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Effects of Selenium with Vitamin E and Melatonin on Cadmium-Induced Oxidative Damage in Rat Liver and Kidneys

Haki Kara · Aydın Cevik · Vahit Konar · Alpaslan Dayangac · Kadir Servi

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Abstract The present study was performed to determine the protective effects of melatonin alone and vitamin E with selenium combination against cadmium-induced oxidative damage in rat liver. A total of 60 male rats were equally divided into five groups, one of which acted as control receiving subcutaneous injections of physiological saline. The remaining four groups were treated with subcutaneous injections of cadmium chloride at a dose of 1 mg/kg weight. The first study group received no treatment. The second group was treated with a combination of 60 mg/kg vitamin E and 1 mg/kg sodium selenite. Group 3 was treated with 10 mg/kg melatonin, and the four group received a combination of vitamin E, sodium selenite, and melatonin at the doses mentioned above. After 1 month, the animals were killed, and liver and kidneys were excised for histopathological inspection and determination of tissue malondialdehyde and the activity of superoxide dismutase. The animals receiving no treatment showed significantly higher malondialdehyde levels and reduced activity of superoxide dismutase ($p<0.05$). Treatment with antioxidants resulted in a significant reduction in malondialdehyde when compared to nontreated animals ($p<0.05$) and increase in the enzyme activity that was almost the same as the controls. The pathological findings were also in parallel with the results of the

H. Kara (✉)
Elazig School of Health Sciences, Firat University, Elazig 23119, Turkey
e-mail: hakikara@yahoo.com

A. Cevik
Sivrice Poultry Breeding High School, Firat University, Elazig 23119, Turkey

V. Konar
Department of Biology, College of Science, Firat University, Elazig 23119, Turkey

A. Dayangac
Department of Biology, College of Science, Ahi Evran University, Kirsehir 40100, Turkey

K. Servi
Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Firat University, Elazig 23119, Turkey

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biochemical analysis. In conclusion, all the agents tested had protective effects against cadmium-induced oxidative damage.

Keywords Cadmium · Melatonin · Selenium · Vitamin E · Oxidative damage · Rat tissue

Introduction

Cadmium (Cd) is a non-essential element with many industrial applications. Its widespread use in batteries, dyes, electrochemistry, and metallurgy has resulted in its accumulation in soils, water, and the general environment [1]. The most important source of Cd in humans is tobacco. In cadmium-exposed mammals, the target organs are the testes, brain, liver, and kidneys, possibly bound to metallothionein [2–4].

It is known that cadmium causes injury to hepatocytes, degeneration in renal proximal tubulus cells, impairments in testes, and various disorders in erythrocytes. Oxidative stress occurs as a result of an increase in Cd-induced peroxidation of membrane lipids in the organs where it accumulates [5–6].

Exposure to cadmium results in decreases of glutathione and of protein-binding –SH groups, which causes an increase in reactive oxygen species like hydrogen peroxide, hydroxyl radicals, and superoxide radical ions that increase lipid peroxidation, change intercellular stability, damage deoxyribonucleic acid (DNA), and result to membrane damage and cell deaths [7]. Tissue levels of malondialdehyde (MDA) and the activity of superoxide dysmutase (SOD) and glutathione peroxidase (GSH-Px) levels are accepted as indicators of the oxidative stress resulting from lipid peroxidation [8–10].

In several studies, lipid peroxidation has been reported as a result of acute or chronic cadmium poisoning resulting in elevated tissue MDA levels, depletion of GSH-Px, and changes of several enzymes such as SOD, alanine transaminase, aspartate aminotransferase blood urea nitrogen, and serum creatinin levels [8, 11–13].

Treatment against cadmium toxicity includes the use of chelating agents, metallothionein, and antioxidant therapy with melatonin, vitamin E, neominophagen, and selenium [8, 11–13]. It is known that various chelator substances as well as antioxidant substances like metallotionein, vitamin E, selenium, neominophagen, and melatonin are used to reduce cadmium toxicity and to lessen oxidative stress [8, 14, 15]. In the present study, we report the effects of individual and combined effects of melatonin, selenium, vitamin E against cadmium-induced liver and kidney damage in rats.

