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Original article

Protective effects of oestradiol against cadmium-induced changes in blood parameters and oxidative damage in rats

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The aim of this study was to investigate the protective effects of oestradiol (E2, 4 mg kg^{-1} b.w. *i.p.*) against cadmium-induced (Cd, 2 mg kg^{-1} b.w. *i.p.*) blood changes in rats. Cadmium induced a significant decline in haemoglobin, haematocrit, and total erythrocyte, lymphocyte, and thrombocyte count, whereas total leukocytes and granulocytes increased. A significant increase was also observed in serum cholesterol, triglycerides, glucose, AST, and ALT activities, whereas total protein and albumin levels dropped significantly. Administration of E2 in combination with Cd alleviated most of these adverse effects. In terms of oxidative stress, Cd significantly increased oxygen-free radicals ($O_2^{\bullet-}$ and H_2O_2) in neutrophils and lipid peroxidation in erythrocytes, whereas E2 treatment reversed these changes to control values. Acute Cd poisoning significantly lowered antioxidant enzyme (SOD and CAT) activity and the level of non-enzymatic antioxidants (GSH and vitamin E), while increasing in GSSG. Treatments with E2 reversed Cd-induced effects on the antioxidant defences and significantly lowered Cd-induced oxidative damage in erythrocytes. This study suggests that exogenous E2 effectively restores redox balance in rat erythrocytes and counters adverse haematological and biochemical effects of Cd poisoning. It also improves the antioxidant capacity of erythrocytes, acting in synergy with endogenous antioxidants.

KEY WORDS: *antioxidant defence system; biochemical parameters; erythrocytes; haematological parameters; heavy metals; oxidative stress; reactive oxygen species; sex hormone*

Exposure to Cd can results in toxic effects on a variety of tissues, but the first to be affected is blood, as Cd binds to the membrane of erythrocytes and plasma albumin and is then transported to the liver (1), causing haematological and biochemical changes (2) and anaemia (3-5). Furthermore, erythrocytes are the most common markers of oxidative stress due to the sensitivity of their cell membranes and antioxidant enzymes to free radicals (6, 7).

The mechanism of acute Cd toxicity involves depletion of glutathione, which results in increased production of reactive oxygen species (ROS) such as superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (•OH) (8, 9). This in turn leads to increased lipid peroxidation, DNA damage, protein oxidation, and eventually to cell dysfunction and necrosis (10).

Female sex hormone oestradiol (17β-oestradiol, E2) inhibits lipid peroxidation caused by free radicals (11) and improves intracellular SOD and CAT activity (12). It can act directly, by scavenging free radicals and by chelating redox-active metal ions or indirectly, by regulating antioxidant enzyme expression (13).

A number of studies have already established its protective role in the cardiovascular and nervous system (14, 15), but little is known about its effects against Cd toxicity in blood *in vivo*.

The aim of this study was to address this gap by investigating how E2 counters the effects of Cd on haematological, biochemical, and oxidative stress parameters, including antioxidant defences in the blood of acutely exposed rats. We started with the assumption that the protective mechanism of E2 against Cd-induced toxicity is based on its antioxidative action and therefore investigated how it counters Cd-induced oxidative burst in neutrophils and redox imbalance in erythrocytes as markers of oxidative stress. Given the complexity of E2 action and difficulties in distinguishing its genomic from nongenomic effects, this study aimed to better characterise its beneficial effects *in vivo*. We hope that our findings will have practical implications for E2 therapy in conditions of metal-induced oxidative stress.

MATERIALS AND METHODS

Chemicals

Chemicals for this study were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and Merck (Darmstadt, Germany). All reagents and chemicals were of analytical grade or higher purity.

Animals

The study included 32 male, eight-week-old Wistar albino rats weighing 200-220 g. As we wanted to investigate non-genomic effects of oestradiol, we used only male rats to avoid interaction between exogenous oestradiol and oestrogen receptors as well as hormonal changes during the oestrous cycle (16). The animals were maintained in plastic cages under standard laboratory conditions (temperature 22±2 °C; 12-hour light/dark cycle). The rats had free access to drinking water and standard rodent laboratory diet. All animal experiments were approved by the institutional ethics committee.

