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EFFECT OF SELENIUM SUPPLEMENTATION ON SERUM AMYLASE, LACTATE DEHYDROGENASE AND ALKALINE PHOSPHATASE ACTIVITIES IN RATS EXPOSED TO CADMIUM OR LEAD

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ABSTRACT. The purpose of the study was to assess the effect of selenium supplementation on serum amylase, lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activities in rats, during subacute exposure to toxic doses of cadmium or lead through the drinking water. The experimental groups (n=6) were: Control, Se (Se^{+4}: 0,2 mg/l), Cd (Cd^{+2}: 150 mg/l), Pb (Pb^{+2}: 300 mg/l), Cd+Se (Cd^{+2}: 150 mg/l; Se^{+4}: 0,2 mg/l) and Pb+Se (Pb^{+2}: 300 mg/l; Se^{+4}: 0,2 mg/l). The animals were sacrificed after 56 days. Amylase, LDH and ALP activities were determined from serum. Se and Pb treatments caused an increase in amylase and LDH activities, when compared to Control group while Cd caused an increase in amylase activity and a decrease in LDH and ALP activities. Cd+Se caused a decrease in amylase activity and an increase in LDH activity, when compared to Cd. Pb+Se caused a decrease in amylase activity in comparison to lead. Selenium supplementation alleviated cadmium or lead induced changes in serum amylase activity. Selenium, coadministered with cadmium, caused a marked increase in serum LDH activity, when compared to cadmium alone or Control group while practically it had no effect on lead induced changes in LDH activity. Cadmium and lead induced disturbances in serum ALP activity were not influenced by selenium supplementation.

Key words: Rats; Lead; Cadmium; Selenium; Blood serum enzyme.

REZUMAT. Efectul suplimentării cu seleniu asupra activităţii amilazei serice, a lactat dehidrogenazei şi a fosfatazei alcaline la sobolani, expuşi la cadmiu sau plumb. Scopul acestui studiu a fost de a evalua efectul suplimentării cu seleniu asupra unor enzime, amilaza, lactat dehidrogenaza (LDH) şi fosfataza alcalină (ALP) la şobolani, în timpul expunerii subacute la doze toxice de cadmiu sau plumb, prin intermediul apei de băut. Loturile experimentale (n=6) au fost: Martor, Se (Se^{+4}: 0,2 mg/l), Cd (Cd^{+2}: 150

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mg/l), Pb (Pb^{+2}: 300 mg/l), Cd+Se (Cd^{+2}: 150 mg/l; Se^{+4}: 0,2 mg/l) şi Pb+Se (Pb^{+2}: 300 mg/l; Se^{+4}: 0,2 mg/l). Animalele au fost sacrificate după 56 de zile. Amilaza, LDH şi ALP au fost determinate din ser. Administrarea de Se sau Pb a determinat o creştere a amilazei şi LDH, în comparaţie cu lotul de control, în timp ce Cd a determinat o creştere a amilazei şi o scădere a LDH şi ALP. Cd+Se au cauzat o scădere a amilazei şi o creştere a LDH, în comparaţie cu Cd. Pb+Se au produs o reducere amilazei, în comparaţie cu plumbul. Suplimentarea cu seleniu a atenuat modificările induse de cadmiu sau plumb asupra amilazei serice. Seleniul, coadministrat cu cadmiul, a cauzat o creştere marcantă a activităţii serice a LDH, în comparaţie cu Cd sau lotul martor, însă nu a avut nici un efect asupra modificărilor produse de plumb asupra activităţii LDH. Tulburările induse de cadmiu şi plumb asupra activităţii serice a ALP nu au fost influenţate de suplimentarea cu seleniu.

Cuvinte cheie: şobolani; plumb; cadmiu; selenium; enzime serice.

INTRODUCTION

Selenium (Se) is an essential microelement with multiple physiological roles. It is a component of several selelenoenzymes with important functions in the body. The most important selenoenzymes are the glutathione peroxidases, deiodinases and thioredoxin reductases (Navarro Alarcon *et* Cabrera-Vique, 2008; Hatfield *et al.*, 2006). In high doses, selenium compounds can be toxic (Reilly, 2006).

Cadmium (Cd) is a toxic metal. It is a potent carcinogen in multiple types of tissues. It has been suggested that Cd toxicity involves the depletion of glutathione and sulfhydryl groups, resulting in the enhanced production of various reactive oxygen species (ROS) such as superoxide ion, hydrogen peroxide and hydroxyl radicals (Liu *et al.*, 2009).