Materials and Methods

Animals

Sixty 14- to 16-week-old male Wistar rats weighing 210–230 g each were obtained for the study. They were housed in a room kept at 22–25°C in 12-h light/dark cycles and maintained on a standard diet and water ad libitum.

After 2 weeks acclimatization, the rats were divided into five groups of 12 animals each. Four groups were treated with subcutaneuos injections of cadmium chloride at a dose of 1 mg/kg for 1 month; the fifth group received saline injections and acted as controls.

The first experimental group was given only cadmium chloride injections. The second group received, in addition to cadmium, a combination of 1 mg/kg sodium selenite and

60 mg/kg vitamin E. The third group was given 10 mg/kg melatonin, and the fourth group a combination of 1 mg/kg sodium selenite plus 60 mg/kg vitamin E and 10 mg/kg melatonin.

At the end of the experiment, the animals were killed by decapitation, and their liver and kidneys were removed and stored −20°C until needed for analysis.

Chemicals

Melatonin (MLT), cadmium chloride ($CdCl_2$), vitamin E (α-tocopherol), and selenium (sodium selenite) were purchased from Sigma (St. Louis, MO, USA). All other chemicals used in the study were in analytical grade and purchased from Merck (Darmstad, Germany).

Determination of Tissue-Free MDA Levels

Collected tissues were homogenized as described in the literature. For determination of MDA levels in tissues, all tissues were homogenization as described for [16]. Then, samples were centrifuged at 3,000×*g* for 5 min at 4°C to separate the sera. To 0.1 ml samples, 0.1 ml 0.5 M $HClO_4$ and 0.8 ml distilled water were added. Addition of acid was necessary to precipitate proteins and release the MDA bound to the amino groups of proteins and other amino compounds. These samples were than centrifuged at 4,500×*g* for 5 min and used for high-performance liquid chromatography analysis.

A Cecil series 1100 choromatograph provided with a cotati 7125 sample injection valve and a Cecil 68174 spectrophotometric dedector was used for the injection of 20 μl of the supernatant for separation in a 250×4.6 mm ID Supelcosil C18 column (5 μm particle and 80 A pore size). The eluent was a mixture of 30 mmol KH_2PO_4 in 65% pH 4 methanol–phosphoric acid at a flow rate of 1.5 ml/min.

Determination of Levels of SOD Activity

Total SOD activity was determined by the method of Sun et al. [17]. The technique is based on inhibition of nitro blue tetrazolium (NBT) reduction by the xanthine–xanthine oxidase system as the superoxide generator. The SOD activity was measured in the ethanol phase of the supernatant after 1 ml of ethanol–chloroform mixture (5:3, *v*/*v*) was added to the same volume of sample and centrifuged. One unit of SOD was defined as the amount of enzyme causing 50% inhibition in the NBT reduction rate. The SOD activity was expressed as U/g protein.

Histopathology

The tissue samples were fixed in 10% formalin, trimmed, embedded in paraffin, and cut into 5-μm sections that were then stained with hematoxylin–eosin for microscopic examination [18].

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) software for Windows, v.10 (SPSS, Chicago, IL, USA), was used for the statistical treatment of the data. The results are expressed as mean values ± standard deviation. Differences between means were established by analysis of variance, with $p<0.05$ considered significant. Significant differences among treatment and control groups were interpreted using the Tukey's honest significant difference post hoc test.

Results

The findings obtained in this study are presented in Table 1. Changes in rat tissues are shown in Figs. 1, 2, 3, 4, 5, and 6. The rats receiving only cadmium chloride had significant increases in MDA levels in the liver and kidneys ($p<0.05$), and significant decrease in SOD levels in all tissues ($p<0.05$). Histological examination revealed picnosis, centrolobular degeneration and necrosis, Kuppfer cell activation in the liver (Fig. 1), severe fibrosis in capsule, degeneration of tubular epithelium cells, and necrosis in kidneys (Fig. 2)

Groups that were administered vitamin E + selenium combination in addition to cadmium were seen to have tissue MDA and SOD values close to the control values. Pathological examination revealed mild centrotubular necrosis with a few picnotic cells in the liver (Fig. 3), focal phagocyte infiltrations, and necrosis in the kidney (Fig. 4),

In the rats given melatonin, the same normalizing trend was observed, but it was less pronounced than that of vitamin E and selenium.

