Full Length Research Paper

Protective effects of ginger (*Zingiber officinale Roscoe*) against cadmium chloride-induced oxidative stress in the blood of rats

Atef, M. M. Attia[1], Fatma, A. A. Ibrahim[1], Noha, A. Abd EL-Latif[1], Samir, W. Aziz[1] and Sherif A. Abdelmottaleb Moussa[1,2]

[1]Biophysical laboratory, Biochemistry Department, Division of Genetic Engineering and Biotechnology, National Research Center, Dokki, Cairo, Egypt.
[2]Department of Physics, College of Science, Al-Imam Muhammad Ibn Saud Islamic University, P. O. Box 90950, Riyadh 11623, Saudi Arabia.

Cadmium is a heavy metal of wide occupational and environmental contamination. In recent years, however, cadmium has been implicated in the pathogenesis of several clinical disorders. Generation of oxidative stress is one of the plausible mechanisms for cadmium-induced diseases. The aim of the study was to investigate the effect of ginger on oxidative stress in rats exposed to cadmium (Cd) of a dose (10 mg/kg b.w.). Ginger was administered orally (500 mg/kg b.w.). After 26 days, significant increases in methemoglobin% (metHb%), carboxyhemoglobin% (HbCO%), glutathione peroxidase (GPx) activity, malondialdehyde (MDA) concentration and hemolysis% were observed in cadmium exposed rats compared to control group ($P < 0.05$), while glutathione-S-transferase (GST), glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PD) showed insignificant changes. Cadmium treatment of rats caused a significant decrease in oxyhemoglobin% (HbO_2%) and total blood hemoglobin (Hb) concentration ($P < 0.05$). Ginger treatment of cadmium exposed rats significantly lowered metHb% ($P < 0.05$), while significantly increased HbO_2% ($P < 0.05$) and total Hb concentration ($P < 0.01$), compared to cadmium alone group. Also ginger treatment significantly increased GPx and G6PD activities of cadmium exposed rats compared to cadmium alone group ($P < 0.05$). The treatment of Cd-exposed animals with ginger lowered MDA concentration and hemolysis% by 20% and 17%, respectively. From these findings it can be concluded that ginger is a strong antioxidant plant that protects the blood of rats against the adverse harmful effects of cadmium chloride exposure as well as cadmium chloride-induced oxidative stress.

Key words: Cadmium, ginger, oxidative stress, erythrocytes, hemolysis, hemoglobin derivatives, rats.

INTRODUCTION

Cadmium (Cd) is an important industrial and environmental pollutant that currently ranks seventh on

the list of hazardous substances developed by the Agency for Toxic Substances and Disease Registry

jointly with the U.S. Environmental Protection agency (EPA) and available at ATSDR website (ATSDR, 2013). In the environment, Cd is dangerous because humans consume both plants and animals that absorb Cd efficiently and concentrate it within their tissues (ATSDR, 1999). The flow of Cd in ecological systems increases through major sources as mining, smelting and industrial use. Sources of human exposure to this metal include food, water, cigarette smoke and alcoholic beverages (Jarup et al., 1998). Normally, absorption of Cd is increased if the animals are on a low calcium diet. Once absorbed, Cd associates with cysteine residues of the low molecular weight protein, metallothionein and accumulates in the body. Since the metabolism of Cd is closely related to zinc metabolism, metallothionein binds and transports both Cd and zinc. Cadmium displaces zinc in many vital enzymatic reactions, such as causing disruption or cessation of enzyme activities (Bauer et al., 1980; Casalino et al., 2002).

Cadmium causes poisoning in various tissues of humans and animals (Swiergosz et al., 1998; Stohs et al., 2000). In the blood, Cd stimulates the formation of metallothioneins and reactive oxygen species (ROS) thus causing oxidative damage in erythrocytes and in various tissues, which results in a loss of membrane functions (Sarkar et al., 1995; Simpkins et al., 1998). It has been reported that chronic treatment with cadmium, induced oxidative damage in erythrocytes of rats, causing destruction of cell membranes and increased lipid per-oxidation, as well as alteration of the antioxidant enzyme system, energy metabolism and the appearance of anemia (Kostič et al., 1993; Zikič et al., 1997; Ognjanovič et al., 2000; Pavlovic et al., 2001; Zikič et al., 2001; Kanter et al., 2005). Rats administered cadmium chloride orally showed decreased values of erythrocyte counts, hemoglobin and hematocrit, as well as decreased mean cell volume (MCV), mean cell hemoglobin (MCH) and reduced mean cell hemoglobin concentration (MCHC), suggestive of microcytic and hypochromic anemia (Bersenyi et al., 2003; Dallak, 2009; Onwuka et al., 2010). Marked alterations of antioxidant system were found in erythrocytes of Cd-treated rats. Activities of catalase and glutathione peroxidase (GPx) as well as the total glutathione (GSH) contents in erythrocytes were significantly decreased, whereas the activity of superoxide dismutase (SOD) was significantly increased (Messaoudi et al., 2010).

Antioxidants are the natural defense mechanism exis-ting in our system and these are capable of scavenging the deleterious free radicals (Matés et al., 2011). A number of dietary antioxidant compounds have been shown to influence the membrane characteristics such as fluidity, stability and susceptibility to membrane oxidative

damage (Halliwell and Gutteridge, 1990). Recently, a great deal of attention has focused on the protective biochemical functions of naturally occurring antioxidants in biological systems against toxic heavy metals (Matés et al., 2010). Thus, it is believed that antioxidant should be one of the important components of an effective the-rapy of Cd poisoning. Ginger, which is the underground stem or rhizome of the plant *Zingiber officinale Roscoe*, contains polyphenol compounds (6-gingerol and its derivatives), which have a high antioxidant activity (Chen et al., 1986; Herrman, 1994). There are more than 50 antioxidants isolated from rhizomes of ginger (Kikuzaki and Nakatani, 1993; Masuda et al., 2004). Among them, 12 compounds exhibited higher antioxidant activity than α-tocopherol. Ginger and its constituents are stated to have antiemetic, antithrombotic, antihepatotoxic, anti-inflammatory stimulant, cholagogue, androgenic and antioxidant effects (Miller et al., 1993; Khaki et al., 2009). The main pharmacological actions of ginger and compounds isolated there, include immuno-modulatory, anti-tumorigenic, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-lipidemic and anti-emetic actions (Ali et al., 2008). It is considered a safe herbal medicine with only few and insignificant adverse/side effects (Ali et al., 2008).