Lead (Pb) is a toxic metal, which can induce various behavioral, biochemical, haematological and histological disorders, both in humans and animals (Ciobanu *et al.*, 2012). The most deleterious effects have been observed on the haemopoietic, nervous, reproductive and renal systems (Papanikolaou *et al.*, 2005). One of the main toxicodynamic mechanisms involved in Pb toxicity is the generation of reactive oxygen species (ROS) (Ercal *et al.*, 2001).

Amylase (α-amylase) is a glycoside hydrolase involved in carbohydrate digestion. Lactate dehydrogenase is an oxidoreductase with important roles in carbohydrate metabolism; in mammals it has five main isoforms. Alkaline phosphatase is a hydrolase involved in dephosphorylation (Murray *et al.*, 2003).

Selenium exerts protective effects against cadmium or lead toxicity (Reilly, 2006; Newairy *et al.*, 2007; Othman *et* Missiry, 1998). There is insufficient and/or inconclusive data regarding the effects of cadmium or lead and the concurrent administration of selenium on serum amylase, lactate dehydrogenase and alkaline phosphatase activities.

The objective of this study was to assess the effect of selenium

supplementation on serum amylase, lactate dehydrogenase and alkaline phosphatase activities during subacute exposure to toxic doses of cadmium or lead through the drinking water, using a rat experimental model.

MATERIALS AND METHODS

All experimental procedures were conducted according to the recommendations set up by 86/609/EEC directive and other applicable regulations. The study protocol was approved by The Ethics Committee for Research of "Grigore T. Popa" University of Medicine and Pharmacy of Iaşi, Romania.

A total of 36 male Wistar rats (initial body weight: 250-400 g) were randomly divided in six groups (n=6) and each group was kept in a single collective cage, maintained in relative constant environmental conditions (temperature 18 - 25^{o} C, light/dark cycle: 12 h/12 h) and had free access to water and dry feed.

Selenium (Se^{+4}), cadmium (Cd^{+2}) and lead (Pb^{+2}) were added in the drinking water by dissolving sodium selenite pentahydrate (Sigma Aldrich), lead acetate trihydrate (Chemical Company, Romania) and cadmium chloride hemipentahydrate (Chemical Company, Romania). The water dispensers were emptied and refilled everyday and the unconsumed volumes of water were measured.

The experimental groups were: Control, Se (Se^{+4}: 0,2 mg/l), Cd (Cd^{+2}: 150 mg/l), Pb (Pb^{+2}: 300 mg/l), Cd+Se (Cd^{+2}: 150 mg/l; Se^{+4}: 0,2 mg/l) and Pb+Se (Pb^{+2}: 300 mg/l; Se^{+4}: 0,2 mg/l).

The weight of the animals was measured every 7 days. The experiment lasted for 56 days. The mean intake of elements reported to body weight (*Table 1*) was approximated based on the daily water consumption of each group, the cumulative body weights and the concentration of the elements in the drinking water.

Table 1 - Estimation of the mean approximate intake of selenium, cadmium and lead throughout the experiment

Group	Se^{+4} (µg/kg b.w.)	Cd^{+2} (mg/kg b.w.)	Pb^{+2} (mg/kg b.w.)
Control	-	-	-
Se	15,48±0,42	-	-
Cd	-	10,48±0,41	-
Pb	-	-	25,34±0,68
Cd+Se	9,81±0,30	7,36±0,23	-
Pb+Se	15,90±0,43	-	23,84±0,64

Values are mean ± standard deviation

The animals were narcotized with 100 mg/kg b.w. of ketamine (injectable solution for veterinary use, administered intraperitoneally) and euthanized at the end of the experiment. Blood was collected in vacutainers without anticoagulant by cardiac puncture. Serum was obtained by centrifugation, after coagulation. Serum activities of amylase, alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were determined

using an Accent 200 biochemical analyzer (Cormay, Poland) and specific assay kits.

Microsoft Office Excel 2007 with Data Analysis add-on was used for statistical processing of experimental data. The results are expressed as mean ± standard error of the mean (SEM). A paired Student's t-test was used ($p < 0,05$ was considered significant).

RESULTS AND DISCUSSION

All three Se, Cd and Pb treatments produced an increase in serum amylase activity, the highest being observed in the Se group. Selenium supplementation of rats exposed to cadmium or lead resulted in a decrease in serum amylase activity, compared to Control group and, respectively, Cd or Pb (*Fig. 1*).

The amount of amylase from serum reflects the balance between the rates of amylase entry and its subsequent removal from the blood. The pancreas and the salivary glands usually account for almost all of the serum amylase, in normal conditions. Hyperamylasemia can be the result of an increased rate of entry into the blood or a decreased rate of clearance. Pancreatic lesions or inflammation can cause hyperamylasemia. A decreased rate of clearance, due to renal impairment, can also cause hyperamylasemia (Solcan *et al.*, 2005).

