THE EFFECT OF SELENIUM ON ANTIOXIDANT DEFENSE SYSTEM IN THE BLOOD OF RATS CHRONICALLY TREATED WITH CADMIUM

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ABSTRACT. The effect of selenium (Se, 7 µg/kg/day, during 30 days) on the antioxidant defense system (AOS): copper zinc containing superoxide dismutase (CuZn SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities in red blood cells (RBC) and glutathione-S-transferase (GST) activity in the plasma, as well as ascorbic acid (AsA) and vitamin E (Vit E) concentrations in the plasma of rats chronically treated with cadmium (Cd, 15 mg/kg/day, during 30 days) was analyzed. All results of experimental groups were compared with control animals. Cd led to increased the activities of CuZn SOD, CAT and GSH-Px, as well as GST activity and Vit E concentration. Se induced a significant increase in GSH-Px and GST activities, as well as concentrations of AsA and Vit E. In animals exposed both to Cd and Se a significant increase of GSH-Px activity was observed. The accumulation of Cd was significantly increased in the plasma of Cd+Se treated and in RBC of Cd treated animals, while it was markedly lower in RBC of Cd+Se treated rats.

The obtained results show, that Cd exhibit toxic effects on the antioxidant defense system in the blood of rats and that Se demonstrates protective role against Cd toxicity.

INTRODUCTION

Cadmium (Cd) is an important environmental pollutant and is mainly ingested by humans through foods [1]. After being intake into the organism Cd enters the blood and binds to plasma albumins and erythrocyte membranes [2] and then it transferred into tissues and organs where it binds to proteins of low molecular mass-producing metallothioneins [3]. Cadmium affects various metabolic processes, such as energy metabolism [4], membrane transport [5] and protein synthesis [6]. Recent studies have shown that Cd stimulates formation of reactive oxygen species, including superoxide anion radicals [7], hydrogen peroxide [8] and hydroxyl radicals [9]. As a consequence, enhanced lipid peroxidation, DNA damage and altered calcium and sulfhydryl homeostasis were noted. A variety of accompanying changes in antioxidant defense system were reported [10]. A key aspect of the

metabolism of Cd is the low-molecular weight metal-binding protein metallothionein, which is synthesized in response to Cd exposure [11]. The chronic toxic effects most clearly associated with Cd exposure occur in the pulmonary system and in the kidneys. Secondarily, chronic Cd exposure has been associated with skeletal system toxicity, hypertension, cardiovascular diseases and carcinogenesis in various tissues [2]. As a result of chronic Cd exposure ″Itai-itai″ disease occur and the disease is characterized mainly by osteomalacia, renal tubular disorder and anaemia which is normocytic, normochromic and nephrogenous [12]. Approximately 95% of ingested Cd will be eliminated through the feces [2].

Selenium (Se) is vital trace element in diet of animals, but in higher concentrations it becomes toxic and even lethal [13]. Se taken in the form of selenite, selenate, selenocysteine and selenomethionine gets the most absorbed in the duodenum [14]. After absorption, increased levels of Se have been recorded in the blood plasma proteins and from there it can be distributed into the tissues where it is incorporated in newly synthesized seleno-proteins [15]. A marked uptake of selenium by erythrocytes were also found [16].

Deficiency of Se causes a variety of pathologies in different mammalian species. Children with a low selenium status develop a cardiomyopathy (Keshan disease) that can be prevented by Se supplementation [17]. In low Se areas of China, adults suffer from an osteoarthropathy (Keshin-Beck disease) characterized by chronic inflammation of the joints, degradation of cartilage and impairment of function [17]. Selenium deficiency also influences neuronal ceroid lipofuscinosis and endemic myxoedematosis cretenism [18]. A possible molecular basis of these Se-responsive diseases is the action of, at least, three seleno-enzymes: ″classical″ (selenium-dependent) glutathione peroxidase (Se GSH-Px), phospholipide hydroperoxide glutathione peroxidase (PH GSH-Px) and thyroxine-5′-deiodinase (T4 5′ D) [19].

It is also known, that Se has a some protective role from the toxic actions of Cd and other heavy metals [20-24]. This protection includes the capability of Se to alter the distribution of Cd in tissues and induces binding of the Cd-Se complexes to proteins, which are similar to metallothioneins [25].

