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VARIATION OF THE DEUTERIUM CONCENTRATION IN RATS` BLOOD AFTER DEUTERIUM DEPLETED WATER ADMINISTRATION AND INTOXICATION WITH CADMIUM

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Abstract: The effects of cadmium pollution are highlighted in many studies, [7,14]. Cadmium, absorbed within the organism, inhibits the action of some antioxidant enzymes, especially those which contain SH and affect especially the liver and kidneys. The level of deuterium in tap water is 150 ppm and in deuterium depleted water are under 80 ppm. Deuterium depleted water with a deuterium level of 30 ppm has an antioxidant effect on the organism [9, 10]. The present study has asses the effects of deuterium depleted water (30 ppm) on the deuterium blood level in the rats exposed to cadmium (20 ppm Cd/kg administered as $CdCl_2$ solution in a single dose). The intoxication with cadmium modified the blood level of deuterium in the animals and the kidney, liver and spleen weight. The blood level of deuterium was determined through weight spectroscopy with the spectrofotometer SMAD 1. We observed a protective effect of deuterium depleted water on the rat organism, for preventive administration, as well as treatment and the important role in eliminating cadmium.

INTRODUCTION

Water is everywhere around us. Without water there is no life. Water on Earth is a mixture of H_2O and D_2O, in certain proportions. The level of deuterium is expressed through the ratio R=D/H in ppm, where D is the number of deuterium atoms and H the number of hydrogen atoms in water. The deuterium level in surface waters is 150 ppm and over 90 ppm in living organisms. Deuterium depleted water is the water that has a deuterium level below 80 ppm. Researches in the last decade have proven, that deuterium depleted water has special influences on cell and tissue developement in living organisms [5, 6, 9,11], the effects being more pronounced when using deuterium depleted water with R=60 ppm or R=30 ppm.

Cadmium is an industrial pollutant. The toxicity of cadmium is well known. There are multiple studies on its carcinogen potential [7, 14]. Unfortunately, the population in big cities and around them is exposed to cadmium pollution by inhaling the industrial smoke, the smoke from cars, dust, through certain edibles or even through water in certain areas. In these areas, people inhale approximately 20-40 µg/day, 5-10% of which are absorbed. After absorption, cadmium is transported in the blood, bound to albumins [9]. Cadmium acts like an inhibitor of some enzymes in the antioxidant system of the organism. The most affected organs to cadmium exposure are liver and kidneys [3, 4]. The purpose of this study is to observe the effects of cadmium intoxication on the deuterium blood level of rats treated with deuterium depleted water (30 ppm) and on the weight of some organs: liver, kidney, spleen.

The research evaluates the way in which deuterium depleted water can protect living organisms against cadmium intoxication. Because deuterium depleted water is non-toxic, the

decreasing of deuterium level in the organism can be accomplished very easy by consuming deuterium depleted water instead of tap water [12].

In this present study we studied the variation of deuterium blood level in rats intoxicated with cadmium and treated with deuterium depleted water. The intoxication with cadmium increases the deuterium blood level. Replacing tap water with deuterium depleted water helps the organism to accumulate less deuterium, thus fighting cell aging [8]. Deuterium depleted water helps to eliminate cadmium from the organism.

MATHERIAL AND METHODS

The experiment was carried on adult male Wister rats with a body weight of 250-300 g. The rats were divided into 6 groups of 8 individuals each. The fodder received was a mixture of cereals (40% wheat, 40% corn, 20% sunflower), mixture which insures the nutritional and energetic value for the animals during the experiment, according to a corresponding diet. The rats were maintained in good physiological conditions. The experiment lasted 61 days and was conducted as following:

L1 – received tap water ad libitum for 61 days.

L2 – received deuterium depleted water (30 ppm) ad libitum for 61 days.

L3 – received tap water ad libitum 30 days; on day 31 of the experiment 20 ppm Cd/kg b.w (as $CdCl_2$), a single dose was administrated by gastric tubing and after 24 hours were sacrificed.

L4 – received deuterium depleted water (30 ppm) ad libitum for 30 days; on day 31 of the experiment 20 ppm Cd/kg b.w (as $CdCl_2$), in single dose, were administrated by gastric tubing and after 24 hours were sacrificed.

L5 - received tap water ad libitum 30 days; on day 31 of the experiment 20 ppm Cd/kg b.w (as $CdCl_2$), in single dose, were administrated by gastric tubing and then for another 30 days they received tap water.

