DOI: 10.7860/JCDR/2017/30297.10841

Original Article

Physiology Section

Activity of Catalase (CAT), ALT and AST in Different Organs of Swiss Albino Mice Treated with Lead Acetate, Vitamin C and Magnesium-L-Threonate

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ABSTRACT

Introduction: Lead is a natural element with toxic properties and is widespread in the environment. Lead toxicity is associated with generation of reactive oxygen and nitrogen species and consumption of antioxidants elements (vitamin E and C, glutathione, thioredoxin and lipoic acid, melatonin, carotenoids and natural flavonoids) in the cell, and unbalancing oxidants-antioxidants levels.

Aim: To evaluate the effects of different chemical combinations (lead acetate, Vitamin C and Magnesium-L-threonate) on antioxidant enzyme activity (catalase-CAT) of liver, kidney, spleen, pancreas and brain, and serum transaminases [Serum Alanine Transaminase (ALT) and Serum Aspartate Transaminase (AST)].

Materials and Methods: Experimental animals (49 male Mus musculus-swiss albino mice) were separated into five different groups. The first group was used as a control, hence the other four groups were treated with sub-lethal doses (90 mg/kg) of lead acetate (group 2), lead acetate (90 mg/kg) and Vitamin C dose 40mg/kg (group 3), lead acetate (90 mg/kg) and Magnesium-L-threonate dose 100 mg/kg (group 4) and only with Magnesium-L-threonate dose 100 mg/kg (group 5), during the treatment period (40 days). Blood samples were taken from the facial vein and used for transaminase analysis. Organ tissue was collected after euthanizing anaesthetized animals with neck dislocation technique.

Results: The results showed that lead acetate treatment has caused significant elevation in the activity of AST (group 2 and 3) and ALT (group 3). Also, CAT activity was significantly ($p<0.05$) increased in groups treated with lead acetate (liver, pancreas, kidney and brain but not in spleen). Treatment of lead intoxicated groups with Vitamin C and Magnesium L-threonate increased significantly CAT activity in brain.

Conclusion: Lead effects by interacting with different molecular systems and increasing enzyme activity (CAT, ALT and AST). Effects on CAT activity of Magnesium-L-threonate and Vitamin C treatment in lead acetate intoxication case are similar. Detoxifying properties of Vitamin C in the brain compared with other organs were very ineffective, because of Blood Brain Barrier (BBB) metabolic competences.

Keywords: Antioxidants, Blood brain barrier, Dose, Toxicity

INTRODUCTION

Lead is one of the most serious global health problems in communities that are involved in activities like lead smelting, gold mining, lead battery manufacturing and recycling [1]. Lead does not have any known biological role or beneficial properties for the organism and also there are no minimal or safe doses of lead exposure [2]. Acute exposure to lead often is followed by symptoms like nausea, headaches, cognitive changes, and emotional disruptions [3]. The ingested lead is stored primarily in bone and soft tissues, but the highest concentration of lead is found in the bone, lung, kidney, teeth, liver, brain and spleen [4,5]. In recent years, there have been changes in legislation about environmental pollution and processing techniques of lead. These developments have reduced a lot of lead pollution, but it is known that lead may be toxic and cause irreversible changes even in low doses [6,7]. Most usual toxic activity of lead is the relationship with oxidative stress, and with Reactive Oxygen Species (ROS) generation [5,8-10]. Most common reaction and damages of reactive oxygen species are oxidation of cell most important molecules (DNA, proteins and lipids), which affect enzyme activity and membrane functions [11-13].

Reactive Oxygen Species (ROS) accumulation is controlled in vivo by two different systems: non-enzymatic antioxidant systems (glutathione-GSH; bilirubin; vitamins A, C, and E) and defence-related enzymes (catalase-CAT, glutathione peroxidase-GPx, and superoxide dismutase-SOD) [14]. The activity of antioxidant enzymes is a proportional response to the accumulation of ROS [13]. If this enzymatic defence system fails to control the increased levels of ROS, it causes the oxidative stress and is capable of damaging membrane lipids, proteins and nucleic acids [10].

The biological functions of vitamin C are based on its ability to provide a reducing equivalent for a large number of biochemical reactions. Due to its reducing properties the vitamin can reduce most ROS that are physiologically relevant [15].