The aim of this study was to evaluate the blood antioxidant defense system in rats chronically treated with Cd, Se and Cd+Se simultaneously. After 30 days of exposure the activities of copper zinc containing superoxide dismutase (CuZn SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6) and glutathione peroxidase (GSH-Px, EC 1.11.1.9) in red blood cells (RBC) and glutathione-S-transferase (GST, EC 2.5.1.18) in the plasma of rats were estimated. Nonenzymatic components of antioxidant defense system, i.e. ascorbic acid (AsA) and vitamin E (Vit E) concentrations in the plasma were also determined, as well as Cd concentration in the plasma and RBC.

MATERIALS AND METHODS

The experiments were carried out with 60 days old male, *Wistar albino* rats weighing 200 ± 10 g at the beginning of experiments. The animals were haused in individual cages in a temperature-controlled environment ($21 \pm 1°C$) and exposed to 12 h light - 12 h dark cycle. The rats were allowed to take food *ad libitum* (chow pellets, Veterinarski zavod, Zemun; 100 μg Se/kg diet, according to declaration). Control rats were drank tap water *ad libitum*. The animals were divided into four groups. The first group of animals was control (C). The second group of rats was treated with cadmium (Cd) and drunk for 30 days by water containing 200 mg $CdCl_2$ x H_2O/L. The third group of animals exposed to selenium (Se) were drank water containing 100 μg Na_2SeO_3/L during 30 days. The last experimental group of animals (Cd+Se cotreated rats), drunk for 30 days water containing 200 mg $CdCl_2$ x H_2O + 100 μg Na_2SeO_3/L. After the treatment an average intake of 15 mg Cd/kg/day was calculated. Since only 6% of perorally taken cadmium may be absorbed by gastrointestinal tract [26], it appears that the rats were actually treated by approximately 1 mg Cd/kg/day, which is significantly less than the LD_{50} value estimated to be 2.2 (1.9-2.6) mg Cd/day [27]. An average intake of 7 μg Se/kg/day was calculated from the water consumed.

After 30 days of treatment the animals were decapitated always between 8 and 10 A.M. to avoid any possible rhythmic variations in the antioxidant level. Fresh blood was immediately collected using heparin (1000 U/mL) as anticoagulant. Aliquots of blood were taken immediately after exanguination and centrifuged for the separation of plasma and blood cells. RBC was washed three times with 3 vol. of cold 155 mmol/L NaCl. Haemolysates containing about 50 g Hb/L were prepared according to McCord and Fridovich [28] and used for the determination of CAT and GSH-Px activities. Measurement of CuZn SOD activity was conducted in the haemolysates of washed RBC, in which Hb was previously removed by the method of Tsuchihashi [29]. CuZn SOD activity in RBC was measured by the epinephrine method [30] based on the capacity of SOD to inhibit autooxidation of adrenaline to adrenochrome. CAT activity in RBC was assayed as suggested by Beutler [31]. The activity of GSH-Px in RBC was evaluated by the method of Maral et al. [32]. For the determination of GST activity in the plasma 1-chloro-2,4-dinitrobenzene (CDNB) was used as a substrate [33]. The AsA concentration in the plasma was measured by the method of Day et al. [34] using 2,4,6-tripyridyl-S-triazine (TPTZ). The concentration of Vit E was evaluated by the method of Desai [35] based on the reduction of Fe^{3+} to Fe^{2+} in the presence of tocopherol and production of colored complex with bathophenantroline. The concentration of Cd in the plasma and RBC was determined by atomic absorption spectrophotometry after digestion with concentrate nitric (17 vol) and perchloric (3 vol) acid in the mixture of oxygen and acetylene [36].

All obtained data were statistically analyzed and expressed as mean $\pm$ SE and differences between experimental and control group were estimated by Student′s *t*-test [37]. The value of $p<0.05$ was taken as the least degree of significance.

RESULTS AND DISCUSSION

The results obtained in our experiments (Figure 1) clearly show that Cd induced a significant increase of CuZn SOD activity in RBC of rats ($p<0.05$). As a consequence of increased superoxide anion radical production induced by elevated CuZn SOD activity, we also obtained a significantly increased activity of CAT in RBC of animals treated with cadmium ($p<0.05$).

