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Effect of Chronic Cadmium Exposure on Antioxidant Defense System in Some Tissues of Rats: Protective Effect of Selenium

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Running title: Selenium protection against cadmium toxicity

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Summary

The effects of selenium (Se) on antioxidant defense system in liver and kidneys of rats with cadmium (Cd)-induced toxicity were examined. Cd (15 mg Cd/kg b.m./day as $CdCl_2$ for 4 weeks) exposure resulted in increase in lipid peroxidation (LP) in both tissues. Vitamin C (Vit C) was decreased in liver, whereas vitamin E (Vit E) was increased in liver and kidneys of Cd exposed animals. Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities were decreased in both tissues, whereas catalase (CAT) activity was decreased only in liver. Glutathione S-transferase (GST) was increased in both tissues. Treatment with Se (0.5 mg Se/kg b.m./day as Na_2SeO_3 for 4 weeks) alone significantly increased liver and kidneys SOD and GSH-Px activities, as well as CAT and GST activities only in liver. In animals exposed to Se both the concentrations of Vit C and Vit E were increased in both tissues. Cotreatment with Se resulted in reversal of oxidative stress with significant decline in analyzed tissues Cd burden. Our results show that Se may ameliorate Cd-induced oxidative stress by decreasing LP and altering antioxidant defense system in liver and kidneys of rats and that Se demonstrates the protective effect from Cd-induced oxidative damage.

Key words

Antioxidant defense system ● Cadmium ● Lipid peroxides ● Selenium ● Rat

Introduction

Cadmium (Cd) is an industrial and environmental pollutant, arising primarily from battery, electroplating, pigment, plastic, and fertilizer industries, and cigarette smoke (Page *et al*. 1986). In the environment, Cd is dangerous because humans consume both plants and animals that absorb Cd efficiently and concentrate it within their tissues (Stohs and Bagchi 1995). Cd shows different mechanisms of toxicity under different experimental conditions and in various species (Iscan *et al.* 1994, Peters *et al.* 1995, Žikić *et al*. 1996, Jamba *et al*. 1997, Waisberg *et al*. 2003). Cd has been demonstrated to stimulate free radical production, resulting in oxidative deterioration of lipids, proteins and DNA, and initiating various pathological conditions in humans and animals (Waisberg *et al*. 2003). Once absorbed, Cd is rapidly cleared from the blood and concentrates in various tissues. Chronic exposure to inorganic Cd results in accumulation of the metal mainly in the liver and kidneys, as well as in other tissues and organs causing many metabolic and histological changes, membrane damage, altered gene expression and apoptosis (Shaikh *et al*. 1999, Casalino *et al*. 2002, Waisberg *et al*. 2003).

Among antioxidant micronutrients, selenium (Se) is an essential dietary trace element which plays an important role in a number of biological processes for humans and many other forms of life. Deficiency of this element induces some pathological conditions, such as cancer, coronary heart disease, and liver necrosis (Saito *et al.* 2003, Wu and Huang 2004, Agay *et al.* 2005). Se taken in the form of selenite, selenate, selenocysteine and selenomethionine gets the most absorbed in the duodenum. 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. A marked uptake of Se by erythrocytes was also found (Combs and Gray 1998).

Se is an essential component of several enzymes such as glutathione peroxidase (GSH-Px), thioredoxin reductase (TR) and selenoprotein P (SeP), which contain Se as selenocysteine. It is also well known that Se is essential for cell culture when a serum-free medium is used (Kim and Combs

1993, Saito *et al.* 2003). It is also known, that Se has a some protective role from the toxic actions of Cd and other heavy metals (Jamall and Sprowls 1987, Ognjanović *et al*. 1995, Žikić *et al*. 1998, Xiao *et al*. 2002). 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 (Viljoen and Tappel 1988, Jamba *et al.* 1997, Combs and Gray 1998).

In the present experiments, the influence of Cd and Se on the antioxidant defense system (AOS), as well as on LP, Cd and Se concentrations in the liver and kidneys of rats were analysed. After 30 days of exposure, the activities of enzymatic (SOD, CAT, GSH-Px and GST) and non-enzymatic (Vit C and Vit E) components of this system were determined. The possible protective role of Se against the toxic effects of Cd has been especially considered.