The combination of melatonin, vitamin E, and selenium resulted in MDA and SOD values that were indistinguishable from those of the control ($p<0.05$). Pathological examination of the tissues taken from the animals in this group showed mild degeneration, diffuse fibrosis and a few picnotic cells in the liver (Fig. 5), mild degeneration, and necrosis in tubular cells in the kidney (Fig. 6).

Discussion

Because of its many industrial applications, cadmium is widely distributed in the environment. The health risk to humans from acute and chronic cadmium exposure has been well documented. Muller reported that single-dose cadmium administration increased lipid peroxidation in the liver and decreased GSH [3]. The decrease in GSH was attributed to its functioning as a lipid peroxidation store. Evidences of peroxidation are the increased MDA levels and decreases in SOD and glutathione activities [12, 19]. Various mechanisms are suggested to be responsible from the cadmium toxicity. One of these mechanisms includes Cd binding to –SH groups from cell membrane proteins, cytoplasmic proteins, and enzymes. In addition, cadmium can reduce activities [20, 21]. Another possible mechanism of cadmium induced in lipid peroxidation is the decrease in GSH. Repeated cadmium

Table 1 Levels of Malondialdehyde and Supeoxide Dismutase in Liver and Kidney Tissues of Cadmium-Treated Male Rats

	Liver MDA (nmol/g)	Kidney MDA (nmol/g)	Liver SOD (U/mg)	Kidney SOD (U/mg)
Control	22.6±2.4	12.4±1.8	72±7	63±5
Cd	110±12.4*	72.4±6.2*	32±6*	25±3*
Cd + VitE + Se	34.8±7.5*·**	24.6±3.8*·**	68±8*·**	60±6**
Cd + MLT	45.4±4.2*·**·***	32.4±3.4*·**·***	65±7*·**	57±6*·**
Cd + VitE + Se + MLT	48.6±5.8*·**·***	38.2±5.2*·**·***	70±9**	61±7**

*$p<0.05$, significant differences compared to control

**$p<0.05$, significant differences compared to Cd-only administration

***$p<0.05$, significant differences compared to other administration groups

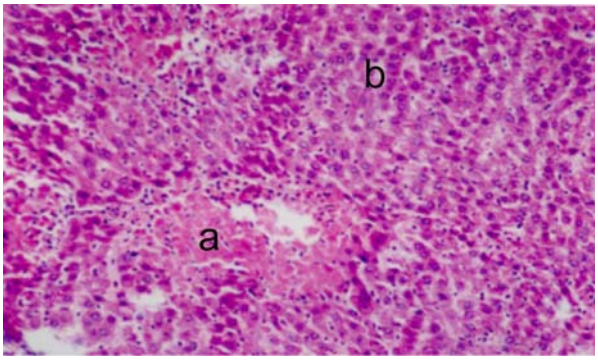

Fig. 1 Severe picnosis (*b*), and centrolobular degeneration and necrosis (*a*) in the liver of male rats given 1 mg/kg cadmium chloride for 1 month (H & E × 66)

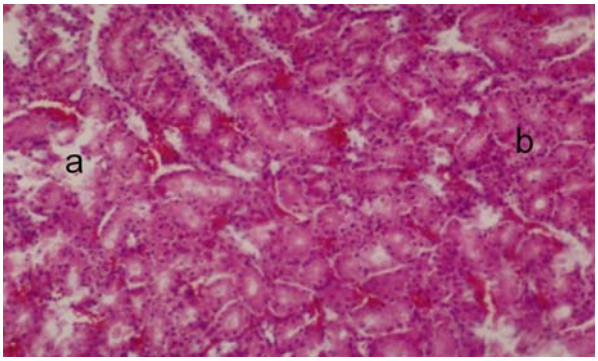

Fig. 2 Severe degeneration of tubular epithelium cells (*b*) and necrosis (*a*) in kidneys of male rats given 1 mg/kg cadmium chloride for 1 month (H & E × 66)

