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THE EFFECT OF OLIVE OIL ON THE PLASMA TRANSAMINASE ACTIVITIES AND BLOOD HEMATOLOGICAL VALUES OF RATS ACUTELY EXPOSED TO CADMIUM

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ABSTRACT. Male *Wistar albino* 3 months old rats were acutely treated with cadmium (Cd, 0.4 mg/kg b.m., i.p., 24[h] before the sacrificing), with olive oil (0.4 mL/kg b.m., i.m., 48[h] before the sacrificing) and Cd+olive oil (in above mentioned amounts). The activities of alanin aminotransaminase (ALT) and aspartat aminotransaminase (AST) in the plasma, as well as hematological values: red blood cells (RBC) count, hematocrite (Ht) value, hemoglobin (Hb) and glucose concentrations were determined in the control and experimental groups of animals.

The activities of ALT and AST in the plasma were significantly increased ($p<0.005$) only in animals treated with cadmium, while in other experimental groups no differences in comparison to controls were found.

Our results show a significant alteration of hematological values. The RBC count ($p<0.005$), as well as Ht value and Hb concentration ($p<0.01$) were significantly decreased in respect to the control animals.

Blood glucose concentration was significantly increased ($p<0.005$) in rats treated with Cd when compared with control rats. However, pretreatment of rats with olive oil diminished the negative effects of cadmium and shows that olive oil prevents or mitigates anemia caused by cadmium.

INTRODUCTION

Cadmium (Cd) is a very toxic heavy metal, an important pollutant of environment (present in soil, water, air, food and in cigarette smoke) which causes poisoning in different organisms (1, 2, 3). Cadmium induces different toxic effects if it is present as a free or non-protein bound (4). After the intake and resorption, Cd enters the blood where it binds to the erythrocyte membranes and proteins of low molecular mass forming metalothioneins (5). Cadmium then transported into the most of tissues and organs in which it also induces the forming of metalothioneins (6, 7, 8, 9). From totally accumulated cadmium in the organism, about 75% is deposited in the liver and kidneys (10, 11, 12). However, Cd is accumulated in most of other tissues and organs, such as pancreas, salivary glands, testes, heart, brain or brown adipose tissue (13, 14, 15, 16, 17, 18).

Binding of cadmium to red blood cells (RBCs) causes their destruction and increased hemolysis, haematological values alterates (decrease of hematocrite value, hemoglobin concentration and total red blood cells count), absorption of intestinal iron is decreased and anemia appears (19, 20, 21). Above mentioned parameters can be taken as the sensitive indicators of cadmium toxicity.

Investigations on different organisms have shown that Cd causes significant metabolic and histological alterations, disturbs biological systems and decreases the body mass growth and mass growth of certain organs (15, 10, 16, 18). Cadmium has negative effects on energy metabolism (20, 22), membrane transport (23, 24) and protein synthesis (25, 10).

Fariss (26) has shown that the scavengers of free radicals and antioxidants may be used in the protection against Cd toxicity. Some antioxidants, such as vitamin E, ascorbic acid, glutathione, selenium and coenzyme Q_{10} exerts the protective effects against oxidative damages in different tissues of animals treated with cadmium (27, 28, 29, 30, 31).

Mediterranean food is rich in vegetables, cereals, fruits and oil (mostly olive oil), so that the problem of coronary and cardiovascular diseases and arteriosclerosis are diminished in the population of Mediterranean countries. On the basis of many literature data it is established that population of Mediterranean countries which use olive oil in the nutrition in comparison to the countries where the sunflower oil is used, suffer from sicknesses caused by reactive oxygen species (32). Olive oil is composed of polyphenol antioxidants and squalen which decreased production of free radicals and prevent some diseases, especially cardiovascular (33). High content of mono unsaturated fatty acids present in olive oil may also protect organism from the appearance of coronary diseases (34, 35). Olive oil contains lipid antioxidants (vitamin E and β-carotene) which inhibit the oxidation of low density lipoproteins and prevent chain reactions of lipid peroxidation (36).

In this work, possible protective effect of olive oil pretreatment on the plasma transaminase activities and some hematological values of rats acutely treated with cadmium were investigated.

MATERIALS AND METHODS

In our experiments male *Wistar albino* rats, 3 months old (weighing 280 ± 30 g) were used. The animals were kept at 21 ± 1°C and exposed to 12 h light - 12 h dark cycle. The animals were injected with $CdCl_2$ (0.4 mg Cd/kg b.m., i.p., 24^h before the sacrificing), with olive oil (0.4 mL/kg b.m., i.m., 48^h before the sacrificing) and with Cd + olive oil (0.4 mg Cd/kg b.m., i.p., 24^h before the sacrificing + 0.4 mL olive oil/kg b.m., i.m., 48^h). Control rats were drunk *ad libitum* by tap water. The exposed rats were oused in individual cages and given a standard diet and water *ad libitum*. Each experimental group consisted of six animals.

The animals were sacrificed by decapitation between 8 and 10 A.M. and fresh blood was immediately collected into heparinized test tubes. RBC count and Ht value were determined by standard hematological techniques (37). The Hb concentration was determined by the cyanmethaemoglobin method (38). The blood glucose concentration was assayed by the ortho-toluidine colorimetric method (39). The activities of ALT and AST in the plasma were determined by spectrophotometric method as suggested by Wooton et al., (40).

Statistical analysis of the results was based on the Student's paired t-test, considering the significance at a level of $p<0.05$ (41).

