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Protective effect of selenium against oxidative stress during ovariectomy and ovariectomy-cadmium exposure on blood parameters and antioxidant enzymes (Peroxidase and catalase) activities in female Rats

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ABSTRACT

Ovariectomized (Ovx) sexually mature adult female rats were exposed to sub chronic treatment of cadmium chloride ($CdCl_2$) at a dose of 6 mg/kg/ body weight/rat then, were orally gavaged daily with 2ml of the treatment for a period of 28 days resulting, significant reduction in Erythrocyte count (RBCs), hematocrit percentage (Hct%) and Hemoglobin value (Hgb), While mean corpuscles hemoglobin concentration (MCHC) was increased. Moreover, hepatic and renal antioxidant enzymes activities, peroxidase and catalase (CAT) were decreased significantly in comparison with the control. The consequences of ovariectomy operation of female rats exhibited almost the same values of all of the above mentioned Ovx-Cd treatment parameters. Both the experimental rat groups (Ovx and Ovx-Cd) were gavaged orally with a 2 ml solution of the dietary supplementation (selenium chloride $SeCl_2$) individually or in combination with $CdCl_2$ at a dose of 6 mg/kg/b.w/rat, resulting recovery effects. Blood parameters and antioxidant enzymatic activities were almost restored to control level except, for peroxidase activity that responded differently in both types of treatment. The present study revealed significant protective action of selenium on the toxicity induced by Ovx or Ovx-Cd in female rats, especially when selenium was subsequently supplemented to both treatments.

KEYWORDS

Ovariectomy;
Cadmium;
Selenium;
Blood parameters;
Peroxidase;
CAT.

INTRODUCTION

Cadmium is one of the most toxic substances in the environment with wide range of organ toxicity. Along elimination half-life, accumulates in blood, stored primarily in the kidney and liver and excreted through glomuerular filtration in the kidney and may have toxic effect on several organs e.g. hematopoietic system, the liver and kidney[1]. It has been proposed that heavy metals acknowledge shortened life span of erythrocytes and inhibit of hemoglobin synthesis[2].

Cadmium has been shown to stimulate the production of intracellular reactive oxygen species (ROS), with membrane protein damage and biomolecules may lead to cellular damage and lipid peroxidation when the rate of ROS generation surpasses the rate of its decomposition by antioxidant defense system,such as the enzymes peroxidase and catalase[3-4]. Several studies have in-

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vestigated the effect of ovariectomy or Cadmium-Ovx on antioxidant system and lipid peroxidation[5-7]. The intervention of cellular antioxidant system is believed to protect the cell to some extent from damages arising from Cd-induced ROS production[8-9].

Cadmium treatment alone or in combination with ovariectomy leads to an important elevation of lipid peroxidation (PLO) and decline of antioxidant enzymatic activities such as catalase[10]. He mechanism by which female rats are protected against metal toxicity may be related to the antioxidant properties of native estrogens[11]. In the literature, protective effects of estrogen are widely described in both animals and human, studies demonstrated that in ovariectomy when estrogen was removed, antioxidant enzymes activity was decreased and the free radicals generation was elevated[12-14].

Few studies investigating the combined effects of cadmium and selenium on antioxidant system and lipid peroxidation are available. Thus, we report here the evaluating and prolonged pairing effect of Cd and Se administration on ovariectomized rats and determing the protective effects of Selenium against both oxidative stress induced by ovariectomy and Cd-ovariectomy.

MATERIALS AND METHODS

Female rats Albino Wistar type weighing (250-300g), were housed in laboratory and maintained at room temperature ($25^0C\pm 2$) and allowed water and food add libitum. Rats were randomly divided to five experimental groups, including (5) animals per group. 1- Normal chow diet with negative control vehicle (intact rat). 2- Normal chow diet with ovariectomized rat (positive control). 3-Normal chow diet with ovariectomized -cadmium chloride at a dose of 6mg/kg/b.w/rats. 4- Normal chow diet with equal mixed volumes of cadmium chloride and selenium chloride at a dose of 6 mg/kg/bw. 5-Normal chow diet with ovariectomized - selenium chloride at a dose of 6 mg/kg/bw. All treatments were gavaged with 2ml solution daily by oral means Rats in groups 2, 3, 5 were anaesthetized with ether and underwent a bilateral ovariectomy via ventral incision.Ovaries were removed and oviducts replaced with minimum disruption to surrounding soft tissues and the incisions were closed with clips. At the end of the experiments, animals were killed by anesthetic overdose using ether. Blood was drawn via the dorsal vena cava and placed into heparinized tubes in spiramix and analyzed using automatic hematological analyzer (Coulter ACT differential analyzer, Documentation, Bakman Coulter, Inc. Industrial Estate, Germany).The red blood cells count (RBCs), hemoglobin value (Hgb), hematocrit percentage Hct% and mean corpuscular hemoglobin concentration(MCHC) were recorded.