Methods

Wistar albino male 60-day-old rats (weighing 200 ± 20 g) were used. The animals were kept at 21 ± 2^{o} C, fed with pellet rat diet, and exposed to 12 h light / 12 h dark cycle. The first group was used as control. The rats of the experimental group were exposed to: (2) Cd (15 mg Cd/kg body mass/day as $CdCl_2$ for 4 weeks), (3) Se (0.5 mg Se/kg body mass/day as Na_2SeO_3 for 4 weeks) and (4) Cd + Se (in above mentioned amounts). Every group consisted of 8 animals. All chemicals were from Sigma (St. Louis, Mo. U.S.A.).

All rats of each group were killed at the end of treatment period. Liver and kidneys were minced and homogenized (10%, w/v) separately in ice-cold saline, sucrose buffer (0.25 M sucrose, 1 mM EDTA and 0.05 M Tris-HCl, pH 7.4) in a Thomas Sci Co. glass-type homogenizer (Teflon pestle). Tissues homogenate from both control and treated rats were used for Vit C and Vit E determination. The homogenate was centrifuged at 100.000 x *g* for 90 min at 4°C and the supernatant was used for antioxidant enzyme assays.

Concentration of Cd in the liver and kidneys was determined by atomic absorption spectrophotometry using a Perkin-Elmer Model 5000 (Shirley *et al*. 1949), while the concentration of Se was determined by fluorimetryc method (Dye *et al.* 1960).

The concentration of LP measured as thiobarbituric acid reactive substances (TBARS) in the tissues of rat was assayed by the method of Ohkawa *et al*. (1979).

Superoxide dismutase (SOD) activity was determined by the epinephrine method (Misra and Fridovich 1972). Catalase (CAT) activity was measured by the method of Beutler (1982). The activity of glutathione peroxidase (GSH-Px) was assayed by following the oxidation of NADPH at 340 nm with t-butyl-hydroperoxide (Tamura *et al*. 1982). Glutathione S-transferase (GST) activity toward 1-chloro-2,4-dinitrobenzene as a substrate was determined according to Habig and coworkers (Habig *et al*. 1974). All enzyme activities were expressed per g of wet tissue (U/g tissue).

Vitamin C (Vit C) concentration was determined spectrophotometrically by dinitrophenyl-hydrazine method at 530 nm (Omaye *et al.* 1979). Vitamin E (Vit E) was measured by the method of Desai (1984) based on the reduction of Fe3+ in Fe2+ in the presence of tocopherol and production of colored complex with bathophenanthroline.

The data were expressed as the mean ± S.E.M. and were analyzed by means of one-way analysis of variance (ANOVA). Statistical evaluation of data was done following Student's *t*-test. A difference was considered significant at $p<0.05$.

Results

The results obtained in this study show that concentration of Cd in liver and kidneys were significantly increased in animals exposed to Cd and in animals exposed to Cd and Se concomitantly ($p<0.005$). Concentration of Se was increased ($p<0.005$) after exposure to Se only and after to concomitant exposure both to Cd and Se ($p<0.005$ and $p<0.01$), (Table 1).

Results indicated that LP concentration was significantly increased in liver ($p<0.005$) and kidneys ($p<0.01$) of rats treated with Cd. Cotreatment with Se was very effective in the prevention of oxidative damage induced by Cd which resulted in significantly lower LP concentration (Table 2).

The data presented in Figs. 1 and 2 shows significant changes in the activity of AOS enzymes during the treatment of rats with Cd, Se and their combination. SOD and GSH-Px activities were significantly decreased ($p<0.05$ and $p<0.005$) in liver (Fig. 1) and kidneys (Fig. 2), whereas CAT activity was decreased ($p<0.005$) only in liver (Fig. 1) in the animals exposed to Cd. Treatment with Cd significantly increased liver ($p<0.005$) and kidneys ($p<0.01$) GST activity (Figs. 1 and 2). Treatment with Se alone significantly increased liver and kidneys SOD ($p<0.01$ and $p<0.005$) and GSH-Px ($p<0.01$ and $p<0.05$) activities, as well as CAT and GST activities only in liver ($p<0.01$). Administration of Cd with Se did not cause significant changes in activity of this enzyme in comparison with control group, while alleviated the harmful effects of Cd (Figs. 1 and 2).