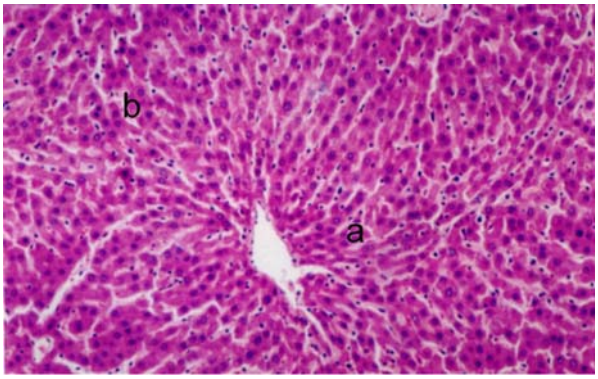

Fig. 3 Centrotubular necrosis (*a*) with a few picnotic cells (*b*) in the rat liver. Treated with a combination of vitamin E and sodium selenite in addition to 1 mg/kg cadmium chloride (H & E × 66)

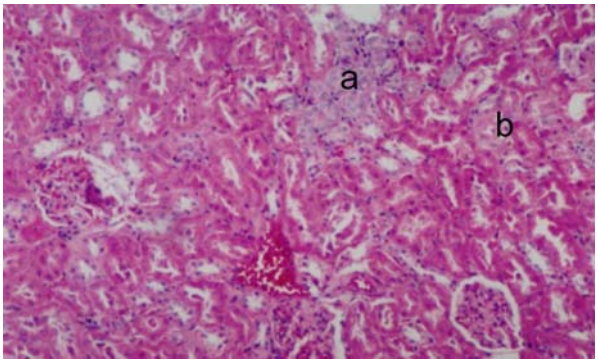

Fig. 4 Focal phagocyte infiltrations and necrosis (*a*, *b*) in the rat kidney. Treated with a combination of vitamin E and sodium selenite in addition to 1 mg/kg cadmium chloride (H & E × 66)

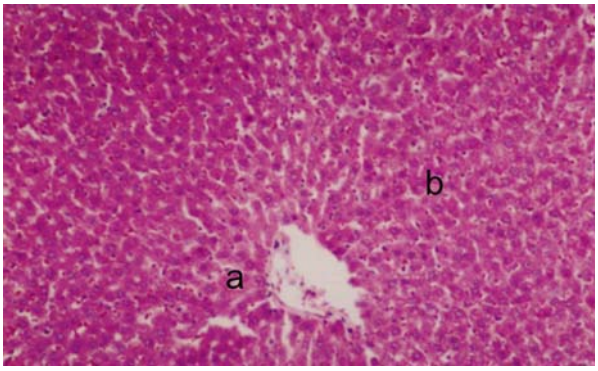

Fig. 5 Centrolobuler degeneration, diffuse fibrosis (*a*) and a few picnotic cells (*b*) in the liver. Treated with a combination of vitamin E and sodium selenite with melatonin in addition to 1 mg/kg cadmium chloride (H & E × 66)

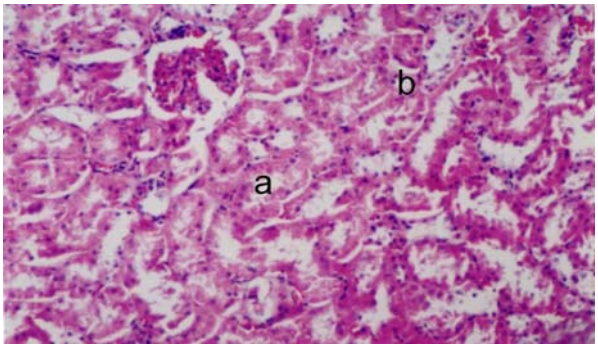

Fig. 6 Degeneration and necrosis in tubular cells in the kidney (*a*, *b*). Treated with a combination of vitamin E and sodium selenite with melatonin in addition to 1 mg/kg cadmium chloride (H & E × 66)

exposures lead to decreases in the activity of glutathione peroxidase enzyme due to the formation of Se–Cd complex in the active part of this enzyme [22].