Magnesium L-threonate is a newly developed supplement that is used to increase brain magnesium and improve memory function. Magnesium L-threonate is formed by two L-threonic acid molecules and magnesium ion, Mg^{2+}. Oral intake of Magnesium has beneficial effects in chronic lead poisoning, inducing decrease of lead body burden and its increased elimination via urine [16]. L-threonic acid is a metabolite of Vitamin C that has unique effects in the body. It is researched for his properties as a chelating agent for different metal ions [17-19], but there is lack of data for its properties as chelating agent of lead. L-threonic acid is identified in the periphery in plasma and the aqueous humor of the eye [20]. Elimination of threonic acid from the body, is not fully explained, but so far it is known that around 10% is excreted with urine [21,22]. Even there is lack of research in safety and toxicity of Magnesium L-threonate, it is known that both magnesium and L-threonic acid have physiological effects on cell metabolism [23].

The aim of the study was to evaluate effects of lead toxicity in antioxidant enzyme activity and effects of treatment with Vitamin C and Magnesium L-threonate of intoxicated animals. Also during

this study, we aimed to evaluate effects of lead on body weight, food and water intake, any behaviour changes during the research. There are many studies that show the effects of lead in antioxidant enzyme activity, ALT and AST [24-26], but there is lack of studies that compare effects of Vitamin C or Magnesium L-threonate treatment in lead toxicity in different organs. The Magnesium L-threonate was used in our research to study effects of magnesium as competitor of lead and chelating properties of L-threonic acid.

MATERIALS AND METHODS

This was an experimental study, project of the PhD thesis in Experimental Biomedicine programme. All the research experiments were conducted in University of Prishtina, Faculty of Natural Science and Math, Department of Biology during the period December 2015 to July 2016.

Trihydrate form of lead acetate was ordered from Läch Ner (Lead acetate trihydrate, reagent grade 99.5%). Vitamin C (L-ascorbic acid 99%), primary and secondary sodium phosphate (99.9%), and hydrogen peroxide (99%) were from Sigma-Aldrich. Magnesium-L-threonate was procured from Swanson Vitamins (Magtein). Double distillate water was used in all experiments. Food for the animals was from Subotica Veterinary Institute (Complete mixture for laboratory rats and mice).

This study started after ensuring that all experimental procedures involving animals were conducted in accordance with Law for Animal Care Nr. 02/L-10 and European Council Directives [27], and approved by Ethics committee of Faculty of Medicine-University of Prishtina (nr. 1278, ref. Nr. 3425).

A total of 49 (six-month-old) male albino mice (Mus musculus-Swiss albino strain) of body weight ranging from (35-40 gram) and were obtained from the vivarium of the Faculty Natural Science and Math, University of Prishtina, Kosovo. Animals were divided into five groups in separate individual cages after two weeks adaptation period and all the conditions in vivarium were as described by the Europian Council Directives. The experimental animals were fed with diet composed of tap water and mixed mouse food. Control group was the first group (N=9) of animals, while the other groups were fed orally with food mixed with sub-lethal doses (90 mg/kg) of lead acetate (group 2, N=8), lead acetate (90 mg/kg) and Vitamin C dose 40mg/kg (group 3, N=8), lead acetate (90 mg/kg) and Magnesium-L-threonate dose 100 mg/kg (group 4, N=12) and only with Magnesium-L-threonate dose 100 mg/kg (group 5, N=12).

The concentration of lead acetate, Magnesium-L-threonate and Vitamin C in the food was calculated every day after measuring body weight of the animal for 40 days of the treatment period to achieve the prescribed dose. After these 40 days of treatment, there was a 20 day wash-up period where animals were fed with normal diet. Animals were sacrificed on the last day of wash up period with neck dislocation after they were anaesthetized with diethyl ether. Samples of organs (liver, brain, kidneys, spleen and pancreas) were taken and put in cold sodium phosphate buffer (concentration 0.05 M and pH 7.0) till they were homogenised. Homogenates were centrifuged at 5000 rpm for 10-15 minutes and then the supernatant was relocated in new sterile sample tubes stored in 4°C. The measurements of enzyme activity were conducted within 24 hours of sample preparation. Protein concentration was measured in dilute organ homogenates using method according to Lowry OH et al., [28]. The enzyme activity was calculated as UI/mg-protein.

During this research, we also collected data for body weight, food intake per day, water intake per day, difference in behaviour of the animals.

Estimation of Catalase Activity

Catalase activity levels are calculated as a mean value of CAT activity for mg protein of the samples. Total CAT activity was determined spectrophotometrically (Shimadzu UV-VIS Mini Spectrophotometer UV-1240) by following the decline in A240 as H_20_2 (E=36 M^{-1} cm^{-1}) was catabolized, according to the method of Nilsson [29]. The method accuracy and sensitivity was estimated by measuring standards of bovine catalase in different activity levels, from 3 to 450 units.