Previous results demonstrated that chronic administration of ginger at the dosages of 500, 1000 and 2000 mg/kg body weight by a gavage method for 35 days was not associated with any mortalities and abnormalities in general conditions, behavior, growth, and food and water consumption in both male and female rats (Rong et al., 2009). Except for dose-related decrease in serum lactate dehydrogenase activity in males, ginger treatment induced similar hematological and blood biochemical parameters to those of controlled animals. Previous results have indicated that ginger is a potent scavenger of a variety of ROS including superoxide anion radicals (O_2^-), hydroxyl radicals ($^.OH$) and peroxyl radicals ($ROO^.$) (Stoilova et al., 2007; Baliga et al., 2013). Ginger reduced H_2O_2-induced apoptotic signals and the levels of intracellular ROS (Oh et al., 2012). Ahmed et al. (2000) and Prabhu et al. (2013), found that ginger significantly lowered lipid peroxidation by maintaining the activities of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase in the blood of healthy rats. Ginger administration has been reported to prevent the lead-induced oxidative stress to erythrocytes in rats (Attia et al., 2013). The protective effects of ginger against lead-induced hepatotoxicity are well documented and have been attributed to its intrinsic antioxidant properties (Attia et al., 2013).

The antioxidant effects of vitamin E, taurine and curcumin against cadmium chloride-induced oxidative

stress to erythrocytes and hematotoxicity in rats have been studied (Ognjanović et al., 2003; Sinha et al., 2008; Kanter et al., 2009). However, effects of ginger, as a powerful antioxidant, on cadmium chloride-induced oxidative stress to erythrocytes and hematotoxicity in rats have not yet been studied. Therefore, the aim of this study was to investigate the antioxidant effects of ginger against cadmium chloride-induced perturbations in oxidative bio-markers of blood such as levels of hemoglobin derivatives, plasma malondialdehyde (MDA) level, GPx, glutathione reductase (GR), glutathione-S-transferase (GST) and glucose-6-phosphate dehydrogenase (G6PD) activities in erythrocytes hemolysate and erythrocytes hemolysis test, in rats.

MATERIALS AND METHODS

Eighteen non-pregnant female albino rats (age: 6 to 8 weeks and about 80 to 105 g body weight) were obtained from the animal house, National Research Center, Cairo, Egypt. All animals were treated in accordance to the principles of Laboratory Animal Facilities of World Health Organization, Geneva, Switzerland (2003). The animals were fed a standard pellet diet and had free access to water. The standard diet contained 50% wheat, 21% corn, 20% soybean, 8% concentrated proteins and 1% a mixture of salts, vitamins and dicalcium phosphate. The nutritional content was 5% fat, 21 % protein, 55% nitrogen free extract and 4% fiber (w/w) with adequate minerals and vitamin contents. The rats were housed in stainless steel cages in a temperature-controlled room (25 ± 2 $^{\circ}$C) with a 12 h light and 12 h dark exposure.

Grouping of animals and treatment

The animals were randomly divided into three groups of 6 animals each, control, cadmium chloride alone, and cadmium chloride with ginger. All groups were given a standard rat chow and water. Rats in cadmium alone and cadmium with ginger groups were given treatments orally by gavage needle for 26 days. Rats of cadmium alone group were given daily 2 ml dose of a solution containing 10 mg/kg body weight of monohydrated cadmium chloride orally. While, rats of cadmium with ginger group were treated orally and daily with 2 ml of an aqueous solution containing 500 mg/kg body weight of ginger in which a concentration of 10 mg/kg body weight of monohydrated cadmium chloride was dissolved. The dose of ginger used in this study was selected on the basis of previous studies (Al-Amin et al., 2006; Rong et al., 2009; Jeena et al., 2011). Ginger, which is the underground stem or rhizome of the plant *Z. officinale Roscoe*, was purchased in a powder form from Elgabry Company for Medicinal Herbs, Giza, Egypt.

Preparation of blood samples

The experiments lasted for 26 days. At the end of the experimental period, blood samples were collected from all animals from the retro-orbital venous plexus. The blood samples were collected into heparinized tubes. The plasma obtained after centrifugation (3000 rpm for 10 min at 4 $^{\circ}$C) was used for MDA determination. Erythrocytes was washed three times in phosphate buffered saline (PBS) solution. Lysed erythrocytes were prepared by addition of four volumes of ice-cold distilled water. Cell membranes were removed by centrifugation at 8,500 rpm for 20 min, and the supernatant was used for the assays of antioxidant enzymes activities.

Biophysical assays

Levels of hemoglobin derivatives (sulfhemoglobin, "SHb", methemoglobin, "metHb", carboxyhemoglobin, "HbCO", and oxyhemoglobin, "HbO$_2$") and the total hemoglobin (Hb) concentration in the blood of rats were determined by the multicomponent spectrophotometric method described previously (Attia et al., 2011a). According to this method, the absorbance measurements for plasma-free diluted Hb solutions were made at four wavelengths (λ = 500, 568, 576 and 620 nm), using a Cary UV/VIS double-beam spectrophotometer (model 100 UV-VIS), manufactured by Varian Inc. company Sydney, Australia. The total blood Hb concentration (in g/dL) was calculated by multiplying the concentration of diluted samples by the dilution factor (DF= 170.66) and the conversion factor for mmol L^{-1} to g dL^{-1} (1.6114). Percentages of erythrocytes hemolysis were determined according to the method of Attia et al. (2011b). According to this method, hemolysis% was determined by the measurement of Hb released from the cells, relative to the total cellular Hb. The absorbance of released Hb was measured at the isobestic point of metHb and HbO$_2$ (λ = 522 nm).