RESULTS AND DISSCUSION

Results depicted in Table 1 show a significant increase of the activities of ALT and AST in the plasma ($p<0.005$) of cadmium exposed rats in comparison with control animals. In other
Experimental groups (olive oil and Cd + olive oil), the plasma transaminase activities were similar to those of controls.

The results obtained in this work are in accordance with results of our previous investigations and point out the damage of the liver and disturbed carbohydrate and protein metabolism (42, 43, 44, 28). Similar results were obtained in our investigations on rats after chronic treatment with cadmium (21, 45). Some authors were also shown that cadmium increased the activities of ALT and AST in serum of rabbits (46) and in the plasma of rats (43, 47, 21, 45, 48). These enzymes have an important role in the processes of

aminoacid and protein metabolism. It is known that ALT and AST are widely spread in some tissues and in normal conditions they show very low activity in serum (plasma). However, stress and influence of different pollutants particularly in liver and heart caused the liberation of transaminases into circulation and increased their activities (49). Our results also show that olive oil pretreatment diminished the harmful effects of cadmium on the activities of transaminases in the plasma.

Table 1: The activities of alanin aminotransaminase (ALT) and aspartat aminotransaminase (AST) in the plasma of rats acutely treated with cadmium (Cd), with olive oil and concomitantly treated with Cd and olive oil in respect to the control animals (C).

PLASMA	ALT (U/mL)	AST (U/mL)
C	14.84 ± 0.34	66.31 ± 1.86
Cd	19.91 ± 0.27 ****	94.93 ± 1.78 ****
olive oil	14.20 ± 0.94	68.34 ± 3.88
Cd+olive oil	15.02 ± 0.90	69.83 ± 2.33

Means ± SEM from 6 animals in each group.
Significantly different from controls: ****$p<0.005$.

RBC count, Ht value, as well as Hb and glucose concentrations were presented in Table 2. Our results show that cadmium treatment induces a significant decrease of RBC count ($p<0.005$), as well as Ht value and Hb concentration ($p<0.01$) in respect to the control rats.

Previous investigations show that cadmium induces oxidative damage in erythrocytes, causing destruction of cell membrane and increase lipid peroxidation, as well as the alteration of the antioxidant defence system and energy metabolism and the appearance of anemia (50, 20, 51, 52).

Table 2: Hematological values: red blood cells (RBC) count, hematocrite (Ht) value, hemoglobin (Hb) and glucose concentration in the blood of rats acutely treated with cadmium (Cd), olive oil and concomitantly treated with cadmium and olive oil (Cd+olive oil) in comparison to the control animals (C).

	RBC (10^{12}/L)	Ht (L/L)	Hb (mmol/L)	Glucose (mmol/L)
C	7.91 ± 0.21	0.45 ± 0.06	8.24 ± 0.11	4.91 ± 0.13
Cd	5.11 ± 0.11 ****	0.41 ± 0.03 ***	7.56 ± 0.10 ***	6.41 ± 0.17 ****
olive oil	7.74 ± 0.15	0.46 ± 0.01	8.42 ± 0.06	5.21 ± 0.15
Cd+olive oil	7.02 ± 0.12 *	0.44 ± 0.01	8.15 ± 0.05	5.17 ± 0.26

Means ± SEM from 6 animals in each group.
Significantly different from controls: * $p<0.05$, ***$p<0.01$ and ****$p<0.005$.

Results obtained in this experiments have confirmed our previous investigations (20, 21) and the results of other authors (19, 50, 51). It is well known, that the presence of cadmium in organism decreases the level of iron in blood (20) and cause the decrease of Hb concentration. The decrease of Ht value in hemolyzed plasma of rats exposed to cadmium indicates the increased destruction of erythrocytes. These results are in accordance with the results of other investigations (19, 50, 20, 51, 48, 52). Cadmium caused the damage of the erythrocyte membrane resulting in hemolyzis in the same way like other metals such as Pb,

Cu and Zn (52). Cadmium induced anemia is characterized by pronounced reticulocytosis and hypochromia (20).

In rats exposed to olive oil hematological values were not significantly changed in comparison to the control values. In rats concomitantly treated with cadmium and olive oil the hematological values such as RBC, Ht and Hb were significantly increased in comparison to the values obtained in rats which received cadmium only. Our results suggest a very important role of pretreatment with olive oil before intoxication with cadmium. Olive oil decreased the toxic effects of cadmium on the hematological values and has the protective role in anemia.

The concentration of glucose was increased ($p<0.005$) in the blood of rats after acute exposure to cadmium, while in rats treated with olive oil and Cd+olive oil the glucose concentration was normalized and was similar to the control values (Table 2).

In animals treated with olive oil before administration of cadmium (pretreated animals) the concentration of glucose was similar to the control values, but it was significantly decreased in comparison to animals which received cadmium only. Pretreatment with olive oil prevents the increase of glucose concentration caused by cadmium.

CONCLUSIONS

Our results show that cadmium causes alterations of carbohydrate and protein metabolism demonstrated as hyperglycemia and increased activities of ALT and AST in the plasma.

Cadmium induced oxidative stress and has an important role in the pathogenesis of anemia and influences oxidative injuries in erythrocytes, their destruction and hemolysis resulting in decreases total red blood cell count, hematocrite value and hemoglobin concentration. These hematological parameters can be taken as sensitive indicators of cadmium toxicity.

It may be concluded, that olive oil exhibit some protective role in the prevention of toxic effects of cadmium on the plasma transaminase activities and some hematological values of rats acutely treated with cadmium.

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