Liver and kidney tissue extraction were prepared based on the method described by (Gerado *et* al2007) method.Briefly, 100 mg tissue was sliced into pieces and homogenized in ice-cold phosphate buffer (50 mmol/l, pH.7.0, containing 0.1 mmol/l EDTA to give a 5 % (w/v) homogenate, thus centrifuged at 3000 g for 10 minutes at $0\ ^0C$. The supernatants used for enzyme assay.

Lipid peroxidation (indicated based on peroxidase activity assay) was performed according to Chance, et al.[15]. Briefly, the reaction mixture contained 5 mL of 50 mM sodium phosphate buffer, PH.7.0, 0.1 mL of 20 mM guaiacol and 0.02 ml of 40 mM hudrogen peroxide (H_2O_2). The reaction started by adding 0.2 ml 0f enzyme extract (prepared as described previously). Enzyme activity determination was performed at 20^0C by measuring the rate of colour development at 470 nm using Shiamdzu-SP1650-double beam spectrophotometer. The coeffeicient (Σ_{470}) of tetraguaicol is 26.6 cm^{-1} mM^{-1}.

Catalase (CAT) activity was determined according to the method described by Aebi[16] 1984 with slight modification.The enzymatic decomposition of H_2O_2 is followed directly by the decrease in absorbance at 240nm. The differences in absorbance per unit time were used as measure of CAT activity. The enzyme activity is given in U/mg/ protein.

RESULTS

The blood parameters, Erythrocyte count (RBCs), Hemoglobin value (Hgb), Hematocrit %(Hct %) and Mean corpuscles hemoglobin concentration (MCHC) are presented in TABLE 1. Regardless of ovariectomy RBCs, Hgb and Hct% were decreased, while MCHC) was increased by Cadmium-ovariectomized exposure in comparison to control. The blood parameters were also changed due to ovariectomy from those of intact females. The above decrease in blood parameters val-

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TABLE 1: Hematological analyses of rat blood after different treatments, Intact (-Ve control), ovariectomized (Ovx), +Ve control, cadmium-ovariectomized Cd/Ovx, cadmium/selenium combination-Ovariectomized Cd+Se/Ovx, ovariectomy-selenium Se/ Ovx

Group	$RBC_S \times 10^6$ / mm^3	Hgb g /dl	HCT %	MCHC (g / dl)
-Ve control (intact)	7.84±0.379	16.2±0.44	46.1±1.89	34.68±2.11
+Ve control (Ovx)	7.31±0.399	13.98±1.875	41.68±2.352	35.54±0.364
Cd / Ovx	6.564*±0.301	12.88*±0.443	39.24*±1.537	38.834*±0.707
Cd+Se / Ovx	6.972±0.628	13.9±0.295	39.88±0.567	35.02±0.356
Se/ Ovx	7.06**±0.223	14.22**±1.132	40.52±2.935	34. **86±270

The data represent mean ± SD. Asterisks (*P < 0.05) indicate significant difference from control. Asterisks (P<0.05) indicate significant difference from Ovx /Cd**

TABLE 2 : Effect of cadmium, cadmium–selenium combination, ovariectomy, ovariectomy -selenium on peroxidase and catalase activity in liver and kidney of rat

Groups	Treatment			
	Peroxidase (U/mg/protein)		CAT (U/mg/protein)	
	Liver	Kidney	Liver	Kidney
-Ve control (intact)	$10.6\times10^{-3}\pm 0.12\times10^{-3}$	$13.7\times10^{-3}\pm 0.4\times10^{-3}$	24.24×1.5	22.38×2.4
+Ve control (Ovx)	$4.8^*\times10^{-3}\pm 0.12\times10^{-3}$	$7.9^*\times10^{-3}\pm 0.3\times10^{-3}$	16.17*×1.3	17.4*×1.4
Cd / Ovx	$1.4^{**}\times10^{-3}\pm 3.6\times10^{-3}$	$1.3^{**}\times10^{-3}\pm 0.17\times10^{-3}$	7.614**×0.2	5.75**×0.25
Cd+Se / Ovx	$3.8^{***}\times10^{-3}\pm 0.14\times10^{-3}$	$6.3^{***}\times10^{-3}\pm 0.32\times10^{-3}$	18.43***±0.39	17.9***2±2
Se/ Ovx	$4.1^{***}\times10^{-3}\pm 0.2\times10^{-3}$	$7.3^{***}\times10^{-3}\pm 0.25\times10^{-3}$	20.543***±1.4	19.79***±1.2

The data represent mean ± SD. Asterisks (*P < 0.05) indicate significant difference from -Ve control. Asterisks (P<0.05) indicate significant difference from Ovx. Asterisks (***P < 0.05) indicate significant difference from Ovx / Cd**

ues was grater in Cadmium-ovariectomized rats as compared with Ovariectomized females, as well as with those animals experimentally supplemented with both Se/Cd combination or Se alone. The MCHC was significantly increased in Cadmium-ovariectomized rats while the change was not significant in both ovariectomized and selenium supplemented group rats either in Cd-combination or alone as compared to intact control rat group.