Biochemical assays

The kits for biochemical parameters were purchased commercially from Biodiagnostic Company, Cairo, Egypt. Plasma MDA concentration was determined spectrophotometrically by the method of Satoh (1978). Thiobarbituric acid (TBA) reacts with MDA in acidic medium at temperature of 95°C for 30 min to form thiobarbituric acid reactive product. The absorbance of the resulting pink product can be measured at 534 nm. GPx activity was determined spectrophotometrically by the method of Paglia and Valentine (1967). The assay is an indirect measure of the activity of cellular GPx. Glutathione disulfide (GSSG), produced upon reduction of organic peroxide by GPx, is recycled to its reduced state by the enzyme GR. The oxidation of NADPH to NADP$^+$ is accompanied by a decrease in absorbance at 340 nm (A$_{340}$) providing a spectrophotometric means for monitoring GPx enzyme activity. To assay GPx, erythrocytes hemolysate is added to a solution containing glutathione, glutathione reductase, and NADPH. The enzyme reaction is initiated by adding the substrate, hydrogen peroxide and the A$_{340}$ is recorded. The rate of decrease in the A$_{340}$ is directly proportional to the GPx activity in the sample.

GST activity was determined spectrophotometrically by the method of Habig et al. (1974). The Biodiagnostic GST assay kit measures total GST activity (cytosolic and microsomal) by measuring the conjugation of 1- chloro- 2,4- dinitrobenzene (CDNB) with reduced glutathione (GSH). The conjugation is accompanied by an increase in absorbance at 340 nm. The rate of increase is directly proportional to the GST activity in the sample. GR activity was determined spectrophotometrically by the method of Goldberg and Spooner (1983). GR catalyzes the reduction of oxidized glutathione (GSSG) in the presence of NADPH, which is oxidized to NADPH$^+$. The decrease in absorbance at 340 nm directly proportional to GR activity is measured. G6PD activity was determined spectrophotometrically by the method of Kornberg (1955). The enzyme activity is determined by measurement of the rate of absorbance change at 340 nm due to the reduction of NADP$^+$.

Statistical analysis

Data were presented as the mean ± standard error (SE) values. One-way ANOVA was carried out, and the statistical comparisons among the groups were performed with Post Hoc and the least significant difference (LSD) tests using a statistical package program (SPSS version 14). $P < 0.05$ was considered as statistically

statistically significant.

RESULTS

Blood hemoglobin derivatives

Table 1 shows the levels of inactive hemoglobins (SHb, metHb, HbCO), active HbO_2 and total Hb concentration in all groups. After 26 days, significant increases in metHb% and HbCO% were observed in cadmium-exposed rats, compared to the control group ($P < 0.05$), while cadmium treatment had no significant effects on SHb%. Cadmium treatment of rats caused a significant decrease in HbO_2% and total hemoglobin (Hb) concentration ($P < 0.05$), compared to controls. Ginger treatment of cadmium exposed rats significantly lowered metHb% ($P < 0.05$), while significantly increased HbO_2% ($P < 0.05$) and total Hb concentration ($P < 0.01$), compared to the cadmium alone group.

Plasma MDA concentration

Table 2 shows the concentration of MDA in plasma of all groups. After 26 days, significant increase in MDA concentration was observed in cadmium exposed rats compared to control group ($P < 0.05$). Ginger treatment of cadmium exposed rats lowered MDA concentration ($P < 0.01$, -20%) compared to cadmium alone group.

Erythrocyte antioxidant enzyme activities

Table 2 shows the activities of GPx, GST, GR and G6PD in erythrocytes of all groups. Marked alterations of antioxidant enzyme system were found in Cd-exposed rats. GPx activity significantly increased ($P < 0.05$) in cadmium compared to control group, while GST, GR and G6PD showed insignificant changes. Ginger treatment significantly increased GPx and G6PD activities of cadmium exposed rats, compared to cadmium alone group ($P < 0.05$), while it has no effect on GR and GST activities. However, GPx, GR and G6PD activities of cadmium+ ginger group are significantly higher ($P < 0.05$) than controls.

Percentages of erythrocytes hemolysis

Figure 1 shows the hemolysis % of erythrocytes in all groups. The hemolysis test indicates that intoxication by cadmium significantly increases the hemolytic effect ($P < 0.05$), whereas after treatment with ginger, it decreases by 17%.

DISCUSSION

Cd is a toxic metal that is widely used in different industries.

Cd promotes an early oxidative stress and afterward contributes to the development of serious pathological conditions because of its long retention in some tissues (Bagchi et al., 2000). The present results have clearly demonstrated the ability of Cd to induce oxidative stress in rat blood as evidenced by increased lipid peroxidation after 26 days of Cd treatment. This finding is in agree-ment with several reports demon-strating that Cd induces oxidative stress by altering the antioxidant status in sev-eral tissues (Ognjanović et al., 2003; Sinha et al., 2008; Kanter et al., 2009; El-Sokkary et al., 2009; Onwuka et al., 2011; Tarasub et al., 2011; Tarasub et al., 2012). The mechanism by which Cd induces ROS is not yet known (Ercal et al., 2001). Pre-vious studies have indicated that treatment of cells with Cd, results in specific mitochondrial alterations (Wang et al., 2004). Cadmium exposure also leads to mitochondrial dysfunction in the renal cortex of rats (Tang and Shaikh, 2001). Cadmium inhibits the mitochondrial electron transfer chain and induces ROS (Wang et al., 2004).

The inactive components of Hb (SHb, metHb and HbCO) are unable to transport oxygen, while HbO_2 is the active Hb. When erythrocytes reach the end of their life due to aging or defects, Hb molecule is broken up and the iron gets recycled. When the porphyrin ring is broken up, the fragments are normally secreted in liver bile. This process also produces one carbon monoxide (CO) molecule for every heme molecule degraded (Hardison, 1996); this is responsible for the normal blood levels of CO and HbCO. This may explain the higher HbCO levels that accompany the higher hemolysis rate after Cd-treat-ment of rats. When the iron atom is in the ferrous form, the protein is active and can bind oxygen reversibly. The oxidation to the ferric form (metHb) leads to an in-active protein. Methemoglobin is unable to carry oxygen. High oxidative stress in red blood cells of cadmium exposed animals can account for the increase in metHb% produced through HbO_2-autoxidation reactions (Waltkins et al., 1985) and its improvement after treatment with ginger can account for the decrease in metHb% and increase in HbO_2% observed in the present study.