Experimental design

Animals were randomly divided into four groups of eight. Group 1 served as control and received saline [0.1 mL kg^{-1} body weight (b.w.)], whereas the remaining three experimental groups received single intraperitoneal (*i.p.*) injections as follows: group 2 - 2 mg kg^{-1} (b.w.) of $CdCl_2$ in 0.1 mL saline; group 3 - 4 mg kg^{-1} (b.w.) of 17β-oestradiol; and group 4 - 17β-oestradiol 24 h after $CdCl_2$ in the above doses. The animals were anaesthetised with ether and decapitated 24 h after treatment.

On the day of sacrifice blood samples were collected in K-EDTA tubes for haematological analysis or in tubes without anticoagulants for other analyses. Haematological and biochemical parameters were measured on the day of sacrifice.

Analytical procedures

Haematological analysis included haemoglobin (Hb), haematocrit (Hct), total erythrocyte (TEC), total leukocyte (TLC), lymphocyte, granulocyte, and platelet (PLT) count using standard methods and was performed with an automated haematology analyser (CELLY70, Biocode Hycel, Massy, France).

Blood biochemistry included serum concentrations of total protein, albumin, total cholesterol, triglycerides, glucose, alanine aminotransferase (ALT), and aspartate aminotransferase (AST), measured on an autoanalyser (Architect C8000, Wiesbaden, Germany) using standard diagnostic kits (Abbott Laboratories, Abbott Park, IL, USA). The rest of the samples were stored at -20 °C until analysis but for no longer than seven days.

To measure oxidative stress parameters, blood samples were centrifuged at 1000 *g* (+4 °C) for 10 min and serum was removed. Erythrocytes were washed three times with an equal volume of cold saline (0.9 %, *v/v*), and 1 mL of washed erythrocytes was lysed on ice in 3 mL of dH_2O (0 °C) for 30 min. To determine the levels of superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), we extracted erythrocyte lysates by adding 0.25 mL of 3 mol L^{-1} perchloric acid and 1 mL of 0.02 mol L^{-1} EDTA to 0.5 mL of lysate. After extraction on ice and centrifugation at 1000 *g* for 10 min, lysate extracts were neutralised with 2 mol L^{-1} K_2CO_3.

The determination of $O_2^{\bullet-}$ was based on the reduction of nitroblue tetrazolium (NBT) in the presence of $O_2^{\bullet-}$ (17). The determination of H_2O_2 was based on the oxidation of phenol red (PR) in the presence of horseradish peroxidase (HRPO) as catalyst (18). Both measurements were done on a JENWAY 6105 UV-Vis spectrophotometer (Bibby Scientific Ltd., Staffordshire, UK) and concentrations are expressed as μmol L^{-1} of erythrocytes.

Lipid peroxidation was determined according to method of Ohkawa et al. (19), based on the reaction of lipid peroxidation products malondialdehyde (MDA) with thiobarbituric acid (TBA). Briefly, haemolysate samples were extracted by adding 0.4 mL of 28 % trichloroacetic acid (TCA) to 0.8 mL of lysate and centrifuged at 1000 *g* for 10 min. Colour reaction was obtained out by adding 0.2 mL of 1 % TBA and incubating the samples in a bath at 90 °C for 15 min. These results are expressed in μmol L^{-1} of erythrocytes using a molar extinction coefficient for MDA.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined using the method of Marklund and Marklund (20), based on pyrogallol oxidation by $O_2^{\bullet-}$ and its dismutation by SOD. Enzyme activity is expressed as units per milligram (U mg^{-1}) of haemoglobin.