Table 3. show the data of Vit C and Vit E concentrations in liver and kidneys. Exposure to Cd caused significant decrease of Vit C in liver ($p<0.005$) and concomitant increase of Vit E in liver ($p<0.005$) and kidneys ($p<0.05$) of rats. In animals exposed to Se both the concentrations of Vit C ($p<0.01$) and Vit E ($p<0.005$) were increased. Concomitant treatment with Cd and Se did not cause significant changes in concentration of this vitamin in both the tissues in comparison with control group.

Discussion

Cd has been recognized as one of the most toxic environmental and industrial pollutants. Cd is an ubiquitous toxic metal that may induce oxidative damage by disturbing the prooxidant-antioxidant balance in the tissues. A significantly increased accumulation of Cd in liver and kidneys were observed in animals treated with Cd (Table 1). Liver, kidney, lung, testes, and heart are the target organs

following Cd exposure, with the severity of their intoxication dependent on the route, dose, and duration of the exposure to the metal (Casalino *et al.* 1997, Štajn *et al*. 1997). In the cell Cd mainly accumulates in the cytosol (70%), followed by the nucleus (15%) and lowest in mitochondria and the endoplasmic reticulum (Casalino *et al*. 1997).

Previous investigations show that peroral intake of Cd induces its great accumulation in red blood cells (Kostić *et al*. 1993), heart (Žikić *et al*. 1998) and the skeletal muscle of rats (Pavlović *et al.* 2001), which is accompanied by marked alterations of enzymatic and nonenzymatic component of AOS. The obtained Cd concentrations are sufficient to induce nephrotoxicity, since it develops at the kidney Cd concentrations of 10-300 μg/g w.m. (Nordberg *et al*. 1992). The same amount of Cd accumulation we found in the liver of these animals (Ognjanović *et al.* 1995). With increasing concentration of Cd in liver and kidneys, the concentration of Se also rises, although it was not administered additionally (Table 1). The increased concentration of Se in liver and kidneys could be explained by its redistribution from other tissues and organs (Jamall and Smith 1985) as well as by forming of Cd-Se protein complexes (Jamba *et al.* 1997, Combs and Gray 1998). By concomitant exposure of rats to both Cd and Se the accumulation of both elements increased in liver and kidneys. All these data indicate that Se diminished the toxic effects of Cd and increased the accumulation of Cd in liver and kidneys (Wahba *et al.* 1993).

Lipid peroxidation is one of the main manifestations of oxidative damage and has been found to play an important role in the toxicity of many xenobiotics (Stohs and Bagchi 1995, Anane and Creppy 2001). The data obtained in our study (Table 2) confirm that chronic intoxication with Cd causes a significant increase of LP concentration in liver and kidneys of rats. Since it causes lipid peroxidation in numerous tissues both in vivo and in vitro (Kostić *et al*. 1993, Casalino *et al.* 1997, Sarkar *et al*. 1998, Ognjanović *et al.* 2003, Tandon *et al.* 2003, El-Demerdash *et al.* 2004), it has been suggested that Cd may induce oxidative stress by producing hydroxyl radicals (O'Brien and Salasinski 1998), superoxide anions, nitric oxide and hydrogen peroxide (Koizumi *et al*. 1996, Waisberg *et al.* 2003).

Moreover, it has been shown that various antioxidants and antioxidant defense systems protect cells from Cd-induced toxicity (Peters *et al*. 1995, Scholz *et al.* 1997, Shaikh *et al.* 1999, Tandon *et al*. 2003, Ognjanović *et al.* 2006).

Cotreatment with Se was very effective in the prevention of oxidative damage induced by Cd which resulted in significantly lower LP concentration in liver and kidneys (Table 2). These results can be explained by the important role of Se in preventing lipid peroxidation and in protection of integrity and functioning of tissues and cells.

The prevention of lipid peroxidation is essential for all aerobic organisms and so the organism is well equipped with antioxidants that directly or indirectly protect cells against the adverse effects of xenobiotics, carcinogens and toxic radicals (Halliwell and Gutteridge 1999). The role of antioxidants in reversing this oxidative stress has been of long-standing interest to basic scientists and clinicians (Matés, 2000).