TABLE 2 shows the antioxidant enzymes status indicated by peroxidase and catalase activities in liver and kidney after sub chronic treatments on Ovariectomized, Ovariectomized-Cd, Ovariectomized/Cd-Se combination and Ovariectomized/Se. Peroxidase was significantly decreased (P<0.05) in both ovariectomized and ovariectomized-Cd exposed rats as compared to the control, although the ovariectomized rats exposed to Cd had a deleterious effect, since peroxidase enzyme activity was dropped sharply as compared to that in ovariectomy. Catalase (CAT) activity was significantly decreased(P<0.05) in both ovariectomized and Ovx-Cd treated rat groups as compared to control animals. The degree of enzymes activity decrease was sever in Ovx-Cd groups in comparison with ovariectomized group. The selenium dietary supplementation alone or simultaneously given in combination with Cd to Ovx and-Ovx cadmium rat groups, increase the enzyme activities significantly. Regarding peroxidase activity was different, since there were not restored to the control level.

DISCUSSION

The effects of ovariectomy and cadmium-Ovx exposure on blood parameters were differ from the intact control rats TABLE 1. The RBC count, Hgb value and Hct % in the experimental animals, either in Ovx or Ovx –Cd treated rats were found to be lower than in intact rat controls. Whereas there was an increase in MCHC value in cadmium-ovariectomized rats, which may be due to the production of inadequate maturation of RBC at rate exceeding the rate of normal RBC production, as described for other metal effect on hematopoiesis process[17].

The decrease in erythrocyte count, hemoglobin values and hemocrit percentage indicated that anemia was less severe in Ovariectomized-selenium treated females in comparison to Ovx-Cd treated group. It is known that metals impair the heme synthesis and is probably related to the inhibition of the hemoglobin synthesis and to various alteration of erythrocyte membrane properties, leading to an increased fragility, deformability, RBC destruction and eventually a shortened life span[2,18,19]. A part from directed inducing, the generation of reac-

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tive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), an element can indirectly induce oxidative stress by increase the vulnerability of membrane to the attack of ROS. The effect of metal on red blood cells membrane in particular have been intensely analyzed because RBC are more vulnerable to oxidative damage than many other cells[20]. One mechanism by which metals produce injury is assumed to be through generation of free radicals and lipid peroxidation. Free radicals in the living body are highly reactive and can cause several disorders[21,22] found that hemolysis induced by hemolytic agents such as heavy metals was associated with a high degree of peroxidation.

Peroxidase is one of the major components of the antioxidant system[23]. In the present study, (TABLE 2) showed an increase amount of lipid peroxidation, due to a sharp decrease in peroxidase and catalase antioxidant enzymatic activities, following both ovariectomy operation and Cd-Ovx treatment, as compared to control. This demonstrated that Ovx and Cd-Ovx treated rats impair the enzymatic antioxidant defense system in liver and kidney tissues.

The decline in peroxidase and CAT activities in Ovx and Cd- Ovx treated rats may be due to inhibition of its ability in the scavenging of free radicals. As it has been reported that Ovariectomy could trigger lipid peroxidation, which in turn reduce the enzymatic antioxidant activities. As a consequence the production of ROS enhanced due to the removal of estrogen produced normally by ovaries[24].

Both enzymatic activities that had restoring effect to the control level in studied parameters after antidotes supplementation may be due to its protection effect against metal-induced oxidative stress. Recent investigations mentioned that antidotes could enhances cadmium transport and decrease its uptake in rat intestine and produce protection against cadmium-induced anemia[25-27]. Apostolski et al.[28] study demonstrate that exogenous administration of selenium have some stimulatory effect on ovarian peroxidase activity.

In the literature, protective effect of estrogen is widely described in both humans and animals[29]. Estrogens may be able to inhibit the generation of free radicals and decrease peroxide levels resulting free radical suppression in rat liver[12,30,31]. Thus the possibility exist that antioxidant such as selenium in the present study, could decrease or prevent some pathological consequences of metal toxicities. Results on other metals effects hypothesized that endogenous estrogen have antioxidant capability[14]. This could explain the differences between intact and ovariectomy in the expression of cadmium exposure effects.

It can concluded that ovariectomy and cadmium/ ovariectomy can increase the production of ROS that results in RBC membrane destruction and function beside the impairment of antioxidant enzymes activities.The antidotes selenium supplementation could replace the act of ovarian estrogen in its protective action.

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