Figure 1. The activities of copper zinc containing superoxide dismutase (CuZn SOD, U/g Hb) and catalase (CAT, U/g Hb) in red blood cells (RBC) of control animals (C), treated with cadmium (Cd, 15 mg/kg/day), treated with selenium (Se, 7 µg/kg/day) and animals concomitantly treated with cadmium and selenium (Cd+Se, 15 mg Cd/kg/day + 7 µg Se/kg/day). The values are means ± SE from seven animals.
Significantly different from control (C): *$p<0.05$.
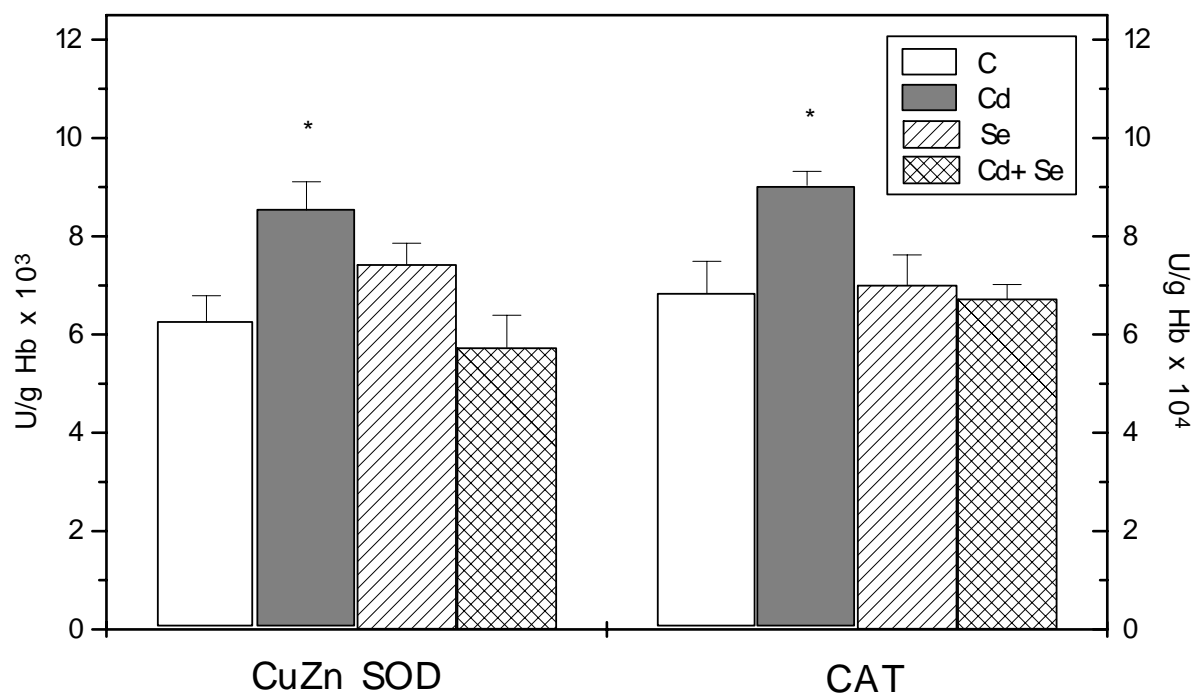
It is well established that Cd lead to increased production of superoxide anion radicals [38, 39] and it is reasonable to expect as a biological response an increased activity of CuZn SOD in RBC of Cd treated animals. Similar results were also obtained for CAT and GSH-Px activities in RBC (Figure 1 and Figure 2). As shown in Figure 2 Cd induced a significant increase of GSH-Px activity in RBC, as well in GST activity in the plasma ($p<0.05$). It is well known that the activity of CAT to be directly proportional to the substrate level, assumed to be increasingly produced by SOD [40]. Similar reasons lead to increased activity of GSH-Px in RBC of rats treated with Cd. Our results also show an increased activity of GST in the plasma of Cd treated animals. The results of other authors also demonstrate that Cd induced elevation of GST activity in the plasma [39], as well as in other tissues

and organs of experimental animals, such as liver and kidneys [22, 23, 41]. GST has a key role in the reaction of conjugation of reduced glutathione with heavy metals and in the reaction of detoxification of lipid peroxides [42], and thus for the protection of integrity and functioning of cells and tissues. Increased activity of GST plays an important role in the prevention of oxidative stress in the plasma of rats caused by cadmium.