The data presented in Figs. 1 and 2 shows significant changes in the activity of AOS enzymes during the treatment of rats with Cd and Se. SOD and GSH-Px activities were decreased in liver (Fig. 1) and kidneys (Fig. 2), whereas CAT activity was decreased only in liver (Fig. 1). This probably is the consequence of the intracellular accumulation of ROS with subsequent development of liver and kidneys injury. Accumulation of Cd and SOD inhibition was highest in liver followed by kidneys, indicating a direct effect of Cd on SOD activity. This suggests a role of free radicals in causing cellular damage with long-term exposed to Cd (Patra *et al.* 1999). The decreased activity of GSH-Px can be explained by competition of Cd-metallothioneins and GSH-Px for sulfur containing aminoacids (Olsson, 1986). Studies of other authors have shown that Cd inhibits the activity of majority of enzymes involved in AOS (Jamall and Sprowls 1987, Sarkar *et al*. 1998, Patra *et al.* 1999, Casalino *et al.* 2002) inducing an increased production of free radicals, lipid peroxidation and destruction of cell membranes (Kostić *et al*. 1993, Casalino *et al.* 1997, Ognjanović *et al.* 2003). Cd also, inhibits the

activities of many enzymes by binding to their sulfhydryl groups or by inhibiting the protein synthesis (Shaikh *et al.* 1999, Waisberg *et al.* 2003).

The increase in the activity of GST in liver and kidneys (Figs. 1 and 2) is in agreement with the finding of our previous investigations which show that exposure to Cd causes an increase the activity of GST in plasma, heart and skeletal muscle (Kostić *et al*. 1993, Pavlović *et al*. 2001, Ognjanović *et al.* 2003, 2006). Other authors showed that Cd exposure increased the activity of this enzyme in different tissues. Indeed an increased hepatic GST activity in rat (Casalino *et al*. 2004) and guinea pig (Iscan *et al*. 1994) has been observed. The GST enzyme has an important role in detoxification of xenobiotics, drugs and carcinogens and thus protects the cells against redox cycling and oxidative stress (Matés 2000, Casalino *et al*. 2004).

Treatment with Se alone significantly increased liver and kidneys SOD and GSH-Px activities, as well as CAT and GST activities only in liver. By concomitant exposure of rats both to Cd and Se, the activities of SOD, CAT and GSH-Px remain at the level of the control values, indicating that Se eliminates the toxic effects of Cd on the activity of these enzymes. However, the activity of GST in liver of these animals was increased. In rats exposed to Cd and Se separately, this can be explained by an important role played by this enzyme in preventing lipid peroxidation and oxidative damage of the liver (Jamall and Smith 1985, Kim and Combs 1993).

The antioxidants such as Vit E, Vit C and GSH protect the erythrocyte membrane from oxidative damage (Beyer 1994, Sarkar *et al.* 1998, Griffith 1999, Ognjanović *et al*. 2003). Shaikh et al. (1999) concluded that oxidative stress appears to play a major role in chronic Cd-induced hepatic and renal toxicity since inhibition of components of the antioxidant defense system accelerated and administration of Vit E protected against Cd toxicity. Also, carotenoids can function directly as antioxidants by reacting with active oxygen species (El-Demerdash *et al.* 2004). In the present study also, Se reduced cellular toxicity caused by Cd-induced ROS and protected the liver (Fig. 1) and kidneys (Fig. 2) antioxidant system. The effects of Se are thought to be related to either the formation of

Cd-Se complexes in association with metallothioneins, or to the changes in tissue Cd distribution (Viljoen and Tappel 1988, Jamba *et al.* 1997).