Cadmium exposure is known to cause interference with antioxidant enzymes, inhibition of energy metabolism, membrane damage, altered gene expression, and apoptosis (Habeebu et al., 1998; Ikediobi et al., 2004). Another target is erythrocytes, which contain hemoglobin that transports oxygen and carbon dioxide between the lungs and all tissues of the body. After exposure, Cd enters the blood, binds the erythrocyte membrane, and stimulates formation of the ROS (Bauman et al., 1993), leading to alterations in the antioxidant system of erythrocytes and imposing oxidative damage upon the membrane (Sarkar et al., 1995; Sarkar et al., 1997; Sarkar et al., 1998; Simpkins et al., 1998). Anemia is one of the characteristic clinical manifestations of chronic Cd intoxication (Horiguchi et al., 1996) as Cd is known to reduce red blood cell count and hematocrit value as well

Table 1. Effects of cadmium chloride and ginger on levels of inactive, active hemoglobins and total Hb concentration in blood of rats.

Parameter (%)	Control	Cadmium chloride	Cadmium chloride+ ginger
SHb	0.235±0.050	0.265±0.068	0.356±0.035
metHb	1.155±0.056	1.480±0.069[a]	1.029±0.074[b]
HbCO	2.418±0.056	2.816±0.041[a]	2.650±0.034
HbO$_2$	96.190±0.127	95.445±0.103 [a]	95.965±0.097[b]
Hb (g/dl)	13.038±0.409	12.210±0.289[a]	13.546±0.210[a,c]

Data are presented as mean ± S.E.[a] significantly different from control- $P < 0.05$. [b] significantly different from cadmium alone treatment group- $P < 0.05$. [c] significantly different from cadmium alone treatment group- $P < 0.01$.

Table 2. Effects of cadmium chloride and ginger on concentrations of plasma MDA, GPx , GST, GR and G6PD activities in erythrocyte hemolysate in rats.

Parameter	Control	Cadmium chloride	Cadmium + ginger
MDA (nmol/ml)	9.9±0.453	10.407±0.360[a]	8.307±0.567[a,d]
GPx (mU/ml)	110.776±12.07	161.463±8.185[a]	187.185±11.766[b,c]
GST (U/L)	456.622±21.443	516.986±101.29	424.753±41.915
GR (U/L)	11.856±0.214	11.883±0.365	12.165±0.221
G6PD (U/g Hb)	8.775±0.359	8.511±0.615	9.793±0.763[a,c]

Data are presented as mean ± S.E [a] significantly different from control- $P < 0.05$.
[b] significantly different from control- $P < 0.01$. [c] significantly different from cadmium alone treatment group- $P < 0.05$. [d] significantly different from cadmium alone treatment group- $P < 0.01$.

as hemoglobin concentration (Ognjanović et al., 2003; Onwuka et al., 2010). The reduction in Hb content may be due to increased rate of destruction or reduction in the rate of formation of erythrocytes. Long-term Cd exposure induces reduction in the rate of formation of erythrocytes in rats through hypo-induction of erythropoietin in the kidneys (Horiguchi et al., 1996). In addition, the reduction in Hb content may be attributed to hyperactivity of bone marrow that leads to production of erythrocytes with impaired integrity that are easily destructed in the circulation (Tung et al., 1975).

This study showed that erythrocytes hemolysis in Cd treated animals is higher than controls. These results are consistent with a previous study (Kanter et al., 2009). This high rate of Cd-induced hemolysis decreases by 17% after ginger treatment. The high rate of hemolysis of cadmium exposed rats can account for the low Hb concentration induced by Cd treatment. On the other hand, the significant decrease in the rate of hemolysis of ginger treated animals can account for the markedly increased concentration of total Hb induced by ginger treatment of Cd exposed rats. Previous results showed that ginger treatment of Cd-exposed rats significantly improved hematological indices (Onwuka et al., 2011). Ginger contains polyphenol compounds (6-gingerol and its derivatives), which have a high antioxidant activity (Chen et al., 1986; Herrman, 1994). This high antioxidant activity of ginger can account for the decrease in lipid peroxidation and erythrocyte hemolysis after ginger treatment of Cd- exposed rats, observed in this study.

The results obtained in our present study show that treatment with Cd, induces an increase level of lipid peroxidation product, MDA, in the blood of rats, which were accompanied by increased formation of ROS (Ognjanović et al., 2003; Sinha et al., 2008; Kanter et al., 2009). As a consequence of enhanced lipid peroxidation, DNA damage, altered calcium and sulfhydryl homeostasis as well as marked marker disturbances of antioxidant defense system occurred (Hiruku and Kawanishi, 1996). Treatment with ginger was effective in decreasing oxidative damage induced by Cd which resulted in markedly lower MDA concentration. Ginger was capable of inhibiting formation of ROS which caused hemolysis, through its high antioxidant activity (Chen et al., 1986; Kikuzaki and Nakatani, 1993; Herrman, 1994; Masuda et al., 2004). It is assumed that except of therapeutic intervention by using potent chelating agents capable to mobilize intracellularly bound Cd (Eybl et al., 1984; Jones and Cherian, 1990), ginger as antioxidants may be important components of an effective Cd intoxication treatment. The inhibitory effect of ginger on lead-induced lipid peroxidation in blood was demonstrated by Attia et al. (2013), who suggested that ginger provided a protection against lipid peroxidation and hemolysis of RBCs induced by lead treatment of rats.

