neutral-buffered formalin for 48 h and then processed for routine paraffin embedding. Five-micron-thick sections were stained with hematoxylin and eosin. Histological slides were examined by light microscopy (objective 40×). For each specimen, the number of mitotic cells was counted in 10 randomly selected reference areas (0.04 mm^2). Their histological images were taken using DP-11 Olympus Digital Camera (Olympus Corp., Tokyo, Japan).

V. *Statistical Processing*

The Student's *t*-test correction was used for comparison of geometric means of cadmium concentration. Nonparametric Kruskal-Wallis and Mann-Whitney tests were applied for the evaluation of variation among mitotic cells in mice liver counts in different groups. Statistical significance was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

I. *Comparison of Cadmium Concentration in Blood, Kidney, Liver, Spleen, Heart, and Skeletal Muscle*

The concentration of Cd^{2+} in blood and internal organs of mice was evaluated after periodical *i.p.* injections with $CdCl_2$ and two different ES concentrations during 6 weeks. Data of Cd^{2+} concentration in blood and organs of control group and exposed to ES mice are presented in Table 1. No significant difference in Cd^{2+} concentration in the organs was observed between the control and ES group. The concentration of Cd^{2+} in the ethanol extract of ES was 0.343 μg /L.

It is of interest that periodical injection of $CdCl_2$ together with ES during 6 weeks caused significant increase of Cd^{2+} concentration in the spleen and skeletal muscle as compared to Cd^{2+} group. The spleen of mice injected with Cd^{2+}+ES 0.05 LD_{50} and Cd^{2+}+ES 0.10 LD_{50} contained higher ($p < 0.001$) Cd^{2+} than mice injected with $CdCl_2$.

The metal concentration in skeletal muscle was significantly higher ($p < 0.02$) only in Cd^{2+}+ES (0.10) group as compared to Cd^{2+} group.

Such increase was also expected in other organs, such as blood, kidney, and liver, but it was not as significant. Only Cd^{2+} concentration in the heart of mice in Cd^{2+}+ES (0.10) group was lower ($p < 0.04$) as compared to Cd^{2+} group.

II. *Expression of the Mitotic Activity of Liver Cells*

The influence of ES on mitotic activity of mice liver cells has also been evaluated (Figure 1). The data showed, that ES extract of concentration 0.05 LD_{50} and 0.10 LD_{50} did not change the number of mitotic liver cells in comparison to the control group. Long term intoxication by cadmium led to significant increase of mitotic activity of liver cells as compared to the control group. The administration of ES together with cadmium decreased number of mitotic cells, which did not differ from the control. The number of mitotic cells was significantly smaller in mice injected with cadmium and ES 0.10 LD_{50} than that in mice injected with $CdCl_2$ only (Figure 2).

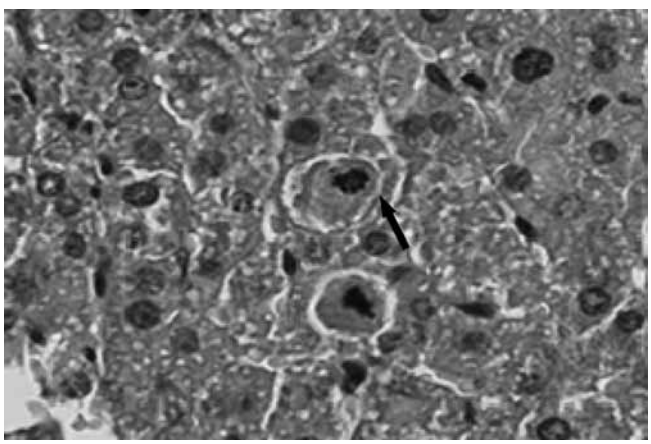

Figure 1. Histology of mouse liver sections. The mice were injected *i.p.* with 0.05 LD_{50} of $CdCl_2$ solution for 6 weeks. The arrow indicates mitotic liver cells. (Haematoxylin and eosin, original magnification × 40).

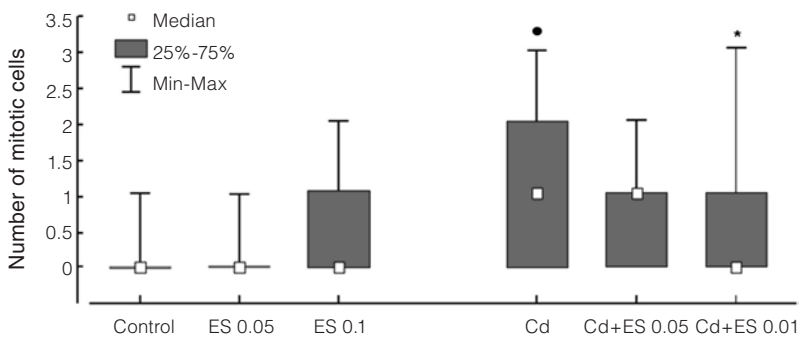

Figure 2. Number of mitotic liver cells of mice after $CdCl_2$ (0.16 mg Cd/kg body mass), after ES (0.55 g ES dry extract/kg body mass); group Cd + 0.05 ES and after ES (1.07 g dry ES extract/kg body mass); group Cd + 0.10 ES injections.
* indicates significant difference at $p < 0.05$ compared to cadmium 6w group (administration of $CdCl_2$ for 6 weeks alone).
• indicates significant difference at $p < 0.05$ compared to control, ES 0.05, ES 0.10 and Cd + ES 0.10.