Catalase (CAT, EC 1.11.1.6) activity was measured with the JENWAY 6105 UV-Vis spectrophotometer at 230 nm (21) as the rate of H_2O_2 degradation by CAT and is expressed in μmol min^{-1} g^{-1} of haemoglobin.

Reduced glutathione (GSH) was determined using the method of Beutler (22), based on GSH oxidation with 5,5'-dithiobis(2-nitrobenzoic acid) and its concentrations expressed as mmol L^{-1} of erythrocytes. The concentrations of oxidised glutathione (GSSG) were determined after enzymatic reaction with glutathione reductase (23) after inhibition of GSH oxidation by *N*-ethylmaleimide, and expressed in μmol L^{-1} of erythrocytes. In both cases, haemolysate samples were extracted by adding 0.4 mL of 0.1 % EDTA and 0.75 mL of precipitation solution (containing 25 % HPO_3, 0.005 mol L^{-1} EDTA and 0.1 mol L^{-1} phosphate buffer) to 0.1 mL of lysate and centrifuging the mix at 1000 *g* for 10 min.

Vitamin E was determined in haemolysate extracts (obtained by adding 0.05 mL of 1 % EDTA to 0.5 mL of lysate) by measuring the reduction of ferric into ferrous ions in the presence of tocopherol and production of coloured complex with bathophenanthroline (24). The absorbance of the produced complex was measured spectrophotometrically (JENWAY 6105 UV-Vis spectrophotometer) at 535 nm. Vitamin E concentrations are expressed in mg L^{-1} of erythrocytes.

Neutrophils were isolated according to the method of Russo-Carbolante et al. (25). Aliquots of blood were collected into plastic tubes containing K-EDTA and Histopaque reagent (1077 mg mL^{-1}) was added. The tubes were centrifuged at 400 *g* for 45 min and the upper phase removed. The lower phase was added 6 % dextran solution (in 0.15 mol L^{-1} NaCl) and PBS and incubated at 37 °C for 20 min. To lyse erythrocytes, the pellet was resuspended in 0.83 % NH_4Cl and after 5 min centrifuged at 480 *g* for 10 min. After washing with PBS, the cells were resuspended in 1 mL of PBS in the concentration of 10^6 cells mL^{-1}. The obtained neutrophils were used to measure $O_2^{\bullet-}$ and H_2O_2 levels using a modified colorimetric NBT test (26) and an ELISA reader (Optic Ivymen System, Model 2100C, Biotech SL, Madrid, Spain). The results are expressed as optical density (OD) of 10^6 cells mL^{-1}.

Statistical analysis

All data were evaluated using the SPSS for Windows software, version 13 (SPSS Inc., Chicago, IL, USA). The results are expressed as mean±standard error of the mean (SEM). Comparisons were made using either factorial analysis of variance (ANOVA) with a post-hoc Bonferroni/Dunnett's multiple analysis or Kruskal-Wallis test (for comparison across several groups) and Mann-Whitney U-test (for comparison between two groups). Differences at $p<0.05$ were considered statistically significant.

RESULTS

Haematological and biochemical findings

Acute poisoning of rats with Cd significantly lowered Hb, Hct, TEC, lymphocyte, and PLT counts and increased TLC and granulocytes. Administration of E2 in combination with Cd alleviated the harmful effects of Cd on most haematological parameters (Table 1).

Table 2 shows significant increase in serum cholesterol, triglycerides, glucose, AST, and ALT, while total protein and albumin levels significantly dropped in the Cd group compared to control. Treatment with Cd+E2 significantly reversed these effects to nearly normal values compared to the Cd group.

