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**EFFECT OF OLIVE OIL ON THE HAEMATOLOGICAL AND  
METABOLIC VALUES IN BLOOD AND PLASMA OF RATS AFTER  
ACUTE CADMIUM INTOXICATION**

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Adult male Wistar albino 3 months old rats were treated by  
acute administration of (1) cadmium (0.4 mg Cd/kg b.m., i.p., 24h  
before the sacrificing), (2) olive oil (0.4 ml olive oil/kg b.m., i.m., 48h  
before the sacrificing) and (3) olive oil + Cd (in above mentioned  
amounts). Haematological values (haematocrit - Htc, haemoglobin  
concentration - Hb and red blood cells count - RBC) and metabolic  
parameters (activity of transaminases - ALT and AST, as well as the  
concentration of glucose) were determined in control and experimental  
groups of animals.

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Our results show significant alterations of haematological values. Haematocrit values and haemoglobin concentrations were decreased ( $p < 0.01$ ) as well as the number of red blood cells ( $p < 0.005$ ) after acute, intraperitoneal (i.p.) administration of cadmium. Experiments with rats receiving olive oil in pretreatment show that olive oil prevents or mitigates anemia caused by cadmium.

The activities of enzymes ALT and AST in plasma were significantly increased only in animals treated with cadmium ( $p < 0.005$ ), where as in other experimental groups there were no differences in comparison to control values. Concentration of blood glucose was significantly increased in rats treated with cadmium ( $p < 0.005$ ) in comparison to control values. However, the pretreatment of rats with olive oil, the negative effects of cadmium were significantly diminished.

*Key words:* olive oil, cadmium, rat, blood

## 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 (SHUKLA and SINGHAL 1984; STOHS and BAGCHI, 1995). After the intake and resorption, cadmium enters the blood where it binds to erythrocytes and proteins of low molecular mass forming metallothioneins (MT) (THORNALLEY and VAŠAK, 1985). Cadmium is then transported into most of tissues and organs in which it also induces the forming of metallothioneins (VILJOEN and TAPPEL, 1988; WORMSER and BEN ZAKINE, 1990). From totally accumulated cadmium in organism, about 75% is deposited in liver and kidneys (OGNJANOVIĆ *et al.*, 1995; ŠTAJN *et al.*, 1997). However, cadmium is accumulated in most of other tissues and organs, such as pancreas, salivary glands, testes, heart, brain or brown adipose tissue (Kostić *et al.*, 1993a; FRIEDMAN and GASEK, 1994; ŠTAJN *et al.*, 1995; SWIERGOSZ *et al.*, 1998; ŽIKIĆ *et al.*, 1998).

Binding of cadmium to erythrocytes causes their destruction and increased hemolysis, haematological values alterates (decrease of haematocrite values, haemoglobin concentration and total red blood cells count), absorption of intestinal iron is decreased and anemia appears (FOX and FRY, 1970; PRIGGE *et al.*, 1977; KOSTIĆ *et al.*, 1993b). Above mentioned parameters can be taken as the sensitive indicators of cadmium toxicity.

Investigations on different organisms have shown that cadmium causes significant metabolic and histological alterations, disturbs biological systems and decreases body mass growth and mass growth of certain organs (OGNJANOVIĆ *et al.*, 1995; ŠTAJN *et al.*, 1995). Cadmium has negative effects in energy metabolism (KOSTIĆ *et al.*, 1993b; PETRONIJEVIĆ *et al.*, 1995), membrane transport (GRABOWSKA and GUMINSKA, 1987) and protein synthesis (DUDLEY *et al.*, 1984; OGNJANOVIĆ *et al.*, 1995).

FARISS (1991) has shown that the scavengers of free radicals and antioxidants may be used in the protection against cadmium toxicity. Some antioxidants, such as vitamin E (Vit E), ascorbic acid (AsA), glutathion (GSH) and selenium (Se) exert protective effects against oxidative damages in different tissues (SHUKLA and CHANDRA, 1989; BEYER, 1994; NIKI *et al.*, 1995).