The results of this study showed that 6-week-long injections of ES ethanol extract of two different concentrations combined with $CdCl_2$ led to significant increase of cadmium concentration in blood, liver, kidney, spleen and skeletal muscle. Once absorbed, the clearance of cadmium from the circulation and deposition into the tissue is rapid. Over 50% of the body burden of cadmium is localized in liver and kidney[24,25].

The accumulation of cadmium in these tissues may be due to their ability to produce large amounts of metallothionein. Typically, the presence of metallothionein within cells will markedly decrease cadmium toxicity[9]. In the present study we also noticed a positive shift in the mitotic index in the mice injected *i.p.* with $CdCl_2$. Cd^{2+} induces apoptosis, mitotic activity of cells and also causes necrotic cell death in certain pathophysiological situations. That may be linked to the replenishment of liver cells, arising out of the toxic $CdCl_2$ effect on chromosome components primarily made up of DNA and protein[25]. It was observed oxidative stress intensification after cadmium administration defined as intensification of liver peroxidation of fats and liver glutathione exhaustion. ES normalizes increased mitotic index of liver cells and protect liver protein synthesizing systems from cadmium toxicity in liver[23].

Chemically, ES is typically either complex phenolics or tetracyclic triterpenoids/steroid. The phenolic compounds include phenylpropanoids and lignans, such as eleutherosid E[14,20]. They are structurally similar to the catecholamines - the mediators of the sympathoadrenal system (SAS) involved in activation of the stress system in the early stages of stress response. The tetracyclic triterpenoids, such as eleutheroside A and, daucosterol, structurally resemble the corticosteroids that act as stress hormones involved in protective inactivation of the stress system[19,21]. The inducement of glutathione and heat shock proteins by ES have been suggested[19,26-28]. ES has protective effects on the activity of superoxide dismutase, catalase, glutathione peroxidase and, glutathione reductase as well the level of glutathione.

Until now we have not been able to describe the precise mechanism of the presented phenomenon, i.e. the administration of cadmium together with ES led to higher concentration of cadmium in the internal organs and blood as compared to the intoxication by cadmium alone. The explanation of this phenomenon requires more detailed investigation in order to reveal mechanisms responsible for this.

CONCLUSIONS

The long-term injections of ethanol extract of ES combined with $CdCl_2$ led to the significant increase of Cd^{2+} concentration in blood, liver, kidney, spleen and skeletal muscle of the experimental mice. ES decreased the mitotic activity of liver cells induced by Cd^{2+}.

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REFERENCES

1. Bremner, I. 1987. Nutritional and physiological significance of metallothionein, in Metallothionein 2nd ed., pp. 81-107. Experientia Suppl., Birkhauser Verlag. Basel, Switzerland.
2. Bernard, A. and Lauwerys, R. 1986. Effects of cadmium exposure in man. In Cadmium toxicology, pp. 135-177. Berlin Heidelberg Springer. New York, U.S.A.
3. Beryllium, cadmium, mercury, and exposures in the glass manufacturing industry 1993. International Agency for Research on Cancer Monographs on the Evaluation of Carcinogenic Risks to Humans, pp. 119-237. IARC Scientific Publications. Lyon, France.
4. Cadmium. 1992. Environmental Health Criteria 134. World Health Organization (WHO). Geneva, Switzerland.
5. Dardimov, I. V. 1976. Ginseng, Eleutherococcus. On the Mechanism of Biological Activity in Nauka. pp. 1-189. Moscow, USSR.
6. Elyakov, G. B. and Ovodov, Y. S. 1972. The glycosides of Araliaceae. Khim. Prir. Soedin. 6: 697-709.
7. Farnsworth, N. R. 1985. Siberian ginseng (Eleutherococcus senticosus) Current status as an adaptogen. In: Wagner, H., Economics and Medicinal Plant Research pp. 155-209. Academic Press. London, U.K.
8. Filaretov, A. A, and Bogdanova, T. S, 1986. Effect of adaptogens on the activity of the pituitary-adrenocortical system in rats. Biull. Eksp. Biol. Med. 101: 573-574.
9. Klaassen, C. D., Liu, J. and Choudhuri, S. 1999. Metallothionein: an intracellular protein to protect against cadmium toxicity. Annu. Rev. Pharmacol. Toxicol. 39: 267-294.
10. Kubiliene , A., Marksiene, R., Kazlauskas, S., Sadauskiene, I., Razukas, A. and Ivanov, L. 2008. Acute toxicity of ibogaine and noribogaine. Medicina. 44: 984-988.
11. Kunimoto, S., Murofushi, W., Kai, H., Ishida, Y., Uchiyama, A., Kobayashi, T., Kobayashi, S., Murofushi, H. and Murakami-Murofushi, K. 2002. Steryl glucoside is a lipid mediator in stress-responsive signal transduction. Cell Struct. Funct. 27: 157-162.
12. Li, X. C. 2001. A new lignan glycoside from Eleutherococcus senticosus. Planta Med. 67: 776-778.
13. Norr, H. 1993. Phytochemical and pharmacological Investigations of the Adaptogens: Eleutherococcus senticosus, Ocimum sanctum, Codonopsis pilosula, Rhodiola rosea and Rhodiola crenulata. In Ph.D. Dissertation, Faculty of Chemistry and Pharmacy. pp. 1-231. Ludwig-Maximillians University, Munich, Germany.