Several researchers stated that acute and chronic cadmium administrations caused damage in the organs particularly the liver and kidney, and that MDA levels increased and GSH-Px and SOD activities decreased as an indication of oxidative stress in these organs [23]. Patra et al. [24] reported that 0.5 mg/kg cadmium chloride administration to rats for 3 months led to increases in MDA levels together with significant decreases in SOD activity in kidneys, liver, and testes. Manca et al. [10] reported that different doses of cadmium administration increased lipid peroxidation in the organs and brought about changes in antioxidant defense systems.

Consistent with previous reports, in the present study, we show that after 1 month treatment with 1 mg cadmium chloride per kilogram weight to male rats caused severe histological changes in the liver and kidney, with evidence of significantly increased MDA and decreased SOD activity ($p<0.05$). The figures display an evident stress in rats that were administered cadmium only. We hold the opinion that the damage in the tissues resulted from the damage in cell membranes and DNA damage, whereas the damage in the erythrocytes stemmed from hemolysis caused by cadmium (Stoh et al. [7]). Reversal of the changes by melatonin, vitamin E, and selenium is consistent with previous results in which various antioxidants were shown to be effective against to cadmium-induced oxidative stress [2, 7, 9, 23, 25].

Several studies have shown that melatonin acts as a free radical scavenger, quenching hydroxyl radicals, superoxide ion radicals, singlet oxygen, and peroxide, and that it seemed to also boost the activities of SOD and GSH-Px [26–28]. In other study, rats that were treated with subcutaneous injection of cadmium at a dose similar to that of our study showed improvement of hepatic damage if treated with 10 mg/kg body weight melatonin for 2 weeks [29]. Melatonin also restored lipid peroxidation caused by cadmium in hamsters [30].

The results of our study are consistent with these reports, demonstrating that melatonin, by itself and combined with vitamin E and selenium, significantly improved damage from cadmium-induced oxidative ($p<0.05$). This effect can be explained by its free-radical scavenging effect as well as its stimulation of the antioxidant enzymes SOD and GSH-Px.

Our results regarding MDA and SOD levels after treatment with selenium and vitamin E are consistent with dose of several researchers. In a recent study by Xiao et al. [20], zinc and selenium showed protective effects against cadmium-induced oxidative stress. In addition, Stajn et al. [14] found that oral administration of 200 ppm Cd and 0.1 ppm Se to rats for 1 month prevented lipidperoxidation caused by cadmium and increased SOD activity. Shaikh et al. [2] reported that such antioxidants as vitamin E or *N*-acetylsysteine were effective against hepato-toxicity or renal toxicity caused by cadmium and reduced oxidative stress. In trace amounts, selenium is of vital importance in mammalians as part of the antioxidant system [25]. Vitamin E, also known as an antioxidant at the cell membrane, functions as a scavenger during the reduction of oxygen molecules causing oxidative stress [31]. GSH-Px has two forms: selenium dependent and selenium independent. We are of the opinion that the increases observed in GSH-Px and SOD activity as a result of exogenous selenium and vitamin E administration can be explained by these effects. Our findings associated with MDA, SOD, and GSH-Px levels obtained after selenium and vitamin E administration are parallel to the results reported by Gupta, Shaikh et al., Xiao et al., and Stajn et al.

It should be noted that the effects of melatonin, vitamin E, and selenium did not significantly differ from those of vitamin E and selenium only. Pathological examinations also gave similar findings.

In conclusion, we saw that melatonin, vitamin E + Se, and vitamin E + Se + MLT administration played a protective role against cadmium-induced lipid peroxidation and liver and kidney injury in rats.

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