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 nutrition in comparison to the countries where the sunflower oil is used, rarely suffer from sicknesses caused by reactive oxygen species. Olive oil is composed of polyphenol oxidants and squalen which decrease induced production of free radicals and prevent some diseases (cardiovascular). High content of monounsaturated fatty acids present in olive oil may also protect organism from the appearance of coronary diseases (MANCINI *et al.*, 1995; MANNA *et al.*, 1997). Olive oil contains lipid antioxidants (Vit E and  $\beta$ -carotene) which inhibit the oxidation of lipoproteins of low density and prevent chain reactions of lipid peroxidation (GERBER, 1997).

In this work, possible protective effect of olive oil pretreatment on haematological and metabolic values in rats after acute intoxication with cadmium was investigated.

#### MATERIALS AND METHODS

Wistar albino male, 3 months old rats (weighing  $280 \pm 30$ g) were used in experiment. The animals were kept at  $21 \pm 2^\circ\text{C}$  and exposed to 12 h light/dark cycle. The animals were injected with (1)  $\text{CdCl}_2$  (0.4mg Cd/kg b.m., i.p., 24<sup>h</sup> before the sacrificing), (2) olive oil (0.4ml/kg b.m., i.m., 48<sup>h</sup> before the sacrificing) and (3) olive oil + Cd (0.4ml o.oil/kg b.m., i.m., 48<sup>h</sup> + 0.4mg Cd/kg b.m., i.p., 24<sup>h</sup> before the sacrificing). Control rats were drunk ad libitum by tap water. The exposed rats were housed in individual cages and given a standard diet and water ad libitum. Each experimental group consisted of six animals.

After the last treatment the animals were sacrificed by decapitation between 8<sup>h</sup> and 10<sup>h</sup> and fresh blood was immediately collected in to heparinized tubes. Number of red blood cells (RBC) and haematocrit (Hct) value were determined by standard haematological techniques (CHANARIN, 1989). The haemoglobin (Hb) concentration was determined by the cyanmethaemoglobin method (DRABKIN and AUSTIN, 1935). The blood glucose concentration was measured by the ortho-toluidine colorimetric method (HULTMANN, 1959). Aliquots of blood for the determination of transaminase were centrifuged to separate plasma and red blood cells. Plasma specimens were used for determination of activities of ALT and AST by spectrophotometry method (WOOTON *et al.*, 1964).

Data are given as mean  $\pm$ SEM. Statistical analysis of the result was based on the Student's paired t-test, considering the significance at a level of  $p < 0.05$  (HOEL, 1966).

## RESULTS

Results depicted in table 1 show that intraperitoneal administration of cadmium induces significant decrease of haematocrite values and haemoglobin concentrations ( $p < 0.01$ ) as well as the number of red blood cells ( $p < 0.005$ ). In rats exposed to olive oil the haematological values were not changed significantly in comparison to control values. In rat pretreated with olive oil before injection of cadmium (olive oil + Cd), haematological values were significantly increased in comparison to values of animals receiving cadmium only.

Table 1: Haematological values (haematocrit values - Hct, haemoglobin concentration - Hb and red blood cells count - RBC) in the blood of rats after exposure to cadmium (Cd), olive oil and olive oil + Cd compared with the controls

	Hct ( <i>l/l</i> )	Hb ( <i>mmol/l</i> )	RBC ( $10^{12}/l$ )
Controls	0.45 $\pm$ 0.06	8.24 $\pm$ 0.11	7.91 $\pm$ 0.21
Cd	0.41 $\pm$ 0.03 ***	7.56 $\pm$ 0.10 ***	5.11 $\pm$ 0.11 ****
Olive oil	0.46 $\pm$ 0.01	8.42 $\pm$ 0.06	7.74 $\pm$ 0.15
Olive oil + Cd	0.44 $\pm$ 0.01	8.15 $\pm$ 0.05	7.02 $\pm$ 0.12 *

Means  $\pm$ SEM from 6 animals in each group.