Oxidative stress parameters

After treatment with Cd, H_2O_2 concentration in erythrocytes dropped significantly compared to control, whereas $O_2^{\bullet-}$ increased, but not significantly. E2 treatment did not affect $O_2^{\bullet-}$ and H_2O_2 in erythrocytes (Figure 1). In contrast, neutrophil concentrations of $O_2^{\bullet-}$ and H_2O_2 significantly increased after Cd

Table 1 *Protective effect of E2 (4 mg kg^{-1} b.w.* i.p.*) treatment on haematological parameters against Cd toxicity (2 mg kg^{-1} b.w.* i.p.*)*

Parameters	Experimental groups (n=8 each)			
	Control	Cd (2 mg kg^{-1})	E2 (4 mg kg^{-1})	Cd+E2
TEC (10^{12} L^{-1})[a]	7.32±0.24	5.14±0.38*	7.02±0.05$^{\#}$	6.72±0.16$^{\#}$
Hb (g dL^{-1})[b]	14.41±0.47	10.22±0.45*	13.84±0.68$^{\#}$	13.06±0.42$^{\#}$
Hct (%)[b]	40.56±0.76	34.70±0.27*	36.22±2.27	37,65±0.19$^{\#}$
TLC (10^{9} L^{-1})[a]	5.34±0.25	17.04±1.05*	6.14±0.52$^{\#}$	13.12±1.15$^{*\#}$
Lymphocyte (%)[b]	68.62±2.24	19.92±0.46*	47.64±2.15$^{\#}$	59.36±1.83$^{\#}$
Granulocyte (%)[b]	22.42±0.34	65.90±3.25*	40.14±2.46$^{*\#}$	29.38±0.65$^{\#}$
PLT (10^{9} L^{-1})[b]	732.4±24.2	519.6±30.9*	640.4±31.4	599.0±24.6*

E2- oestradiol; TEC - total erythrocyte count; Hb- haemoglobin; Hct - haematocrit; TLC- total leukocyte count; PLT - platelets
Each value represents mean±SEM * $p<0.05$ *compared to control;* $^{\#}$ $p<0.05$ *compared to Cd-treated group ([a]according to ANOVA; [b]according to the Kruskal-Wallis test)*

Table 2 *Protective effect of E2 (4 mg kg^{-1} b.w.* i.p.*) treatment on biochemical parameters against Cd toxicity (2 mg kg^{-1} b.w.* i.p.*)*

Parameters	Experimental groups (n=8 each)			
	Control	Cd (2 mg kg^{-1})	E2 (4 mg kg^{-1})	Cd+E2
Total protein (g L^{-1})[a]	62.70±1.96	53.25±1.32*	64.62±1.57$^{\#}$	57.48±2.84$^{\#}$
Albumin (g L^{-1})[b]	15.2±0.38	10.4±0.16*	15.8±0.24$^{\#}$	12.7±0.28$^{*\#}$
Cholesterol (mmol L^{-1})[a]	1.35±0.08	2.08±0.11*	1.17±0.09$^{\#}$	1,14±0.03$^{\#}$
Triglycerides (mmol L^{-1})[a]	0.55±0.03	0.87±0.04*	0.68±0.06$^{\#}$	0.59±0.02$^{\#}$
Glucose (mmol L^{-1}) [a]	4.76±0.22	7.14±0.29*	5.67±0.15$^{\#}$	6.37±0.23$^{\#}$
AST (U L^{-1})[a]	194.8±2.64	312.6±2.45*	176.4±2.17$^{\#}$	201.8±2.45$^{\#}$
ALT (U L^{-1})[b]	70.3±1.78	139.6±1.24*	73.6±1.82$^{\#}$	91.7±1.65$^{\#}$

E2- oestradiol;AST-aspartate aminotransferase; ALT- alanine aminotransferase
Each value represents mean±S.E.M. * $p<0.05$ *compared to control;* $^{\#}$ $p<0.05$ *compared to Cd-treated group ([a]according to ANOVA; [b]according to the Kruskal-Wallis test)*