Our data (Table 3) show that Cd significantly decreases Vit C concentration in the liver of rats, which is in accordance with the results of other investigators (Chatterjee *et al.* 1973, Shukla and Chandra 1989). It is known that increased accumulation of Cd in the liver induces lipid peroxidation and increases the production of malondialdehyde (MDA), (Tandon *et al.* 2003), which consequently inhibits the enzyme L-gulonolactone oxidase (Chatterjee *et al.* 1973, Shukla and Chandra 1989, Hudecova and Ginter 1992) necessary for the synthesis of Vit C which is a potent scavenger of free oxygen radicals and its deficiency results in intracellular oxidative damage in the guinea-pig (Nagyova *et al.* 1994). In rats exposed to increased concentrations of Se, a new opposite effect was obtained (Table 3), so that the concentrations of Vit C in liver and kidneys were significantly increased. From our results it can be concluded that the inhibitory effect of Cd on the concentration of Vit C is more marked than the stimulatory effects of Se, since the concentration of Vit C in the liver was significantly lower in rats concomitantly exposed to both Cd and Se than in control animals. Both Cd and Se cause a significantly increase of concentration of Vit E in liver and kidneys of rats (Table 3). Vit E is a major free radical chain-breaking antioxidant, and can also interfere with the initiation and progression of Cd-induced oxidative damage. Vit E is the primary liposoluble antioxidant, which may have an important role in scavenging free oxygen radicals and in stabilizing the cell membranes, thus maintaining its permeability (Beyer 1994). Moreover, it is known that antioxidants, such as Vit E, coenzyme Q, Vit C, beta-carotene, GSH and Se may act synergically, preventing lipid peroxidation and cell destruction (Beyer 1994, Scholz *et al.* 1997, Navarro *et al.* 1999, El-Demerdash *et al.* 2004). It is well known that Se and Vit E shown compensative effects and that a deficiency of both elements causes massive injury in some cases (Saito *et al.* 2003).

Our previous investigations showed that chronic treatment with Cd induces a decrease of Vit C concentration in plasma, liver (Žikić *et al*. 1995) and kidneys (Štajn *et al*. 1997) of young and adult

rats, while Cd increases the concentration of Vit E in rat heart (Ognjanović *et al*. 2006), kidneys (Štajn *et al*. 1997) and plasma (Kostić *et al*. 1993, Ognjanović *et al*. 2003). Thus, a number of studies have been carried out to determine the protective effects of Se in different biological models of injury (Kim and Combs 1993, Scholz *et al.* 1997, Combs and Gray 1998, Agay *et al.* 2005).

From the present study it can be concluded that Cd accumulation in liver and kidneys of rats, due to chronic dietary intake of Cd, is associated with marked alterations enzymatic (SOD, CAT, GSH-Px and GST) and non-enzymatic components (Vit C and Vit E) of AOS. Data suggest that lipid peroxidation was associated with Cd toxicity in both the tissues. Our results showed that the nutritional antioxidant Se ameliorated oxidative stress and loss of cellular antioxidants and suggest that Se efficiently protect liver and kidneys from Cd-induced oxidative damage. 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.

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Table 1. Cadmium (Cd) and selenium (Se) concentrations in liver and kidneys of control and rats treated with cadmium (Cd), selenium (Se) and their combination (Cd+Se).

Parameters	Experimental groups			
	Control	Cd	Se	Cd+Se
Cd (μg /g tissue)				
Liver	**0.32 ± 0.01**	**21.26 ± 1.72** ***	**0.24 ± 0.02**	**24.53 ± 1.12** ***
Kidneys	**0.42 ± 0.01**	**23.44 ± 1.68** ***	**0.47 ± 0.03**	**23.25 ± 1.31** ***
Se (μg /g tissue)				
Liver	**0.72 ± 0.04**	**0.64 ± 0.08**	**1.83 ± 0.09** ***	**1.43 ± 0.05** ***
Kidneys	**0.52 ± 0.02**	**0.25 ± 0.02** *	**1.28 ± 0.06** ***	**0.75 ± 0.06** **

Data are expressed as mean ± S.E.M. $n = 8$ for each groups.

Significant different from controls: *p<0.05, ** p<0.01, ***p<0.005.

Table 2. Concentration of lipid peroxides (LP) in liver and kidneys of control and rats treated with cadmium (Cd), selenium (Se) and their combination (Cd+Se).

LP (nmol/ g tissue)	Control	Cd	Se	Cd + Se
Liver	**27.43 ± 2.64**	**42.61 ± 3.84** ***	**24.36 ± 2.17**	**29.82 ± 2.72**
Kidneys	**20.56 ± 1.18**	**27.63 ± 1.75** **	**16.38 ± 0.64** *	**23.15 ± 1.26**

Data are expressed as mean ± S.E.M. $n = 8$ for each groups.