Marked alterations of antioxidant system were found in erythrocytes of Cd-treated rats. In animals exposed to Cd, the activity of GPx in RBC was significantly increased (Table 2). These results are consistent with previous studies demonstrating that Cd induced an increase in SOD
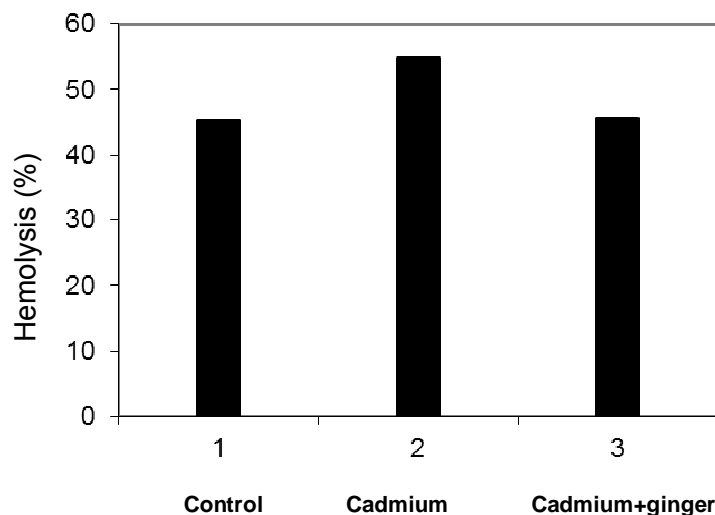
Figure 1. Percentages of erythrocytes hemolysis in all groups. Hemolysis test was carried out after incubation of erythrocytes in phosphate buffered saline (PBS), pH 7.4, for 3 days at room temperature. [a] significantly different from control- $P < 0.05$, [b] significantly different from cadmium alone treatment group- $P < 0.05$.

and GPx activities in erythrocytes of Cd-exposed rats (Zikić et al., 2001; Ognjanović et al., 2003; Kanter et al., 2005; Kanter et al., 2009; Attia et al., 2014). It is known that Cd induces the formation of superoxide anion radicals in erythrocytes and it is reasonable to expect an increased activity of SOD. Cadmium induced an increase in GPx activity which may be explained by their influence on hydrogen peroxide as substrate which is formed in the process of dismutation of superoxide anion radicals (Shaikh et al., 1999). The treatment with ginger of Cd exposed rats caused an increased GPx activity, indicating that this substance eliminates the toxic effects of Cd on the activity of this enzyme. The treatment with antioxidants helped to elevate the GPx activity and erythrocyte GSH content (Sinha et al., 2008). In erythrocytes and other tissues, the enzyme GPx, containing selenium as a prosthetic group, catalyzes the destruction of H_2O_2 and lipid hydroperoxides by GSH, protecting membrane lipids and hemoglobin against oxidation by peroxides (Murray et al., 1996). Thus, the increase in GPx activity induced by ginger treatment of Cd-exposed rats can account for the ginger-induced reduction of Hb oxidation and oxidative damage of RBC observed in this study. GPx is a preventive antioxidant, which reduces the rate of chain initiation of lipid peroxidation reaction (Murray et al., 1996). The increase in its activity can account for the decrease in the level of lipid peroxidation product, MDA, in the blood of rats induced by ginger treatment.

Preclinical studies carried out in the past decade have shown that ginger possesses cytoprotective effects against diverse xenobiotics such as alcohol, acetaminophen, fungicides, heavy metals, and organophosphorus compounds. The protective actions are shown to be mediated through its free radical-scavenging antioxidant, cytoprotective, and anti-inflammatory effects. In contrast to the results of this study, ginger was shown to induce the detoxifying enzymes GST in the presence of xenobiotics, thus offering protection to the liver (Shivashankara et al., 2013). GST catalyzes the conjugation of GSH, via a sulfhydryl group, to electrophilic centers on a wide variety of substrates in order to make the compounds more soluble (Douglas, 1987; Oakley, 2011). This activity detoxifies endogenous compounds such as lipid peroxides and also enables the breakdown of xenobiotics. GST efficiently conjugates the end products of lipid peroxidation (Leaver and George, 1998), reducing the level of MDA and thus contributing to the protection of the cell integrity (Grose et al., 1987; Ognjanović et al., 1995). GR catalyzes the reduction of GSSG to the sulfhydryl form glutathione (GSH), which is a critical molecule in resisting oxidative stress and maintaining the reducing environment of the cell (Meister, 1988; Deponte, 2013). Therefore, the increase in GR activity can account for the ginger-induced reduction in oxidative stress, as observed in this study. G6PD is in the pentose phosphate pathway, a metabolic pathway that supplies reducing energy to cells (such as erythrocytes) by maintaining the level of the co-enzyme, nicotinamide adenine dinucleotide phosphate (NADPH). The NADPH in turns maintains the level of GSH in these cells that helps protect the erythrocytes against oxidative damage, which causes acute hemolytic anemia. G6PD reduces nicotinamide adenine dinucleotide phosphate (NADP) to NADPH while oxidizing glucose-6-phosphate (Aster et al.,

2010). G6PD deficiency is very common worldwide, and causes acute hemolytic anemia (Cappellini and Fiorelli, 2008). Therefore, the increase in G6PD activity can account for the ginger-induced reduction in oxidative damage and hemolysis of erythrocytes, as observed in this study.

The high antioxidant activity of ginger is attributed to its high content of polyphenol compounds (6-gingerol and its derivatives), which have a high antioxidant activity (Chen et al., 1986; Herrman, 1994). The isolated antioxidants of ginger are divided into two groups; gingerol related compounds and diarylheptanoids. The nonvolatile fraction of the dichloromethane extract of ginger rhizomes exhibited a strong antioxidant activity. The fraction was purified by chromatographic techniques to provide five gingerol related compounds and eight diarylheptanoids (Kikuzaki and Nakatani, 1993). Among them, 12 compounds exhibited higher antioxidant activity than α-tocopherol. The activity was probably dependent upon side chain structure and substitution patterns on the benzene ring. The oleoresin (gingerols and shogaols), responsible to the pungent flavor of ginger, varies from 4.0 to 7.5% and also possesses substantial antioxidant activity (Kikuzaki and Nakatani, 1993; Balachandran et al., 2006). Ginger is a strong anti-oxidant plant and may either mitigate or prevent generation of free radicals. The alcohol ginger extract inhibited the hydroxyl radicals ($\cdot$OH) 79.6% at 37 °C and 74.8% at 80 °C, which showed a higher antioxidant activity than quercetin (Stoilova et al., 2007). All major active ingredients of ginger such as zingerone, gingerdiol, zingibrene, gingerols and shogaols have antioxidant activity (Zancan et al., 2002; Min-Ji et al., 2012; Baliga et al., 2013). Zingerone scavenges superoxide anion radicals ($O_2^{\cdot-}$). 6-gingerol and zingerone are reported to be good scavengers of peroxyl radicals ($ROO^{\cdot}$). 6-shogoal also inhibited the production of nitric oxide (NO). 6-Gingerol is the major bioactive constituent responsible for the antiinflammatory, antitumour and antioxidant activities of ginger (Nagendra et al., 2013).