Figure 2. The activities of glutathione peroxidase (GSH-Px, nmol NADPH/min/g Hb) in red blood cells (RBC) and glutathione-S-transferase (GST, nmol GSH/min/mL) in the plasma of control animals (C), treated with cadmium (Cd, 15 mg/kg/day), treated with selenium (Se, 7 μg/kg/day) and animals concomitantly treated with cadmium and selenium (Cd+Se, 15 mg Cd/kg/day + 7 μg Se/kg/day). The values are means ± SE from seven animals.
Significantly different from controls (C): *$p < 0.05$; **$p < 0.02$; ***$p < 0.01$.
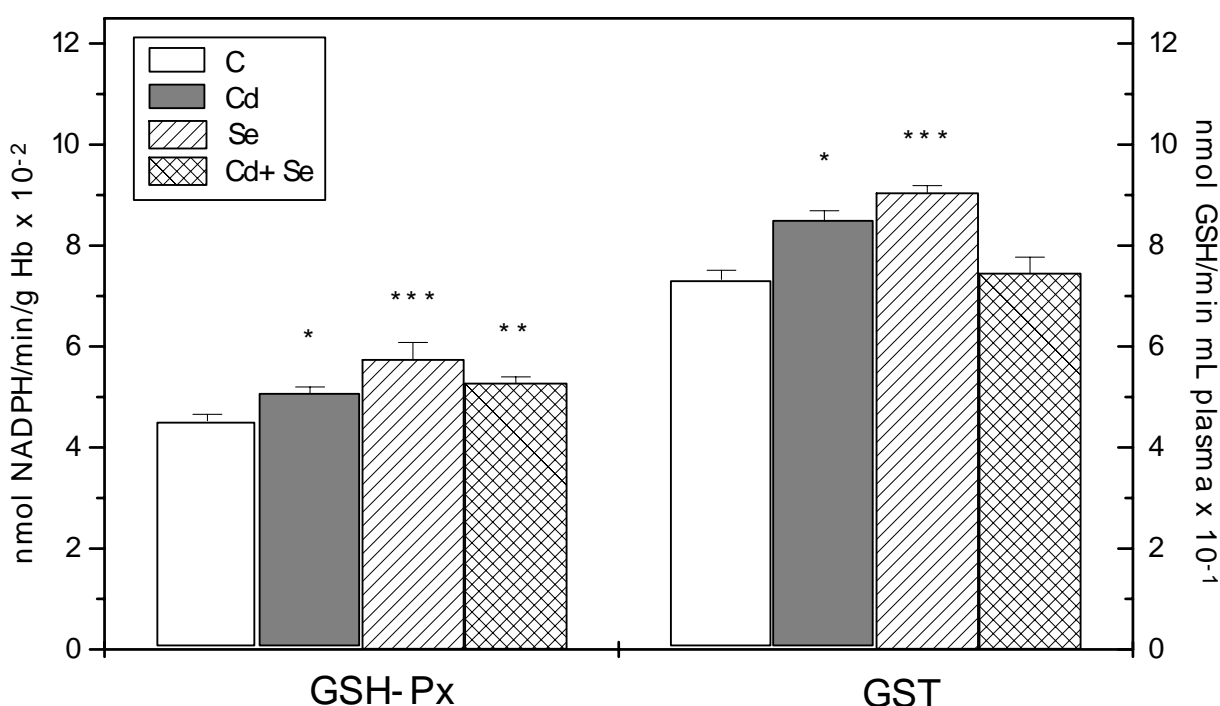

No changes of AsA concentration in the plasma of rats treated with Cd ws detected (Figure 3). The concentration of Vit E in the plasma was significantly higher ($p < 0.05$) in animals exposed to this heavy metal. Our results (Figure 3) show that Cd induced a significant increase of Vit E concentration in the plasma, but not in the plasma AsA concentration. These results are in accordance with the findings considering the influence of Cd on Vit E concentration in the plasma and in other tissues and organs of rats [23, 43]. The increased concentration of Vit E in the plasma could be explained by its protective role against the toxic influence of Cd on the erythrocyte membranes. In previous investigations of other authors [44] it was shown that plasma Vit E was in dynamic equilibrium with Vit E in erythrocyte membranes, maintaining thus the stability and permeability of these membranes. Increased concentration of Vit E in the plasma is accompanied with increased activity of plasma GST and together they act efficiently in the prevention of oxidative stress induced by Cd.