14. Munck, A., Guyre, P. M. and Holbrook, N. J. 1984. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr. Rev. 5: 25-44.
15. Ovodov, Y. S., Frolova, G. M., Nefedova, M. Y. and Elyakov, G. B. 1965. Glycosides of Eleutherococcus senticosus. II. Structure of Eleutherosides A, B1, C and D. Khim. Prir. Soedin 1: 63-64.
16. Ovodov, Y. S., Ovodova, R. G., Solovyova, T. F., Elyakov, G. B. and Kochetkov, N. K. 1965. Glycosides of Eleutherococcus senticosus. I. Isolation and some properties of Eleutherosides B and E. Khim. Prir. Soedin 1: 3-7.
17. Panossian, A., Wikman, G., Kaur, P. and Asea, A. 2009. Adaptogens exert a stress protective effect by modulation of expression of molecular chaperons. Phytomedicine 16: 617-622.
18. Panossian, A. and Wikman, G. 2009. Evidence-based efficacy of adaptogens in fatigue, and molecular mechanisms related to their stress-protective activity. Current Clin. Pharmacol. 4: 198-219.
19. Pearce, P. T. 1982. Panax ginseng and Eleutherococcus senticosus extracts: in vitro studies on binding to steroid receptors. Endocrinol. Jpn. 29: 567-573.
20. Phillipson, J. D. and Anderson, L. A. 1984. Ginseng-quality, safety and efficacy? Pharm. J. 232: 161-165.
21. Prodius, P. A., Manukhina, E. B., Bulanov, A. E. , Wikman, G. and Malyshev, I. I. 1997. Adaptogen ADAPT modulates synthesis of inducible stress protein HSP 70 and increases organism resistance to heat shock. Biull. Eksp. Biol. Med. 123: 629-633.
22. Schlemmer, G. 1989. Analyse von biologischem Material mit der Graphitrohrofen - AAS. In: Instrumentalized Analytical Chemistry and Computer Technology. pp. 561-568. GIT VERLAG: Darmstadt, Germany.
23. Smalinskiene, A., Lesauskaite, V., Zitkevicius, V., Savickiene, N., Savickas, A., Ryselis, S., Sadauskiene, I. and Ivanov, L. 2009. Estimation of the Combined Effect of Eleutherococcus senticosus Extract and Cadmium on Liver Cells. Ann. N. Y. Acad. Sci. 1171: 314-320.
24. Sunderman, F. W. 1978. Carcinogenic effects of metals. Fed. Proc. 37: 40-46.
25. Stanevicienė, I., Smalinskiene, A., Lesauskaite, V., Ivanoviene, L., Liekis, A., Sadauskiene, I., Abdrachmanovas, O. and Ivanov, L. 2006. Influence of long-term cadmium ions exposure on mitotic and apoptotic activities and protein synthesis in mouse liver. Biologija. 3: 118-122.
26. Steinmann, G. G., Esperester, A. and Joller, P. 2001. Immunopharmacological in vitro effects of Eleutherococcus senticosus extracts. Arzneimittelforschung 51: 76-83.
27. Wiegant, F. A., Surinova, S., Ytsma, E., Langelaar-Makkinje, M., Wikman, G. and Post, J. A. 2009. Plant adaptogens increase lifespan and stress resistance in C. elegans. Biogerontology 10: 27-42.
28. Zapesochnaya, G. G., Kurkin, V. A., Boyko, V. P. and Kolkhir, V. K. 1995. Phenylpropanoids–promising biologically active compounds of medicinal plants. Khim. Farm. Zh. 29: 47-50.