Conclusion

It can be concluded from the presented results that cadmium induced oxidative damage in erythrocytes, leading to loss of membrane function by enhanced lipid peroxidation as well as alteration of the activity of GPx antioxidant enzyme. Moreover, the results of multi-component spectrophotometric analysis showed an increase in the level of inactive methemoglobin (metHb) and a decrease in HbO$_2$% and total Hb concentration. Ginger expressed protective role against toxic influence of cadmium on all affected parameters in rats. Ginger may exert its protective actions against cadmium-induced hematotoxicity in rats possibly through its antioxidant mechanisms. The results raise the possibility of ginger being considered as one of the component of the regular diet of the people in the areas, where they may have

chances of exposure to cadmium occupationally or environmentally.

Conflict of interest

Authors declare no conflict of interest.

REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR) (1999). Toxicological profile for cadmium. US Department of Human and Health Services.

Agency for Toxic Substances and Disease Registry (ATSDR) (2013). Available at: http://www.atsdr.cdc.gov/spl/index.html.

Ahmed RS, Steh V, Banerjee BD (2000). Influence of dietary ginger (*Zingiber officinales Rosc*) on antioxidant defense system in rat, comparison with ascorbic acid. Indian J. Exp. Biol. 38:604-606.

Al-Amin ZM, Thomson M, Al-Qattan KK, Peltonen-Shalaby R, Ali M (2006). Anti-diabetic and hypolipidaemic properties of ginger (*Zingiber officinale*) in streptozotocin-induced diabetic rats. Brit. J. Nutr. 96:660-666.

Ali BH, Blunden G, Tanira MO, Nemmar A (2008). Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinales Roscoe*): a review of recent research. Food Chem. Toxicol. 46:409-420.

Aster J, Kumar V, Robbins SL, Abbas AK, Fausto N, Cotran RS (2010). Robbins and Cotran pathologic basis of disease. Saunders/Elsevier. pp. 33340-33341.

Attia AMM, Ibrahim FAA, Nabil GM, Aziz SW (2013). Antioxidant effects of whole ginger (*Zingiber officinale Roscoe*) against lead acetate-induced hematotoxicity in rats. J. Med. Plants Res. 7:1108-1113.

Attia AMM, Nabil GM, Ibrahim FA, Hassan NS (2011a). Multi-component spectrophotometric determination of inactive and active haemoglobins in rat and mice blood. Rom. J. Biophys. 21:267-276.

Attia AMM, Sayed AM, Ibrahim FA, Mohammed AS, El-Alfy MS (2011b). Effects of antioxidant vitamins on some hemoglobin properties and erythrocytes in homozygous beta-thalassemia. Rom. J. Biophys. 21:1-16.

Bagchi D, Bagchi M, Stohs SJ, Ray SD, Kuszynski CA, Pruess HG (2000). Free radicals and grape seed proathocyanidin extract: importance in human health and disease prevention. Toxicol. 148:187-197.

Balachandran S, Kentish SE, Mawson R (2006). The effect of both preparation method and season on the supercritical extraction of ginger. Sep. Purif. Technol. 48:94-105.

Baliga MS, Latheef L, Haniadka R, Fazal F, Chacko J, Arora R (2013). Ginger (*Zingiber officinale Roscoe*) in the treatment and prevention of Arthritis. Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases. pp. 529-544.

Bauer R, Demeter I, Hasemann V, Johansen JT (1980). Structural properties of the zinc site in Cu, Zn-superoxide dismutase; perturbed angular correlation of gamma ray spectroscopy on the Cu, 111Cd-superoxide dismutase derivative. Biochem. Biophys. Res. Commun. 94:1296-1302.

Bauman JW, Liu J, Klaassen CD (1993). Production of metallothionein and heat-shock proteins in response to metals. Fundam. Appl. Toxicol. 21:15-22.

Bersenyi A, Feket SG, Szocs Z, Berta E (2003). Effect of ingested heavy metals (Cd, Pb, Hg) on hematology and serum biochemistry in rabbits. Acta Vet. Hung. 51:297-304.

Cappellini MD, Fiorelli G (2008). Glucose-6-phosphate dehydrogenase deficiency. Lancet 371:64-74.

Casalino E, Calzaretti G, Sblano C, Landriscina C (2002). Molecular inhibitory mechanisms of antioxidant enzymes in rat liver and kidney by cadmium. Toxicology 30:37-50.

Chen Ch, Kuo M, Wu Ch, Ho Ch (1986). Pungent compounds of ginger (*Zingiber officinale (L) Rosc*) extracted by liquid carbon dioxide. J. Agric. Food Chem. 34:477-480.

Dallak M (2009). Camel's milk protects against cadmium chloride-induced hypochromic microcytic anemia and oxidative stress in red blood cells of white albino rats. Am. J. Pharmacol. Toxicol. 4:136-143.

Deponte M (2013). Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes. Biochim. Biophys. Acta. 1830:3217-3266.

Douglas KT (1987). Mechanism of action of glutathione-dependent enzymes. Adv. Enzymol. Relat. Areas Mol. Biol. 59:103-167.

El-Sokkary GH, Nafady AA, Shabash EH (2009). Melatonin ameliorates cadmium-induced oxidative damage and morphological changes in the kidney of rat. Open Neuroendocrinol. J. 2:1-9.

Ercal N, Gurer-Orhan H, Aykin-Burns N (2001). Toxic metals and oxidative stress. Part I. Mechanisms involved in metal-induced oxidative damage. Curr. Trop. Med. Chem. 1:529-539.

Eybl V, Sykora J, Koutensky J, Caisova D, Schwartz A, Mertl E (1984). Interaction of chelating agents with cadmium in mice and rats. Environ. Health Perspect. 54:267-273.

Goldberg DM, Spooner RJ (1983). Methods of enzymatic analysis (Bergmeyen HV. Ed.), 3rd edn. Vol 3, Verlag Chemie, Deerfield beach, Fl. pp. 258-265.