In our experiments it is also found that perorally administered Se markedly increased the activities of GSH-Px in RBC and GST in the plasma of rats. These results are similar to the results of our previous investigation on the heart of rats treated with Se [24]. Highly significant correlation between GSH-Px activity and Se concentration have been found in the liver, lung, myocard, muscles and erythrocytes of the rats. This is in accordance with the fact that Se is the constituent of this enzyme molecule [45]. Investigations with Se deficiency have been shown marked depletion of GSH-Px activity in the liver of experimental animals [46]. At the same time an inverse correlation between GSH-Px activity and the concentration of lipide peroxides have been reported [47]. Under our experimental conditions rats actually received 100 µg Se in the basal diet and 100 µg Se in drinking water. These amounts are sufficient to satisfy requirements of RBC GSH-Px, achieved a plateau at 180 µg of dietary Se [48]. Increased activities of RBC GSH-Px, as well as plasma GST has a protective role in the blood and increase biological defense against free radical mediated injuries.

Figure 3. The concentrations of ascorbic acid (AsA, mg%) and vitamin E (Vit E, µg/mL) in the plasma of control animals (C), treated with cadmium (Cd, 15 mg/kg/day), treated with selenium (Se, 7 µg/kg/day) and animals concomitantly treated with cadmium and selenium (Cd+Se, 15 mg Cd/kg/day + 7 µg Se/kg/day). The values are means ± SE from seven animals.
Significantly different from controls (C): *$p < 0.05$; **$p < 0.02$.
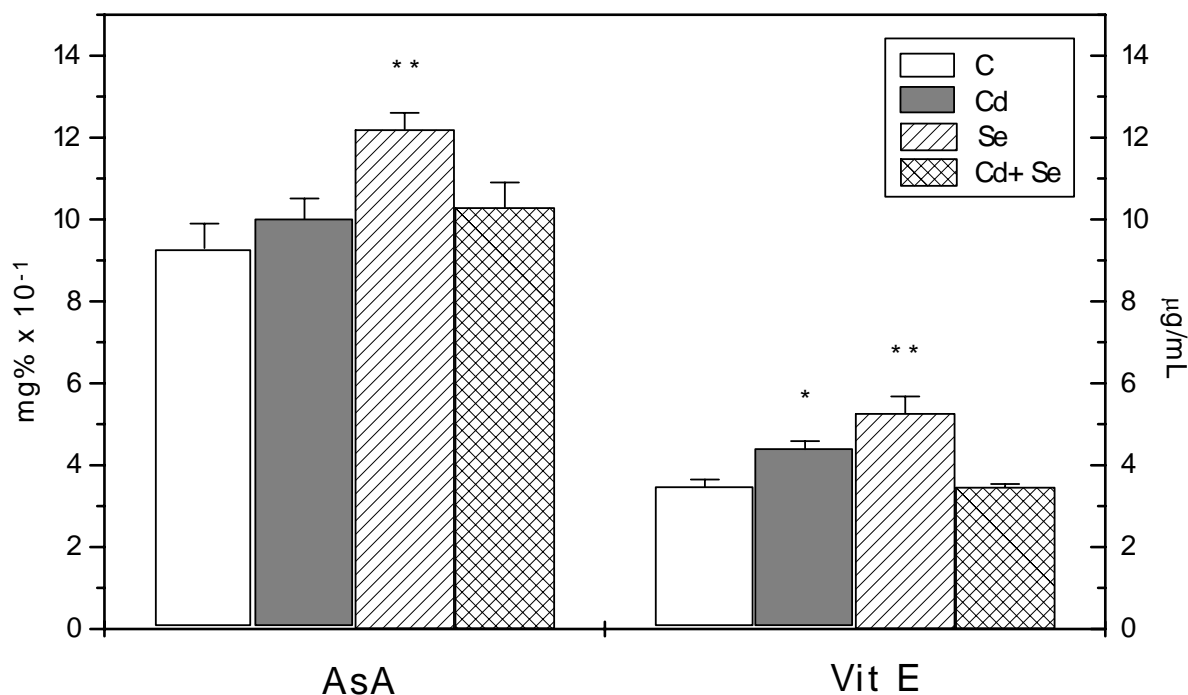
Results presented in Figure 2 show that Se supplementation induced a significant elevation of RBC GSH-Px activity and in plasma GST activity ($p < 0.01$). At the same time Se expressed no significant effect in CuZn SOD and CAT activities in RBC of animals. Treatment with Se also induced a significant increase in AsA and Vit E concentrations ($p < 0.02$) in the plasma (Figure 3).