Significantly different from controls: \*  $p < 0.05$ , \*\*\* $p < 0.01$  and \*\*\*\* $p < 0.005$

The concentration of glucose was increased in blood of rats after i.p. administration of cadmium ( $p < 0.005$ ), whereas in rats treated with olive oil and olive oil + Cd the concentration was normalized and was similar to control values (Table 2). In rats receiving olive oil + Cd the concentration of glucose was significantly decreased in comparison to the values in cadmium treated rats ( $p < 0.01$ ).

Table 2: Metabolic values (concentration glucose in the blood, and the activities of alanin amino -transaminase - ALT and aspartat aminotransaminase - AST in the plasma) of rats after exposure to cadmium (Cd), olive oil and olive oil + Cd compared with the controls.

	Glucose ( <i>mmol/l</i> )	ALT ( <i>U/ml</i> )	AST ( <i>U/ml</i> )
Controls	4.91 $\pm$ 0.13	14.84 $\pm$ 0.34	66.31 $\pm$ 1.86
Cd	6.41 $\pm$ 0.17 ****	19.91 $\pm$ 0.27 ****	94.93 $\pm$ 1.78 ****
Olive oil	5.21 $\pm$ 0.15	14.20 $\pm$ 0.94	68.34 $\pm$ 3.88
Olive oil + Cd	5.17 $\pm$ 0.26	15.02 $\pm$ 0.90	69.83 $\pm$ 2.33 *

Means  $\pm$ SEM from 6 animals in each group.

Significantly different from controls: \*  $p < 0.05$  and \*\*\*\* $p < 0.005$

Significant increase of the activity of transaminases (ALT and AST) in the plasma ( $p < 0.005$ ) of cadmium exposed rats was all shown in our experiments (Table 2). In rats receiving only olive oil and in rats pretreated with olive oil (olive oil + Cd), the activities of ALT and AST in plasma were similar to control values, but were significantly decreased in comparison to animals which receiving cadmium only.

#### DISCUSSION

Previous investigations show that cadmium induces oxidative damages in erythrocytes, causing their destruction, damage of cell membrane and increase of lipid peroxidation, as well as the alteration of the system of antioxidative defence and energy metabolism and the appearance of anemia (KUNIMOTO *et al.*, 1986; KOSTIĆ *et al.*, 1993b; SARKAR *et al.*, 1997; HAMADA *et al.*, 1998).

Results obtained in this work have confirmed our previous investigations (KOSTIĆ *et al.*, 1993b) and the literature data as well (FOX and FRY, 1970; PRIGGE *et al.*, 1977; KUNIMOTO *et al.*, 1986; SARKAR *et al.*, 1997). It was shown in this experiment that acute i.p. administration of cadmium causes decrease of RBC count ( $p < 0.005$ ), haematocrite values and haemoglobin concentrations ( $p < 0.01$ ) and appearance of anemia (Table 1). It is well known that the presence of cadmium in organism decreases the level of iron in blood (KOSTIĆ *et al.*, 1993b), which may cause the decrease of haemoglobin concentration. The decrease of haematocrite value in haemolysed plasma of rats exposed to cadmium indicates the increased destruction of erythrocytes. These results are in accordance with the results of other investigations (PRIGGE *et al.*, 1977; KUNIMOTO *et al.*, 1986; KOSTIĆ *et al.*, 1993b; SARKAR *et al.*, 1997; ŽIKIĆ *et al.*, 1997; HAMADA *et al.*, 1998), which show that cadmium damages the membrane of erythrocytes and leads to haemolysis in the same way like other metals such as Pb, Cu and Zn (HAMADA *et al.*, 1998). Anemia, which appears in cadmium - treated rats is characterized by pronounced reticulocytosis and hypochromia (KOSTIĆ *et al.*, 1993b).

In rats exposed to olive oil the haematological values were not significantly changed in comparison to control values. In rats which received olive oil before cadmium (olive oil + Cd), the haematological values (Htc, Hb, RBC) were significantly increased in comparison to the values 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 haematological values and has the protective role in anemia.