Grose EC, Richards JH, Jaskot RH, Menache MG, Graham JA, Dauterman WC (1987). Glutathione peroxidase and glutathione transferase activity in rat lung and liver following cadmium. Toxicology 44:171-179.

Habeebu SS, Liu J, Klaassen CD (1998). Cadmium-induced apoptosis in mouse liver. Toxicol. Appl. Pharmacol. 149:203-209.

Habig W, Pabst M, Jakoby W (1974). Colorimetric determination of glutathione-S-transferase activity. J. Biol. Chem. 249:7130-7139.

Halliwell B, Gutteridge JMC (1990). Role of free-radicals and catalytic metal-ions in human-disease-an overview. Method Enzymol. 186:1-85.

Hardison RC (1996). A brief history of hemoglobins: plant, animal, protest, and bacteria. Proc. Natl. Acad. Sci. USA. 93:5675-5679.

Herrman K (1994). Antioxidativ wirksame Pflanzenphenole sowie Carotinoide als wichtige Inhaltsstoffe von Gewürzen. Gordian 94:113-117.

Hiruku Y, Kawanishi S (1996). Mechanism of oxidative DNA damage induced by delta-aminolevulinic acid in the presence of copper ion. Cancer Res. 56:1786-1793.

Horiguchi H, Sato M, Konno N, Fukushima M (1996). Long-term cadmium exposure induces anemia in rats through hypoinduction of erythropoietin in the kidneys. Arch. Toxicol. 71:11-19.

Ikediobi CO, Badisa VL, Ayuk-Takem LT, Latinwo LM, West J (2004). Response of antioxidant enzymes and redox metabolites to cadmium-induced oxidative stress in CRL-1439 normal rat liver cells. Int. J. Mol. Med. 14:87-92.

Jarup L, Alfven T, Persson B, Toss G, Elinder CG (1998). Cadmium may be a risk factor for osteoporosis. Occup. Environ. Med. 55:435-439.

Jeena K, Liju VB, Kuttan R (2011). A preliminary 13-week oral toxicity study of ginger oil in male and female Wistar rats. Int. J. Toxicol. 30:662-670.

Jones MM, Cherian MG (1990). The search for chelate antagonists for chronic cadmium intoxication. Toxicology 62:1-25.

Kanter M, Aksu B, Akpolat M, Tarladacalisir YT, Aktas C. Uysal H (2009). Vitamin E protects against oxidative damage caused by cadmium in the blood of rats. Eur. J. Gen. Med. 6:154-160.

Kanter M, Coskun O, Gurel A (2005). Effect of black cumin (Nigella sativa) on cadmium-induced oxidative stress in the blood of rats. Biol. Trace Elem. Res. 107:277-287.

Khaki A, Fathiazad F, Nouri M, Khaki AA, Chelar C, Ozanci C, Ghafari-Novin M, Hamadeh M (2009). The effects of ginger on spermatogenesis and sperm parameters of rat. Iranian J. Reprod. Med. 7:7-12.

Kikuzaki H, Nakatani N (1993). Antioxidant effects of some ginger constituents. J. Food Sci. 58:1407-1410.

Kornberg A (1955). Methods in Enzymology Vol. 1. Academic Press, New York. P 323.

Kostić MM, Ognjanović B, Zikić RV, Stajn A, Rosic GL, Zivkovic RV (1993). Cadmium-induced changes of antioxidant and metabolic status in red blood cells of rats: in vivo effects. Eur. J. Haematol.

51:86-92.

Laboratory Animal Facilities, Laboratory Biosafety Manual, second edition (revised), (2003). World Health Organization, Geneva, Switzerland. pp. 22-24.

Leaver MJ, George SG (1998). A piscine glutathione S-transferase which efficiently conjugates the end-products of lipid peroxidation. Mar. Environ. Res. 46:71-74.

Masuda Y, Kikuzaki H, Hisamoto M, Nikatani N (2004). Antioxidant properties of gingerol related compounds from ginger. BioFactors 21:293-296.

Matés JM, Segura JA, Alonso FJ, Márquez J (2010). Roles of dioxins and heavy metals in cancer and neurological diseases using ROS-mediated mechanisms. Free Radic. Biol. Med. 49:1328-1341.

Matés JM, Segura JA, Alonso FJ, Márquez J (2011). Anticancer antioxidant regulatory functions of phytochemicals. Curr. Med. Chem. 18:2315-2338.

Meister A (1988). Glutathione metabolism and its selective modification. J. Biol. Chem. 263:17205-17208.

Messaoudi I, Hammouda F, El Heni J, Baati T, Said K, Kerkeni A (2010). Reversal of cadmium-induced oxidative stress in rat erythrocytes by selenium, zinc or their combination. Exp. Toxicol. Pathol. 62:281-288.

Miller JK, Brzezinska-Slebodzinska E, Madsen FC (1993). Oxidative stress, antioxidant and animal function. J. Dairy Sci. 76:2812-2823.

Min-Ji B, Seon O, Mira J, Woo-Sik J (2012). 6-shogaol-rich extract from ginger up-regulates the antioxidant defense systems in cells and mice. Molecules 17:8037-8055.

Murray RK, Granner DK, Mayes PA, Rodwell VW (1996). Lipids of physiologic significance: Harper's Biochemistry, 24th edition. Appleton and Lange, Norwalk, Connecticut, Los Altos, California. pp. 119, 155.

Nagendra Chari KL, Manasa D, Srinivas P, Sowbhagya HB (2013). Enzyme-assisted extraction of bioactive compounds from ginger (Zingiber officinale Roscoe). Food Chem. 139:509-514.

Oakley A (2011). Glutathione transferases: a structural perspective. Drug Metab. Rev. 43:138-151.

Ognjanović BI, Pavlović SZ, Ikić Tajan RVA, Maletić SD, Saičić ZS, Petrović VM (2000). The enzyme activities and blood hematological values of rats exposed to cadmium. Kragujevac J. Sci. 22:93-99.

Ognjanović BI, Pavlović Z, Maletić SD, Zikić RV, Štajn AŠ, Radojičić RM, Saičić ZS, Petrović VM (2003). Protective influence of vitamin E on antioxidant defense system in the blood of rats treated with cadmium. Physiol. Res. 52:563-570.