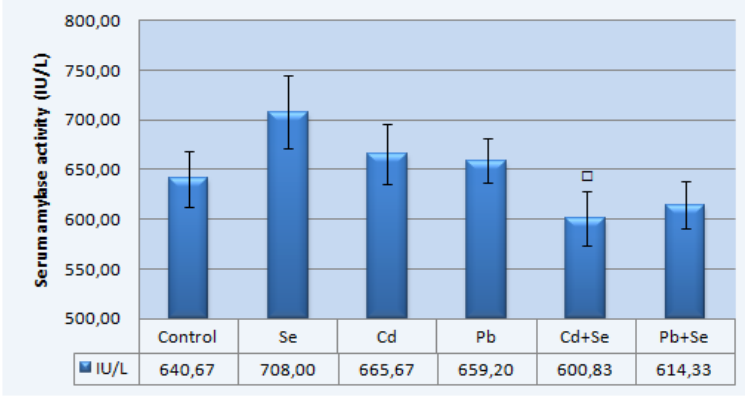

Figure 1 - Serum amylase activity. Error bars are SEM; ■ = significantly different compared to Control group ($p<0,05$); □ = significantly different compared to Se ($p<0,05$); ● = significantly different compared to Cd ($p<0,05$); ▲ = significantly different compared to Pb ($p<0,05$)

Pancreatitis has many ethiopathogenic causes. Intoxication with various organic or inorganic substances can be a cause (Khurana *et* Barkin, 2001; Reed *et* Gorelick, 2014). Hyperamylasemia has a low specificity in the diagnosis of pancreatitis, but is routinely used for this purpose, together with amylase and other markers (Yadav *et al.*, 2002).

Cadmium administered for a shorter period of time (1-5 days) and at lower doses (1 mg/kg b.w. subcutaneously) did not cause a marked change in serum amylase activity in mice (Shimada *et al.,* 2012). Cadmium stimulated *in vitro* release of amylase when added alone in the incubation medium of pancreatic lobules from guinea pig (Linari *et al.,* 2001). Cadmium administered through the drinking water (in sublethal doses, as $CdCl_2$) for 26 weeks to rats caused a slight increase in serum amylase (Lukacinova *et al.,* 2012).

Mayland *et al.* (1986) reported that concurrent administration of selenium and lead to sheep increased serum amylase activities, as compared to the administration of lead alone. Other parameters also indicated that the coadministration of lead and selenium was more toxic than lead.

In the present, study the degree of pancreatic, renal or other type of damage caused by Cd or Pb treatments was apparently not sufficiently intense enough to cause a marked increase in serum amylase activity. Selenium alone apparently was more harmful than cadmium or lead in this case. The increase in serum amylase activity for the Se, Cd and Pb groups was not significant.

Coadministration of selenium with cadmium or lead apparently reduced their toxicity and even decreased serum amylase activity below the mean value of the Control group. This decrease lacks statistical significance when referring to the Control group. The mechanisms of interaction between selenium and cadmium or lead which could explain this decrease are not known.

Both Se and Pb treatments caused an increase in LDH serum activity when compared to Control group, while Cd treatment apparently caused a decrease. Simultaneous intake of cadmium and selenium resulted in a high increase in LDH serum activity in comparison to Cd (*Fig. 2*).

LDH is involved in the terminal step of anaerobic glycolysis pathway, which occurs in the cell cytoplasm. LDH regulates the byproducts of mitochondrial oxidative or reductive stress conditions by maintaining lactic acid levels at normal values, converting it to pyruvate (Haffor and Alhazza, 2007).

LDH is used as a diagnostic marker for various heart and liver pathological conditions, haematological conditions or some types of tumours, although it has a low specificity in some cases (Huijgen *et al.,* 1997).

In vitro, addition of cadmium (as $CdCl_2$) in the culture medium of mouse hepatocytes significantly enhanced LDH and ALP leakage and increased reactive oxygen species (ROS) production (Pal *et al.,* 2011).

In other studies, cadmium caused an increase in plasma or serum LDH activity in rats (Abbes *et al.,* 2007; Borges *et al.,* 2008; Pari and Shagirtha, 2012) and mice (Brandao *et al.,* 2009). Coadministration of diphenyl diselenide (an

organoselenium compound) with cadmium significantly reduced plasma LDH activity, almost to control values (Borges *et al.,* 2008; Brandao *et al.,* 2009). It has been previously shown that lead cause an increase in serum or plasma LDH activity (Haffor and Alhazza, 2007; Waggas, 2012).