L6 - received deuterium depleted water (30 ppm) ad libitum for 30 days; on day 31 of the experiment 20 ppm Cd/kg b.w (as $CdCl_2$), a single dose was administrated by gastric tubing and then for another 30 days they received deuterium depleted water.

24 hours after the cadmium intoxication, blood samples were collected on heparin by cardiac punction and then the rats were sacrificed as follows: the rats from groups L1, L2, L3 and L4. At the end of the experiment (day 61), blood samples were collected from L5 and L6, which were then sacrificed. Blood and tissue samples were taken under general narcosis. The investigations were carried out with the approval of the Local Ethics Committee according to the Romanian law 205 /2004, art.7, 18, 22 and the regulations no. 143/400/2002 and 37/2002, concerning with the protection of vertebrate animals used for experimental and other scientific purposes. The data are presented as means ± S.D. values. ANOVA, TTest, MINITAB and the nonparametric test Mann-Whitney were used. The blood samples were distillated under vacuum, and the resulting water was analyzed for the deuterium level with the weight spectrofotometer SMAD 1. The precision of its determination is $\pm 2‰$. *The organ weight was determined by weighing them on the analitical scale Kern, Gmbh, Germany. DDW was obtained in accordance with a contract between the Faculty of Veterinary Medicine Timisoara, Romania and the heavy water plant ROMAG Turnu Severin, Romania.*

RESULTS AND DISCUSIONS

Throughout the experiment, variations of the deuterium blood level of the tested animals were registered. The results are presented in table 1, figure 1. At the rats which

received tap water (L3 – H_2O + Cd) and were intoxicated with cadmium (20 ppm Cd/kg), after 24 hours, the deuterium blood level increased from 146,79 to 157,78 ppm (7,49% as opposed to the control L1 – H_2O). At group L2 (DDW), after 30 days of deuterium depleted water (30 ppm) pretreatment, a decrease of the deuterium blood level from 146,79 to 90,78 ppm (38,16% as opposed to the control L1) was registered.

The pre-treated group with deuterium depleted water (L4 –DDW + Cd), 24 hours after the intoxication with cadmium chloride suffered an increase of the deuterium blood level from 90,78 to 104,77 ppm (11,4% as opposed to group L2). After another 30 days of DDW treatment, the deuterium blood level continueed to increase from 104,77 to 133,47 ppm (47,025% as opposed to group L2), the average value remaining smaller than the one from group L1. The deuterium level of the tested rats, which consumed tap water, had very similar values to the deuterium blood level in humans, which in some studies was 149-150 ppm and was with 30‰ higher than the deuterium level of tap water in Central and Eastern Europe [1, 13]. The high blood level of deuterium, immediately after administrating cadmium chloride can be a clue and a favourising factor of disturbing cellular processes, „aging" of intracellular water, by decreasing its level of structure. This results were according to the literature data, which showed an increase of the deuterium blood and urine level along with aging and have a negative influence of deuterium on metabolic processes [2, 8, 14,15].

Table 1

The deuterium blood level in male Wistar rats intoxicated with Cd, pre-treated and treated with deuterium depleted water (30 ppm)

	L1 H_2O	L2 DDW	L3 H_2O +Cd	L4 DDW+Cd	L5 H_2O+Cd+ H_2O	L6 DDW+Cd+DDW
R (ppm)	146,79	90,78	157,78	104,77	149,67	133,47
d(‰)	-57,6	-417,15	-115.4	-327,3	-59,5	-143

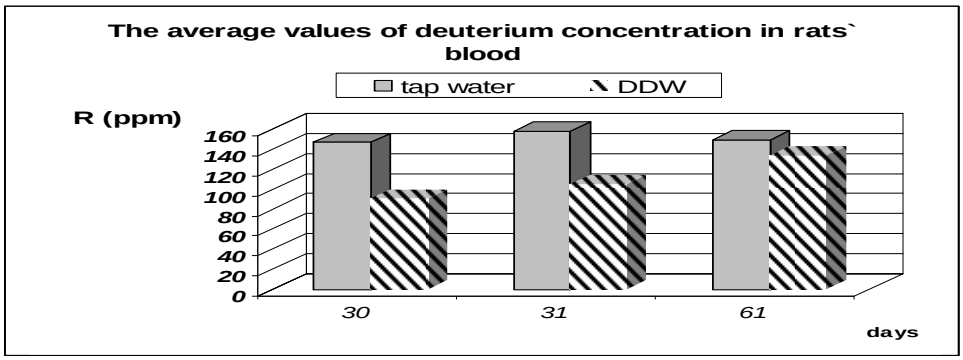

Fig.1. The deuterium blood level in male Wistar rats intoxicated with Cd, pre-treated and treated with deuterium depleted water (30 ppm)

If there were significant variations of the deuterium's level at the time of intoxication, it is necessary to take into consideration the measuring of the deuterium level as a method for detecting intoxication with heavy metals and evaluating their severeness.Such a method was proposed in other studies as well as for diagnose human cancer [1, 14].