On the basis of previous investigations [22, 23, 43, 49] it is known that Se decreases the utilization of Vit E in the gastrointestinal tract, as well as increases the GSH-Px activity. Therefore, increased concentrations of Vit E are retained [50]. Beside the synergism between AsA and Vit E an increased concentration of AsA in the plasma was obtained [51].

As mentioned above, Cd induced a significant increase in CuZn SOD and CAT activities in RBC of animals (Figure 1). In animals exposed to Cd+Se simultaneously, CuZn SOD and CAT activities in RBC were not significantly altered. These results clearly show that Se completely eliminated the effects of Cd on these enzyme activities in RBC of rats. The effects of Se are thought to be related to either the formation Cd-Se complexes in association with metallothioneins, or changes in Cd distribution [23]. Some authors proposed that Se prevent Cd toxicity through a mechanism that does not involve induction of metallothionein synthesis [52]. Sasamura and Suzuki [53] suggested that in the blood stream transition metals and Se compounds form a metal-Se/S complex which then bound to a plasma protein named selenoprotein P (Sel P) to form a ternary complex (metal-Se/S)-Sel P. The molar rations of Cd to Se in this complex was 1:1. Another reason for the protective role of Se against Cd toxicity is the influence of Se on the redistribution of Cd from a protein of lower molecular mass into a protein of higher molecular mass [54]. At the same time RBC GSH-Px activity in Cd+Se cotreated animals (Figure 2) was statistically significant increased ($p < 0.02$), while plasma GST activity was normalized and reached control values. No changes in the plasma AsA and Vit E concentrations in animals treated both with Cd+Se were observed.

Table 1. The concentration of cadmium in the plasma (Cd, ng/mL plasma) and red blood cells (RBC, ng/mL cells) of control animals (C), treated with Cd (15 mg/kg/day), treated with selenium (Se, 7 μg/kg/day) and animals concomitantly treated with cadmium and selenium (Cd+Se, 15 mg Cd/kg/day + 7 μg/kg/day). The values are means ± SE from seven animals.
Significantly different from controls (C): *$p < 0.05$; **$p < 0.02$.

	Plasma (ng Cd/mL plasma)	RBC (ng Cd/mL cells)
C	680 ± 40	2800 ± 120
Cd	650 ± 35	7720 ± 232 **
Se	770 ± 38	2760 ± 136
Cd+Se	1190 ± 41 **	1560 ± 126 *

In addition to the results considering antioxidant defense enzyme activities and concentration of vitamins, Cd concentration in the plasma and RBC of control and treated groups of animals was depicted in Table 1. These results show that Cd concentration was significantly increased in the plasma of Cd+Se cotreated rats ($p < 0.02$) and in RBC of rats exposed to Cd ($p < 0.02$). In contrast, in RBC of animals exposed to Cd+Se simultaneously the concentration of Cd was significantly decreased

(p<0.05). This is in accordance with the fact that Cd enters the blood and binds to plasma albumins and erythrocyte membranes [2]. At the same time, Se binds to Cd and forms Cd-Se complexes [52]. From there Cd can be transferred into the tissues and organs and redistributed among proteins of various molecular mass [54]. This explain decreased concentration of Cd in RBC of Cd+Se cotreated rats.

CONCLUSIONS

It can be concluded that Cd exerts toxic effects on the antioxidant defense system in the blood of rats by influencing the enzyme activities and concentrations of nonenzymatic scavengers. Selenium *per se* improved antioxidant status by inducing the increase of RBC GSH-Px and plasma GST activities, as well as plasma Vit E concentration, but not induced a changes in CuZn SOD and CAT activities in RBC. Exposure both to Cd+Se elevated RBC GSH-Px activity and normalized changes of CuZn SOD, CAT and GST activities, as well as Vit E concentration induced by Cd. Therefore, Se significantly decreased Cd accumulation in RBC of rats.

Selenium in the dosage administered exerts protective effects on enzymatic and nonenzymatic components of antioxidant defense system in the blood of rats chronically treated with cadmium.

ACKNOWLEDGMENTS

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