Estimation of Transaminases

Alanine Aminotransferase (ALT) and Aspartate aminotransferase (AST) was assayed by the IFCC method without pyridoxal phosphate using Elitech kit. ALT and AST activity levels are calculated in UI/L at 37°C. ALT and AST measurement were done using spectrophotometer (Shimadzu UV-VIS Mini Spectrophotometer UV-1240), wavelength 340 nanometres, in quartz cuvette with 1 cm path length, for 175 seconds after 50 seconds of incubation in 37°C.

STATISTICAL ANALYSIS

Data analysis have been done with Microsoft excel Data analyser 2016. Data were analysed by One-way analysis of variance (ANOVA) followed by a post-hoc test, the least significant difference. Results are reported as mean±SD. Statistically significant change was considered in probability level of <0.05.

RESULTS

There was a different response to catalase activity for different organs. In liver, we observed significant ($p<0.05$) increase in CAT activity between control (blank feed) and group 2. Also, the group 2 showed significant increase of CAT activity in other organs {kidney ($p<0.05$), pancreas ($p<0.05$) and brain ($p<0.05$) compared to control group. A higher activity of CAT was registered (liver, pancreas and kidney) for group 2 when it was compared with groups 3 and 4 [Table/Fig-1]. These results can be explained by the protective effect of Vitamin C in toxicity of the organism. Brain CAT activity is increased more in group 4 compared with the group 2. Increased effect of lead acetate toxicity in the brain when it was given with vitamin C and Magnesium-L-threonate, is shown by increased levels of CAT activity in the brain tissue, compared with the group treated only with lead acetate. The same effect was registered in spleen tissue [Table/Fig-1].

Group Organ	Group 1 (N=9)	Group 2 (N=8)	Group 3 (N=8)	Group 4 (N=12)	Group 5 (N=12)
Liver	232.8± 56.89	498.3± 64.94*	218.9± 89.16	294.4± 110.87	140.6± 45.46
Pancreas	22.6±7.17	45.5±27.97*	30.1±12.10	35.8±24.11	41.1±12.33
Spleen	53.0± 18.51	44.7± 9.52	68.4± 19.92	77.4± 16.53*	53.6± 18.67
Kidney	128.7± 42.14	244± 46.74*	142.4± 34.62	89.7± 50.18	126.3± 64.83
Brain	4.9±2.25	7.7±2.45*	8.4±2.88*	8.2±2.51*	5.4±2.56

[Table/Fig-1]: Catalase activity level (CAT-UI/mg protein) in liver, spleen, kidney, pancreas and brain tissue of mice (Mus musculus-Swiss albino) treated for 40 days. *p<0.05 comparing with control. One-way ANOVA single factor showed significance (*p<0.05) in five organs, Least Significant Difference test was done as a post-hoc test.

Group Parameter	Group 1 (N=9)	Group 2 (N=8)	Group 3 (N=8)	Group 4 (N=12)	Group 5 (N=12)
ALT	68.5±15.6	72.1.04±33.7	87.8±31.9*	45.9±20.7	71.2±27.5
AST	87.1±32.1	223.8± 52.8*	134.9± 31.8*	119.5± 45.3	114.5± 39.2

[Table/Fig-2]: ALT and AST activity level (UI/L at 37°C) in serum of mice (Mus musculus-Swiss albino) for 40 days. *p<0.05 comparing with control.

Our results showed that AST activity levels in the group 2 and 3 were significantly higher ($p<0.05$) compared with the control group. Similar results are obtained also from ALT, but with a significant increase in activity of the enzyme in a group 3 compared with control group [Table/Fig-2].

Body weight and food intake of the animals was measured everyday also and, did not have any significant change during the 40 days of treatment. Behaviour of the animals was checked for abnormal patterns (increased activity, feeding, sleeping and increased aggressiveness during handling of the animal), and during the time of the research no abnormal behaviour was registered.