Changes in the organs` weight (liver, kidney and spleen) were also studied. The results are presented in table 2, figures 2, 3, 4.

Table 2

Average values of the organs` weight (liver, kidney, spleen) in male Wistar rats, intoxicated with Cd (20ppm), pre-treated and treated with deuterium depleted water (30 ppm) or H_2O

Group	L1 H_2O	L2 DDW	L3 H_2O +Cd	L4 DDW+Cd	L5 H_2O+Cd+H_2O	L6 DDW+Cd+DDW
liver (g)	9,909± 0.35	9,763± 1.52	10,129± 0.98	9,867± 0.84	9,978± 0.98	8,783± 1.11
kidney(g)	2,327± 0.12	2,167± 0.21	2,375± 0.18	2,184± 0.09	2,455± 0.30	2,379± 0.19
spleen(g)	1,179± 0.13	0,985± 0.10	1,007± 0.15	1,172± 0.22	1,225± 0.45	1,379± 0.26

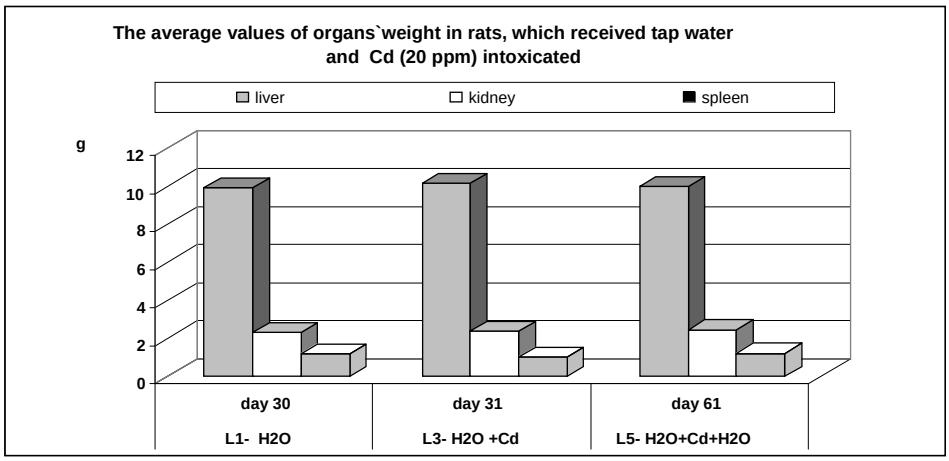

Fig.2 Variation of the average values of the organs` weight in male Wistar rats with tap H_2O administration and intoxicated with Cd (20ppm), in single dose

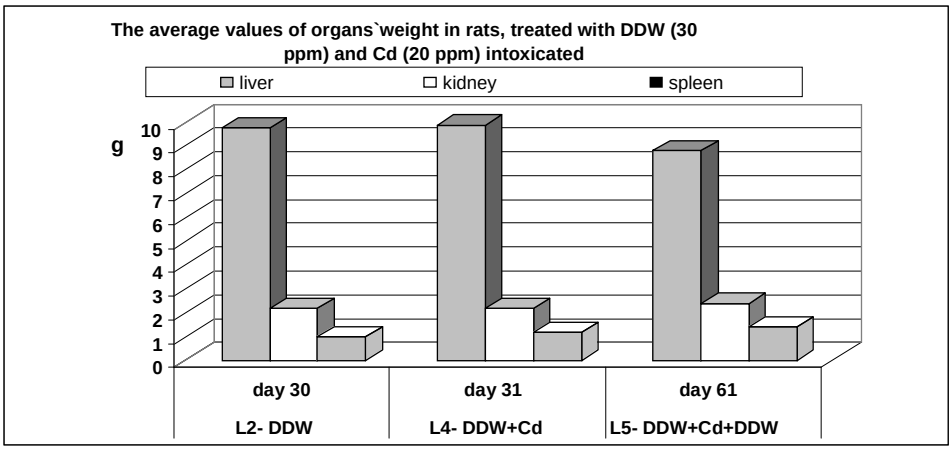

Fig.3 The average values of organs` weight in male Wistar rats, intoxicated with Cd (20 ppm) in single dose, pre-treated and treated with DDW(30 ppm)

At L2 group (deuterium depleted water), the average values of the weight were smaller then those of the control on all studied organs. After 24 hours from the cadmium intoxication at L3 group (H_2O + Cd), the liver weight increased from 9,909 to 10,129 g (11,4% as opposed to the control L1); the kidney and spleen weight increased with 2,06%, and 14,6%, as opposed to the control L1. At L4-DDW + Cd, pre-treated with DDW, the liver weight was

increasing only with 1,06% as opposed to group L2 and the kidney weight increased from 2,167 to 2,1845 g (0,81% as opposed to group L2); the spleen weight increased from 0,9885 to 1,172 g (15,9% as opposed to group L2) remaining smaller than group L1.