The results obtained in this work (Table 2) show that the concentration of blood glucose was significantly increased ( $p < 0.005$ ) after acute administration of cadmium. The increased activity of transaminase enzymes (ALT, AST) in plasma was also observed ( $p < 0.005$ ). These results are in accordance with results obtained in previous investigations and point to the damage of liver and disturbed carbohydrate and protein metabolism (CHAPATWALA *et al.*, 1980; RAJANNA *et al.*, 1984). Similar results were obtained in our previous investigations in rats after

chronic treatment with cadmium (ŠTAJN *et al.*, 1993), where it was shown that cadmium caused hyperglycemia and proteinemia.

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

In previous investigations it was also shown that cadmium increased the activity of transaminase enzymes (ALT, AST) in serum of rabbits (PISCATOR and AXELSSON, 1970) and in plasma of rats (RAJANNA *et al.*, 1984; ŠTAJN *et al.*, 1993). These enzymes have an important role in the processes of aminoacid and protein metabolism. It is known that these enzymes are widely spread in tissues, and that in normal conditions they show very low activity in serum (plasma). However, in stress condition and also due to the influence of different pollutants the damages of tissues occur, particularly in liver and heart, causing the liberation of transaminases into circulation, increasing their concentration and activity (HENRY ET AL., 1974). The activities of ALT and AST in rats receiving olive oil and olive oil + Cd were not changed significantly in comparison to control values, whereas they were significantly decreased in comparison to values in rats which received cadmium only. Our results show that olive oil pretreatment diminished the harmful effects of cadmium on the activity of these enzymes.

#### CONCLUSION

Our results suggest that cadmium induced oxidative stress has an important role in the pathogenesis of anemia. Cadmium induces oxidative injuries in erythrocytes, their destruction and haemolysis, decreases haematocrite values, haemoglobin concentration and total red blood cell count. These parameters can be taken as sensitive indicators of cadmium toxicity. Cadmium causes alterations in carbohydrate and protein metabolism demonstrated as hyperglycemia and increased activity of transaminases in plasma. Possible protective role of olive oil pretreatment (and the role of antioxidants present in oil) in the prevention of toxic effects of cadmium on the haematological and metabolic values in blood and plasma of rats was pointed out in this work.

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EFEKT MASLINOVOG ULJA NA HEMATOLOŠKE I METABOLIČKE  
PARAMETRE U KRVI I PLAZMI PACOVA POSLE AKUTNE  
INTOKSIKACIJE KADMIJUMOM

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Mušjaci pacova *Wistar albino*, stari tri meseca, akutno su tretirani (1) kadmijumom (0.4 mg Cd/kg t.m., i.p., 24<sup>h</sup> pre žrtvovanja), (2) maslinovim uljem (0.4 ml maslinovog ulja/kg t.m., i.m., 48<sup>h</sup> pre žrtvovanja) i (3) maslinovim uljem + Cd (u navedenim količinama). Kod kontrolnih, kao i kod eksperimentalnih grupa životinja, određivane su hematološke vrednosti (hematokritska vrednost - Hct, koncentracija hemoglobina - Hb i broj eritrocita - RBC), kao i metabolički parametri (aktivnost alanin aminotransaminaze - ALT i aspartat aminotransaminaze - AST u plazmi i koncentracija glukoze u krvi).

Naši rezultati pokazuju značajne promene hematoloških vrednosti. U odnosu na kontrolne vrednosti, značajno je smanjena hematokritska vrednost i koncentracija hemoglobina ( $p < 0.01$ ), kao i broja eritrocita ( $p < 0.005$ ) kod pacova posle akutnog, intraperitonealnog (i.p.), davanja kadmijuma. Eksperimenti sa pacovima koji su u predtretmanu dobijali maslinovo ulje, pokazuju da maslinovo ulje sprečava ili ublažava kadmijumom - izazvanu anemiju.

Aktivnosti enzima ALT i AST u plazmi su značajno povećane samo kod životinja tretiranih kadmijumom ( $p < 0.005$ ), dok kod ostalih eksperimentalnih grupa nema razlike u poređenju sa kontrolnim vrednostima. Koncentracija glukoze u krvi je značajno povećana ( $p < 0.005$ ) kod pacova tretiranih kadmijumom u poređenju sa vrednostima kontrolnih životinja. Međutim, predtretman pacova maslinovim uljem značajno je umanjio negativne efekte kadmijuma.

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