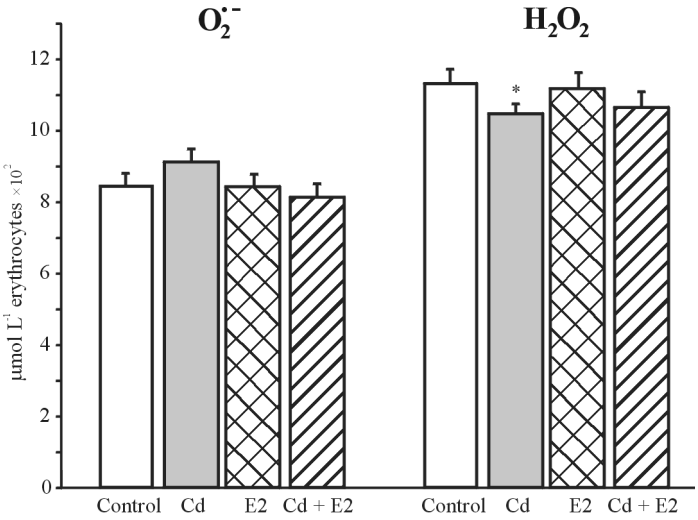

Figure 1 *Protective effect of E2 (4 mg kg^{-1} b.w.* i.p.*) treatment on superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) concentrations in erythrocytes of rats against Cd toxicity (2 mg kg^{-1} b.w.* i.p.*). E2- oestradiol. Each value represents mean±SEM (n=8 animals)* * $p<0.05$ *compared to control (according to the Kruskal-Wallis test)*

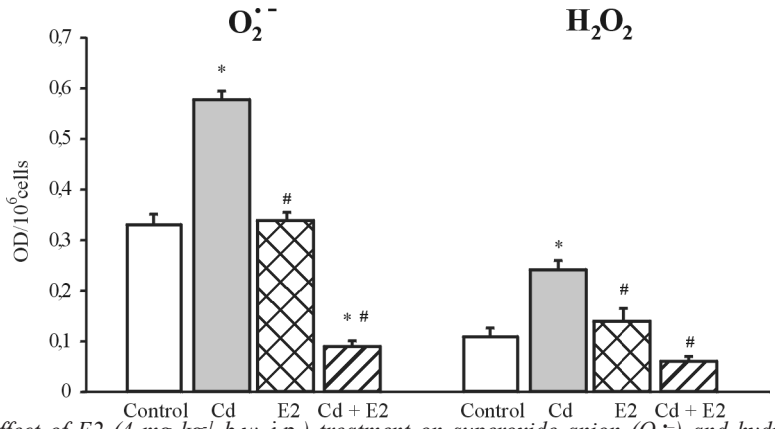

Figure 2 *Protective effect of E2 (4 mg kg^{-1} b.w.* i.p.*) treatment on superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) concentrations in neutrophils of rats against Cd toxicity (2 mg kg^{-1} b.w.* i.p.*). E2- oestradiol. Each value represents mean±SEM (n=8 animals)* * $p<0.05$ *compared to control;* $^{\#}$ $p<0.05$ *compared to Cd-treated group (according to the Kruskal-Wallis test). OD - optic density*

treatment, while E2 lowered them significantly (Figure 2). Treatment with E2 reversed Cd-induced changes in these oxidative stress parameters.

Cadmium significantly increased LPO in erythrocytes, whereas treatment with E2 restored it to control levels (Figure 3).

Antioxidant defence enzymes SOD and CAT significantly dropped in Cd-treated rats, whereas, again, E2 treatment restored these to control values (Figure 4).

Table 3 shows changes in GSH, GSSG, and vitamin E. As with other parameters, E2 significantly reversed Cd-induced changes.

DISCUSSION

Our findings agree with earlier reports (3, 5, 27-29) and confirm the ability of Cd to induce oxidative stress in rat erythrocytes. ROS production and lipid peroxidation induced by acute Cd treatment resulted in erythrocyte haemolysis and anaemia, in the activation of immune system, and in disorders of carbohydrate and lipid metabolism in hepatocytes.

Oestradiol seems to efficiently counter the adverse haematological and biochemical effects of Cd in rats and may substantially reinforce endogenous protection against oxidative stress.