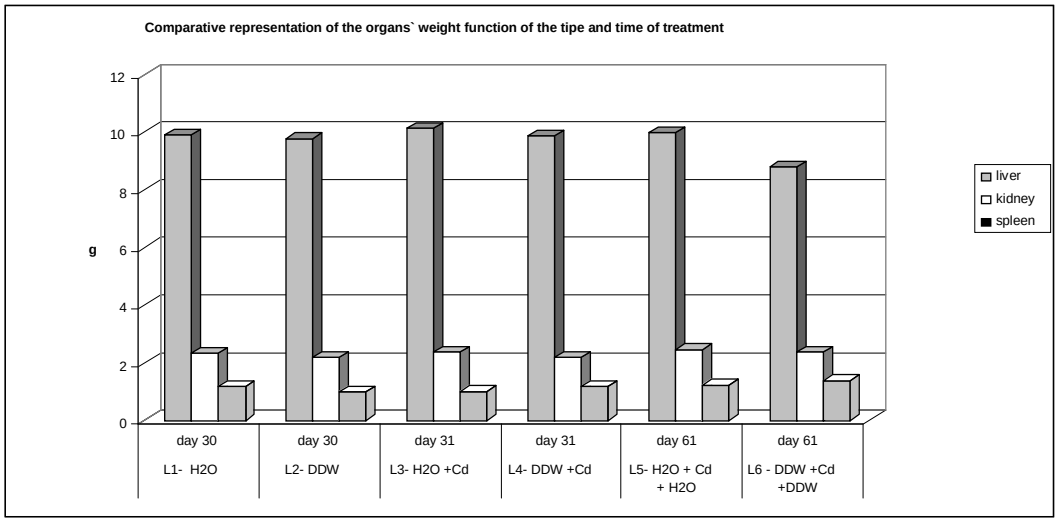

Fig.4 Comparative representation of the organs` weight depending of the type and time of treatment

After 30 days cadmium (20 ppm) intoxication, in single dose, was performed.

At L5 (H_2O + Cd + H_2O), the liver weight decreased from 10,129 to 9,978 g (1,525% as opposed to the control L1), the kidney weight increased from 2,3755 to 2,455 g (3,35% as opposed to group L3). At L6 (DDW + Cd + DDW), the liver weight decreased from 9,867 to 8,783 g (10,98% as opposed to group L4); the kidney weight increased from 2,1845 to 2,3795 g (8,92% as opposed to group L4), and in spleen, from 1,172 to 1,3798 g (17,76% as opposed to group L4) and even as opposed to the control L1. The variation of the organs` weight leads to the conclusion, that for the pre-treated and treated group with deuterium depleted water, cadmium accumulated after 24 hours in the liver but in the next 30 days, following a treatment with deuterium depleted water, Cd is eliminated from the liver, increasing in kidney and spleen, and eliminate from the organism. At the group that consumed tap water, the accumulation of cadmium in the liver was higher (the weight difference was bigger at group L3 than L4, 24 hours after the intoxication); 30 days consecutive the intoxication, the liver weight decreases less at group L5 than at group L6, and the kidney and spleen weight increased less at group L5 than at group L6, which means, that deuterium depleted water helps to more rapidly eliminate cadmium from the organism than tap water throughout 30 days after the intoxication. Pre-treatment with deuterium depleted water offers the organism protection against cadmium intoxication (the liver is much less affected than when using tap water).

CONCLUSIONS

- Cadmium intoxication leads to an increasing of deuterium concentration in blood. Replacing tap water with deuterium depleted water may help the organism to accumulate less deuterium
- Replacing tap water with deuterium depleted water may help to maintain a small deuterium blood level after intoxication with cadmium, which is benefic for a good cell function.
- Pre-treatment with deuterium depleted water protects organs against cadmium intoxication.
- Pre-treatment and treatment with deuterium depleted water may help to scavenge cadmium from the organs.

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