Significant different from controls: *p<0.05, ** p<0.01, ***p<0.005.

Table 3. Vitamin C (Vit C) and vitamin E (Vit E) concentrations in liver and kidneys of control and rats treated with cadmium (Cd), selenium (Se) and their combination (Cd+Se).

Parameters	Experimental groups			
	Control	**Cd**	**Se**	**Cd + Se**
Vit C (mg %)				
Liver	**37.60 ± 0.84**	**30.01 ± 0.39** ***	**45.23 ± 1.58** **	**34.53 ± 0.61**
Kidneys	**21.90 ± 0.53**	**20.87 ± 0.91**	**25.84 ± 1.71** **	**23.68 ± 1.23**
Vit E (μg /g tissue)				
Liver	**13.41 ± 0.69**	**23.32 ± 0.65** ***	**26.97 ± 1.50** ***	**16.04 ± 0.48**
Kidneys	**15.73 ± 0.87**	**17.96 ± 0.53** *	**27.01 ± 1.46** ***	**15.82 ± 0.80**

Data are expressed as mean ± S.E.M. $n = 8$ for each groups.

Significant different from controls: *p<0.05, ** p<0.01, ***p<0.005.

FIGURE LEGENDS

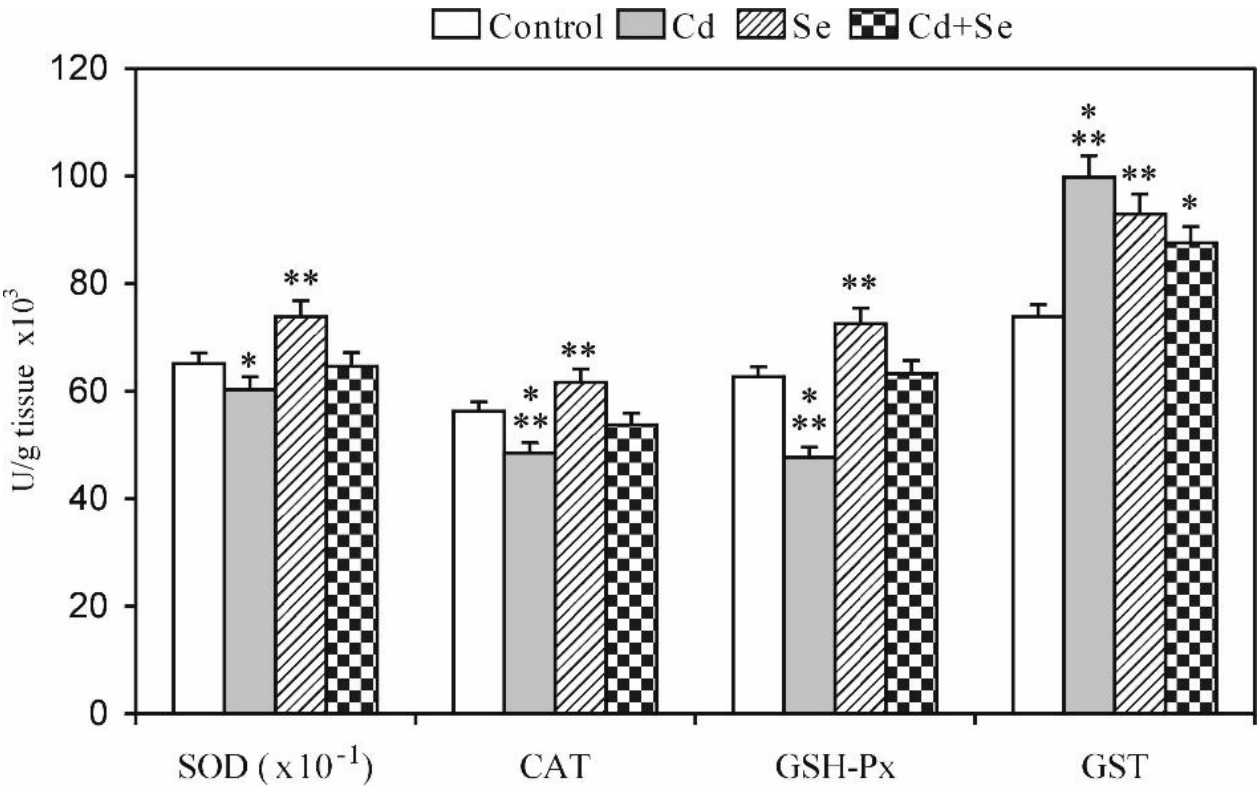

Fig. 1. The activity of antioxidant enzymes (SOD, CAT, GSH-Px and GST) in liver of control and rats treated with cadmium (Cd), selenium (Se) and their combination (Cd+Se). Data are expressed as mean ± S.E.M. n = 8 for each groups. Significant different from controls: *p<0.05, ** p<0.01, ***p<0.005.