The most important findings of this study are briefly discussed below. Consistent with other studies (30, 31), E2 showed beneficial effects on carbohydrate metabolism as it lowered high blood glucose in Cd-treated rats. The observed drop in AST and ALT may indicate protective activity of E2 against hepatocyte damage. In addition, E2 alleviated inflammation. According to previous reports (32, 33), this was probably mediated by reduced spontaneous secretion of inflammatory cytokines and migration of leukocytes into inflamed areas.

Our results show that Cd increased granulocyte count and induced neutrophil oxidative burst. Some of the molecular mechanisms of Cd toxicity, as reported in a review by Waisberg et al. (1), include release of a large amount of $O_2^{\bullet -}$ and H_2O_2 from activated neutrophils into circulation, which leads to the influx of ROS in erythrocytes. In our study, acute

Table 3 *Protective effect of E2 (4 mg kg^{-1} b.w.* i.p.*) treatment on GSH, GSSG, and Vit E against Cd toxicity (2 mg kg^{-1} b.w.* i.p.*)*

Parameters	Experimental groups(n=8 each)			
	Control	Cd (2 mg kg^{-1})	E2 (4 mg kg^{-1})	Cd+E2
GSH (mmol L^{-1})[a]	4.59±0.52	3.16±0.18*	4.27±0.18$^{\#}$	3.91±0.27$^{\#}$
GSSG (μmol L^{-1})[b]	0.34±0.10	0.63±0.03*	0.43±0.05	1.19±0.13$^{*\#}$
Vit E (mg L^{-1}) [b]	31.93±0.33	24.26±0.37*	35.61±3.16$^{\#}$	32.54±4.70$^{\#}$

E2- oestradiol; GSH-reduced glutathione; GSSG-oxidized glutathione; Vit E-vitamin E
Each value represents mean±S.E.M. * $p<0.05$ *compared to control;* $^{\#}$ $p<0.05$ *compared to Cd-treated group ([a]according to ANOVA; [b]according to the Kruskal-Wallis test)*

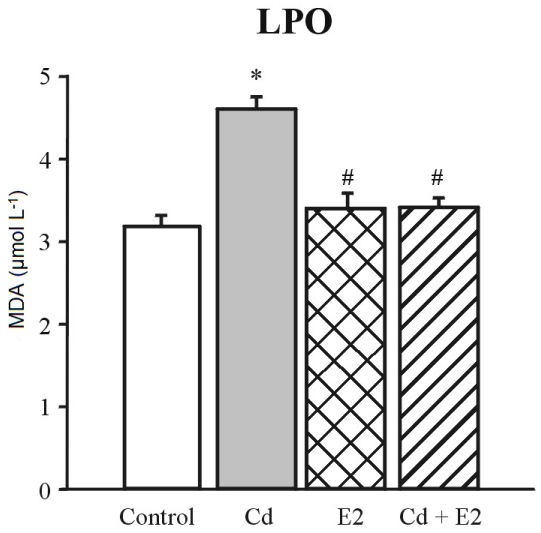

Figure 3 *Protective effect of E2 (4 mg kg^{-1} b.w.* i.p.*) treatment on lipid peroxide (LPO) concentration in erythrocytes of rats against Cd toxicity (2 mg kg^{-1} b.w.* i.p.*). E2 - oestradiol. Each value represents mean±SEM (n=8 animals) * p<0.05 compared to control; # p<0.05 compared to Cd-treated group (according to the Kruskal-Wallis test). MDA - Malondialdehyde*