In the current study, LDH serum activity was abnormally lower in the Cd group, compared to Control group, but it was not significant statistically. This result does not correlate well with the results of other studies. It would have been expected that cadmium toxicity would cause an increase in serum LDH activity. One particular of this experiment is that the interindividual variance (expressed by SEM) of LDH activity in the Control group was very high. The cause of this variability was not identified. It is possible that the actual theoretical mean of the Control group should have been lower. Coadministration of selenium with cadmium was apparently more toxic than cadmium alone. Lead and selenium, coadministered with lead, apparently did not exert significant toxic effects on LDH activity.

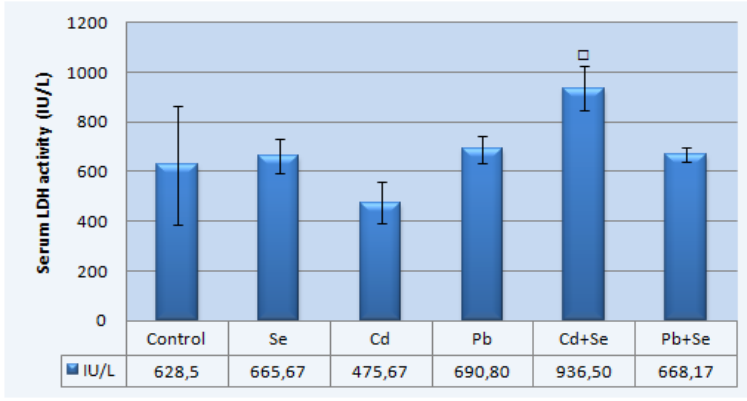

Figure 2 - Serum LDH activity. Error bars are SEM; ■ = significantly different compared to Control group (p<0,05); □ = significantly different compared to Se (p<0,05); ● = significantly different compared to Cd (p<0,05); ▲ = significantly different compared to Pb (p<0,05)

Cadmium caused a significant decrease in serum ALP activity, compared to Control group. Concomitant intake of selenium and cadmium did not result in a marked change in serum ALP activity, compared to Cd group. In the case of Pb and Pb+Se groups a slight, insignificant increase in serum ALP activity was observed when compared to Control group (*Fig. 3*).

Changes in serum ALP activity of diagnostic importance result from an increased entry of the enzyme into the circulation. This can be the result of an increased osteoblastic activity in

the case of bone disease or an increased synthesis of alkaline phosphatase by hepatocytes in the case of hepatobiliary disease (Solcan *et al.,* 2005). Other researchers showed that cadmium caused an increase in ALP serum activity when compared to controls (Wen *et al.,* 2002; Pari and Shagirtha, 2012). From this point of view, the results of the current study are contradictory; the exact cause was not identified.

Lead causes significant increase in serum ALP activities in comparison to non-treated animals (Shalan *et al.,* 2005; Mehana *et al.,* 2012). In this experiment, neither cadmium nor lead caused toxicity on ALP activity; selenium coadministration did not affect toxicity.

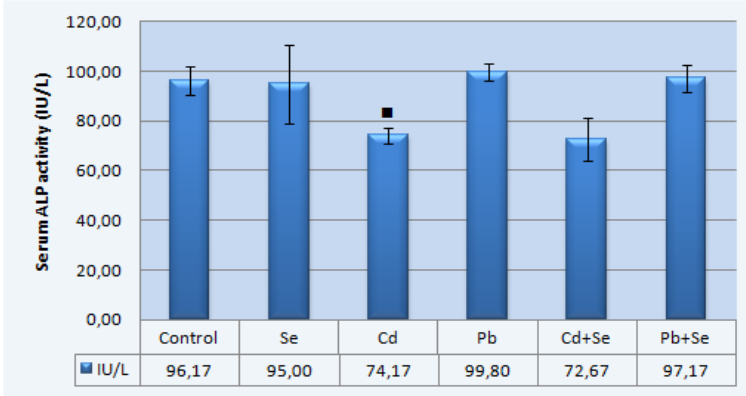

Figure 3 - Serum ALP activity. Error bars are SEM; ■ = significantly different compared to Control group (p<0,05); □ = significantly different compared to Se (p<0,05); ● = significantly different compared to Cd (p<0,05); ▲ = significantly different compared to Pb (p<0,05)

CONCLUSIONS

Selenium supplementation alleviated cadmium or lead induced changes in serum amylase activity. Selenium coadministered with cadmium caused a marked increase in serum LDH activity, when compared to cadmium alone or control while practically it had no effect on lead induced changes in LDH activity. Cadmium and lead induced disturbances in serum ALP activity were not influenced by selenium supplementation.

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