Ognjanović BI, Zikić RV, Štajn AŠ, Saičić ZS, Kostić MM, Petrović VM (1995). The effects of selenium on the antioxidant defense system in the liver of rats exposed to cadmium. Physiol. Res. 44:293-300.

Oh SY, Lee J, Lee JS, Seo S, Ma J (2012). An aqueous extract of Zingiber officinale Roscoe protects mouse primary hepatic cells against hydrogen peroxide-induced oxidative stress. Biotechnol. Bioprocess. Eng. 17:168-175.

Onwuka FC, Erhabor O, Eteng MU, Umoh IB (2010). Ameliorative effect of cabbage extract on cadmium-induced changes on hematology and biochemical parameters of albino rats. J. Toxicol. Environ. Health Sci. 2:11-16.

Onwuka FC, Erhabor O, Eteng MU, Umoh IB (2011). Protective effects of ginger toward cadmium-induced tests and kidney lipid peroxidation and hematological impairment in albino rats. J. Med. Food. 14:817-821.

Paglia DE, Valentine WN (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70:158-169.

Pavlovic SZ, Ognjanović BI, Stajn AS, Zikić RV, Saičić ZS, Petrović VM (2001). The effect of coenzyme Q10 on blood ascorbic acid, vitamin E, and lipid peroxide in chronic cadmium intoxication. J. Environ. Pathol. Toxicol. Oncol. 20:133-140.

Prabhu AN, Shivashankara AR, Haniadka R, Palatty PL, Prabhu D, Baliga MS (2013). Antiatherogenic effects of ginger (Zingiber officinale Roscoe): Scientific observations and ethnomedicinal validation. Bioactive Food as Dietary Interventions for Cardiovascular Disease. P. 693-704.

Rong X, Peng G, Suzuki T, Yang Q, Yamahara J, Li Y (2009). A 35-day gavage safety assessment of ginger in rats. Regul. Toxicol. Pharm. 54:118-123.

Sarkar S, Yadav P, Bhatnagar D (1997). Cadmium-induced lipid peroxidation and the antioxidant system in rat erythrocytes: the role of antioxidants. J. Trace Elem. Med. Biol. 11:8-13.

Sarkar S, Yadav P, Bhatnagar D (1998). Lipid peroxidative damage on cadmium exposure and alterations in antioxidant system in rat erythrocytes: a study with relation to time. Biometals 11:153-157.

Sarkar S, Yadav P, Trivedi R, Bansal AK, Bhatnagar D (1995). Cadmium-induced lipid peroxidation and the status of the antioxidant system in rat tissues. J. Trace Elem. Med. Biol. 9:144-149.

Satoh K (1978). Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin. Chim. Acta. 90:37-43.

Shaikh ZA, Vu TT, Zaman K (1999). Oxidative stress as a mechanism of chronic cadmium-induced hepatotoxicity and renal toxicity and protection by antioxidants. Toxicol. Appl. Pharmacol. 154:256-263.

Shivashankara AR, Haniadka R, Fayad R, Palatty PL, Arora R, Baliga MS (2013). Bioactive food as dietary interventions for liver and gastrointestinal disease. pp. 657-671.

Simpkins C, Lloyd T, Li S, Balderman S (1998). Metallothionein-induced increase in mitochondrial inner membrane permeability. J. Surg. Res. 75:30-34.

Sinha M, Manna P, Sil PC (2008). Taurine protects the antioxidant defense system in the erythrocytes of cadmium treated mice. BMB Rep. 41:657-663.

Stohs SI, Bagchi D, Hassoun E, Bagchi M (2000). Oxidative mechanisms in the toxicity of chromium and cadmium ions. J. Environ. Pathol. Toxicol. Oncol. 19:201-213.

Stoilova I, Krastanov A, Stoyanova A, Denev P, Gargova S (2007). Antioxidant activity of a ginger extract (*Zingiber officinale*). Food Chem. 102:764-770.

Swiergosz R, Zakrzewska M, Kapusta KS, Bacia K, Jankowska I (1998). Accumulation of cadmium and its effect on bank vole tissues after chronic exposure. Ecotoxicol. Environ. Safe 41:130-136.

Tang W, Shaikh ZA (2001). Renal cortical mitochondrial dysfunction upon cadmium metallothionein administration to Sprague-Dawley rats. J. Toxicol. Environ. Health 62:221-235.

Tarasub N, Junseecha T, Tarasub C, Devakul Na Ayutthaya W (2012). Protective effects of curcumin, vitamin C, or their combination on cadmium-induced hepatotoxicity. J. Basic Clin. Pharm. 3:273-281.

Tarasub N, Tarasub C, Devakul Na Ayutthaya W (2011). Protective role of curcumin on cadmium-induced nephrotoxicity in rats. J. Environ. Chem. Ecotoxicol. 3:17-24.

Tung HT, Cook FW, Wyatt RD, Hamilton PB (1975). The anemia caused by aflatoxin. Poultry Sci. 54:1962-1969.

Waltkins AJ, Kawanishi S, Caughey WS (1985). Autoxidation reactions of hemoglobin A free from other red cell components: A minimal mechanism. Biochem. Biophys. Res. Commun. 132:742-751.

Wang Y, Fang J, Leonard SS, Rao KM (2004). Cadmium inhibits the electron transfer chain and induces reactive oxygen species. Free Radic. Biol. Med. 36:1434-1443.

Zancan KC, Marques MO, Petenate AJ, Meireles MA (2002). Extraction of ginger (Zingiber officinale Roscoe) oleoresin with CO_2 and co-solvents: a study of the antioxidant action of the extracts. J. Supercrit. Fluid 24:57-67.

Zikić RV, Stajn A, Ognjanović B, Pavlovic SZ, Saicic ZS (1997). Activities of superoxide dismutase and catalase in erythrocytes and transaminases in the plasma of carps (Cyprinus carpio L.) exposed to cadmium. Physiol. Res. 46:391-396.

Zikić RV, Stajn A, Pavlovic SZ, Ognjanović BI, Saicic ZS (2001). Activities of superoxide dismutase and catalase in erythrocytes and plasma transaminases of goldfish (*Carassius auratu*s Gibello Bloch.) exposed to cadmium. Physiol. Res. 50:105-111.