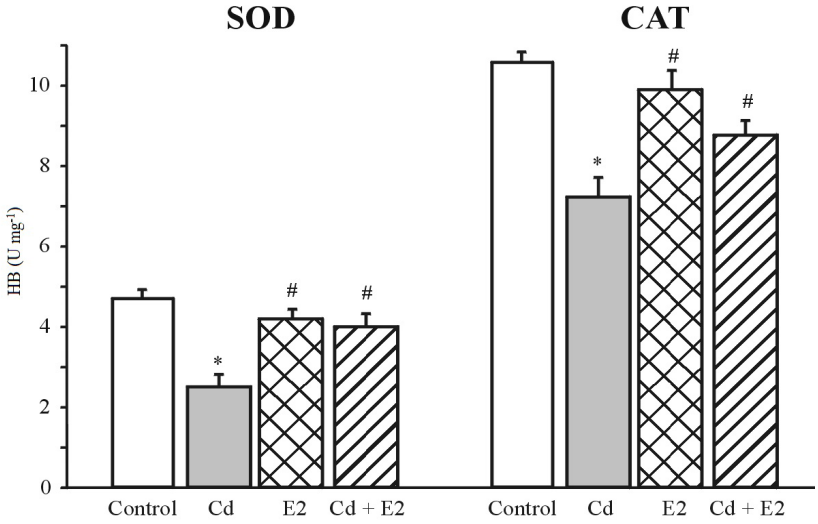

Figure 4 *Protective effect of E2 (4 mg kg^{-1} b.w.* i.p.*) treatment on superoxide dismutase (SOD) and catalase (CAT) activities in erythrocytes of rats against Cd toxicity (2 mg kg^{-1} b.w.* i.p.*). E2- oestradiol. Each value represents mean±SEM (n=8 animals) * p< 0.05 compared to control; # p<0.05 compared to Cd-treated group (according to the Kruskal-Wallis test)*

Cd treatment resulted in significantly lower H_2O_2 concentration and increased LPO concentration in erythrocytes. These cells are capable of producing and storing nitric oxide (NO) (34). Being faster than SOD, NO scavenges $O_2^{\bullet-}$ to form cytotoxic peroxynitrite ($ONOO^-$), which in turn mediates protein nitration and lipid peroxidation (6, 7). Since increased LPO disrupts the normal function or destroys the membrane of erythrocytes, the final outcome of these events is a decrease in haematocrit level, and eventually anaemia, which was also observed in our study.

We found that treatment with E2 significantly reduced the concentration of both $O_2^{\bullet-}$ and H_2O_2 in neutrophils, suggesting that E2 effectively counteracted oxidative burst induced by Cd. This is in line with the study by Priyanka et al. (35), who have reported that this hormone inhibits production and release of large amounts of $O_2^{\bullet-}$ and H_2O_2 from neutrophils that

can pass erythrocyte membranes and induce oxidative stress.

Oestrogens protect cells against oxidative stress in direct or oestrogen receptor-independent manner by decreasing ROS production and preventing intracellular H_2O_2 accumulation (15, 36, 37). By transferring the hydrogen atom from its phenol-hydroxyl ring to $O_2^{\bullet-}$, E2 scavenges oxyradicals and blocks their intracellular accumulation, thus preventing the formation of $ONOO^-$ and lipid peroxidation (11, 13).

Our results have confirmed that E2 counters Cd toxicity by inhibiting LPO in erythrocytes and by preventing haemolysis. Previous studies (11, 13) suggest that this is mediated by the phenol hydroxyl group of E2, which donates hydrogen atoms to a lipid-derived radical. The resulting oestrogen phenoxyl radical is stabilised by delocalisation of unpaired electrons in the aromatic ring (13, 14). Oestrogens thus prevent LPO by sacrificing themselves to oxidation, turning into quinol, which can be formed directly from E2 and ·OH without the participation of metabolic enzymes, and by converting back to E2 using NADPH as the reducing agent without the production of ROS (13, 38). In the erythrocytes of Cd-exposed rats we found lower SOD and CAT activity and a significant depletion of non-enzymatic antioxidants (GSH and vitamin E). Since Cd is able to substitute divalent metals in metalloenzymes (1), the lowering effect of Cd on SOD activity is the consequence of interactions between Cd and Zn or Cu in the SOD molecule. Lower SOD activity reflects on lower H_2O_2 concentration, which finally results in lower CAT activity, whose substrate is H_2O_2 (6, 7).