DISCUSSION

The objective of this study was to evaluate the effects of different chemical combinations of lead acetate with Vitamin C and Magnesium-L-threonate on antioxidant enzyme activity (catalase-CAT) of liver, kidney, spleen, pancreas and brain, and serum transaminases (ALT and AST). Most of the data for lead intoxication in other research are linked with liver or blood lead level [30,31]. Time of the intoxication, dosage of lead and supplements, age and sex of the animals are some of the factors that defined the differences in some of our results when they were compared with other authors [30-32]. Our research results are in coherence with other author's research. Compared with normal rats, in the group treated with lead, was registered decreased in the activity of superoxide dismutase and increased the activity of catalase, increased levels of glutathione and peroxidation of lipids [25]. Similar to our result, Adegbesan BO et al., registered an increase of CAT activity in liver of rats after acute lead intoxication [33]. Effects of lead intoxication in spleen tissue are registered by many authors [34-36]. Spleen tissue catalase activity level were increased in groups 3 and 4 compared with control group or group 2 but significant results were found in group 4 only. In our study, changes in catalase activity levels in spleen tissue did not follow the trend of other organs (liver, kidney and pancreas), as other authors registered [37]. Correa M et al., registered increase in mice brain catalase activity after acute lead acetate administration [38]. The activity of catalase is induced upon metal exposure to animal species by some environmental parameters and chemicals pollution [39]. Acute lead intoxication cause accumulation of delta amino levelunic acid, and increased lipid peroxidation level, decreasing levels of glutathione and levels of antioxidative enzyme activity [40]. As an antioxidant, Vitamin C decreased toxicity effects of lead on some organs (liver, pancreas and kidney), but did not have any protective role in the brain. This lack of effect of Vitamin C is proven by higher levels of CAT activity in the brain in the group 3 compared with group 2. Vitamin C has main role in Nramp2 (DMT1) overexpression [41], and the substrate profile of DMT1 includes metals, Fe^{2+}, Cd^{2+}, Co^{2+}, Mn^{2+}, Ni^{2+}, VO^{2+}, Pb^{2+}. Divalent metal transporter 1 (DMT1), which is a divalent cation membrane transporter, was involved in the transcellular transport of lead across the blood brain barrier-BBB [42].

Hepatocytes and erythrocytes are cells that have registered the highest catalase activity [43]. Hepatotoxicity of heavy metals comes by their ability to reduce levels of proteins and glutathione and increasing synthetisation of ROS, such as H_2O_2, OH^- and O_2^{2+}. These products by peroxidation of lipids can cause damage to the cell membrane and then outpouring into the blood some of the liver enzymes. Aminotransferases activity will be increased in serum when the liver is damaged in acute or chronic form. Transferring the α-amino group from aspartate or alanine to the α-keto group of ketoglutaric acid, which generates pyruvic acid and respectively oxalacetic acid that are very important components of citric acid cycle, is the role of ALT and AST [26].

Our result shows that lead acetate exposure had effects on increasing level of AST and ALT activity in serum. The activity of AST was significantly increased in the group 2 and 3. Mazumdar I et al., registered increased ALT and AST levels in significant portion during a chronic period of lead exposure [24]. Ibrahim NM et al., results showed that lead intoxication highly and significantly increased the activity of AST and ALT in serum [25]. The results from other research show a correlation of lead exposure and transaminase activity levels in serum. Compared with the control group the levels of serum ALT, AST, Alkaline Phosphatase (ALP), and γ-glutamyltransferase (γ-GT) activities were elevated in lead acetate treated animals with statistically significant differences ($p<0.05$) [44]. One of our most important interests during this study was to show that lead intoxication has a major variety of implications in body metabolism, antioxidative-oxidative molecular balance in different organs and plasma. Changes in enzyme activity are one of the first indicators of organism intoxication.

LIMITATION

In this article, results are not from human participants and cannot be used as a reference values for humans without further research.

Vitamin C is one of the most prescribed vitamins by the medical professionals, hence our results gives a warning in usage of these supplements in cases of lead intoxication. In addition, Magnesium L-threonate as a supplement for learning memory enhancement should be carefully taken until further research is done.

CONCLUSION

Antioxidative supplement (Vitamin C) had a protective effect on some organs and tissue (liver, kidney and pancreas) of mice intoxicated with lead. This effect of vitamin C was not seen in brain and spleen of the same animals. Magnesium L-threonate as a substance which is used, as a supplement for cognitive improvement, did not cause any adverse effect on other organs of the body, on the contrary, due to the content of L-threonate it had antioxidant effect in groups treated with lead similar to the vitamin C. Effects of Vitamin C and Magnesium L-threonate were different in brain and spleen compared to other organs (liver, kidney and pancreas). Although, many studies are done about lead and vitamin C, there is much to be done.

Funding

All the funds in this research are provided by the Faculty of Math and Natural Science-University of Prishtina (dt.23.01.2015, ref nr. 1570).

ACKNOWLEDGEMENTS

The dean of the Faculty of Math and Natural science in University of Prishtina, Prof. Dr. Tahir Arbneshi, who has made possible to use all the resuorces and laboratories of the faculty.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **May 22, 2017**
Date of Peer Review: **Jun 26, 2017**
Date of Acceptance: **Sep 25, 2017**
Date of Publishing: **Nov 01, 2017**