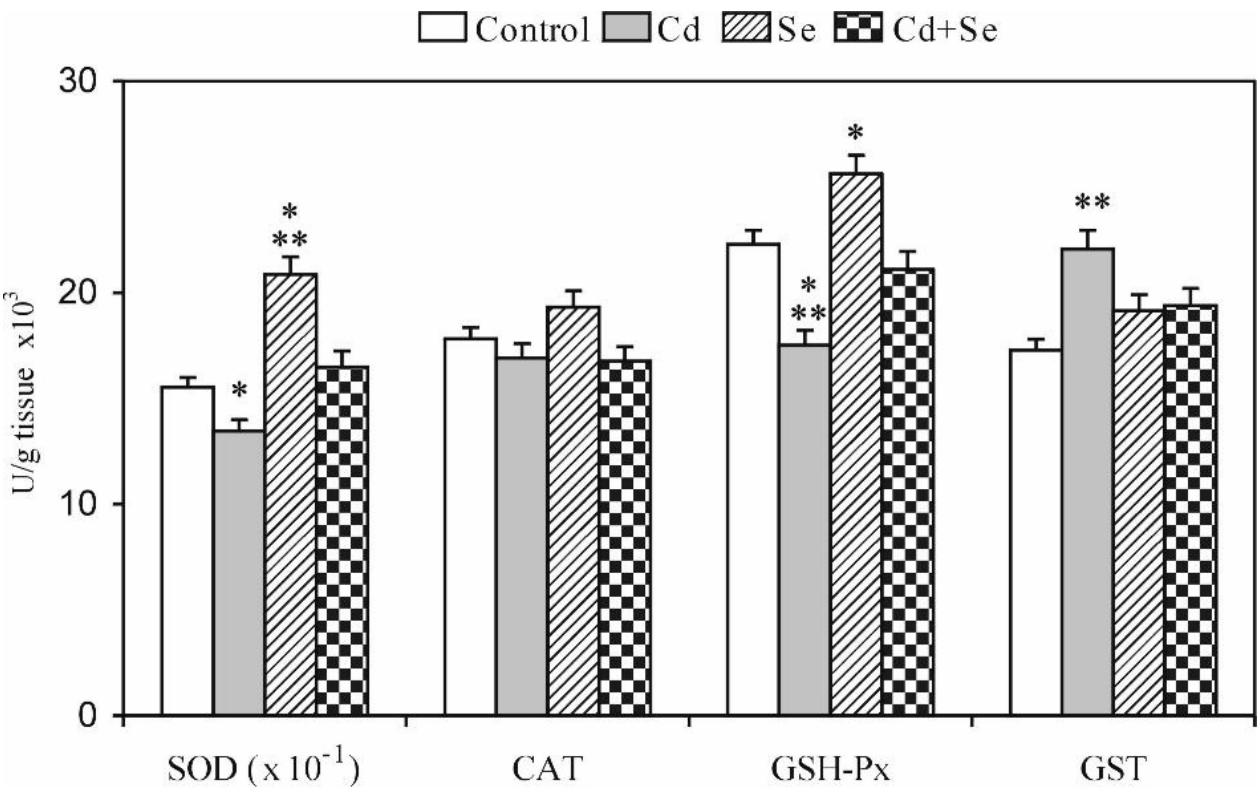

Fig. 2. The activity of antioxidant enzymes (SOD, CAT, GSH-Px and GST) in kidneys of control and rats treated with cadmium (Cd), selenium (Se) and their combination (Cd+Se). Data are expressed as mean ± S.E.M. n = 8 for each groups. Significant different from controls: *p<0.05, ** p<0.01, ***p<0.005.