The degradation of H_2O_2 is also potentiated by vitamin E and GSH (6, 7). Our results confirm the protective effects of E2 against changes in both enzymatic and non-enzymatic antioxidants in rat erythrocytes. These findings are in line with Hambden et al. (30), who found that E2 enhanced SOD and CAT activities in the liver of diabetic rats. Similar protective effects were observed in cultured rat hepatocytes (39), where E2 inhibited ROS generation, lipid peroxidation, as well as the loss of SOD and GSH-Px activities. The results of our study suggest that E2 improves erythrocyte antioxidant capacity due to either direct scavenging of ROS or induction of antioxidant enzymes.

To prevent LPO and cell damage, exogenous antioxidants may act in synergy with endogenous antioxidants (5, 27, 40, 41). Here we investigated two endogenous antioxidants - GSH and vitamin E - and found that E2 treatment increased their concentrations. Molecules rich in –SH groups, such as GSH, have a key role in binding Cd and reducing its toxicity. Dlugosz et al. (38) have suggested that E2 can increase the levels of –SH groups.

CONCLUSION

The results of this study show that exogenous E2 effectively coped with the oxidative overload in rat neutrophils, erythrocyte redox imbalance, and adverse haematological and biochemical effects of acute Cd poisoning. E2 improved erythrocyte antioxidant capacity by acting synergistically with endogenous antioxidants.

We hope that our results will shed more light on direct, nongenomic effects of E2 *in vivo* and provide important information about the protective mechanism of E2 in Cd-induced toxicity. They may also have broader implications for the therapeutic use of E2, especially in postmenopausal women.

Conflicts of interest

The authors declare no conflict of interest.

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Sažetak

Zaštitno djelovanje estradiola protiv promjena krvnih parametara i oksidativnog stresa u štakora izazvanih akutnim trovanjem kadmijem

Cilj ovog istraživanja bio je ispitati moguće zaštitne učinke estradiola (E2, 4 mg kg^{-1} tjelesne težine *i.p.*) protiv kadmijem izazvanih (Cd, 2 mg kg^{-1} tjelesne težine *i.p.*) promjena u krvi štakora. Kadmij je značajno smanjio vrijednosti hemoglobina, hematokrita, eritrocita, limfocita i trombocita te povećao vrijednosti ukupnih leukocita i granulocita. Također je uočen značajan rast razina AST i ALT, serumskog kolesterola, triglicerida i glukoze te pad razina ukupnih proteina i albumina. Primjena E2 u kombinaciji s Cd ublažila je većinu tih štetnih učinaka Cd. U pogledu oksidacijskog stresa, Cd je značajno potaknuo ne samo nastanak slobodnih kisikovih radikala ($O_2^{\bullet-}$ i H_2O_2) u neutrofilima nego i lipidnu peroksidaciju u eritrocitima, a primjena E2 te je promjene svela na kontrolne vrijednosti. Akutno otrovanje kadmijem osjetno je smanjilo aktivnost antioksidativnih enzima SOD i CAT te razinu neenzimskih antioksidansa GSH i vitamina E, a povećalo razinu GSSG. Primjena E2 preokrenula je promjene u sustavu antioksidacijske obrane i značajno spriječila oksidacijska oštećenja u eritrocitima izazvana kadmijem. Prema rezultatima ovog istraživanja, E2 se učinkovito bori s disbalansom redoks statusa eritrocita i negativnim hematološkim i biokemijskim učincima nastalima nakon akutnog trovanja štakora kadmijem. Egzogeno primijenjen E2 pridonosi poboljšanju antioksidativnog kapaciteta eritrocita, djelujući sinergistički s endogenim antioksidansima.

KLJUČNE RIJEČI: *antioksidativni obrambeni sustav; biokemijski parametri; eritrociti; hematološki parametri; spolni hormon; reaktivni oblici kisika; teški metali*

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