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Full Length Research Paper

Antioxidant effects of curcumin against cadmium chloride-induced oxidative stress in the blood of rats

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Humans are exposed to a number of toxic elements in the environment. Cadmium, widely used in industry, is a great environmental health problem of both humans and animals. Effects of reactive oxygen species (ROS) generation have been postulated to be major contributors to cadmium-exposure related disease. The aim of this study was to investigate the effect of curcumin on oxidative stress in rats exposed to cadmium. Curcumin was administered orally (600 mg/kg body weight). After 24 days, significant increases in methemoglobin percentage (metHb%), superoxide dismutase (SOD), glutathione peroxidase (GPx) activity, malondialdehyde (MDA) concentration and hemolysis test were observed in cadmium exposed rats compared to control group (P < 0.05), while GSH concentration showed insignificant change. Curcumin treatment of cadmium exposed rats significantly lowered metHb%, while significantly increased oxyhemoglobin percentage (HbO₂%), compared to cadmium alone group (P < 0.05). Also curcumin treatment significantly increased GPx activity of cadmium exposed rats as compared to cadmium alone group (P < 0.05). Curcumin treatment of cadmium exposed rats lowered MDA concentration and hemolysis percentage by 10 and 9%, respectively. The findings of this study suggest that curcumin elevated the GPx activity of cadmium exposed rats and had ameliorative effect on lipid peroxidation and erythrocytes hemolysis. Moreover, the results of multicomponent spectrophotometric analysis suggest that curcumin treatment of lead exposed rats lowered the levels of inactive metHb level and elevated the level of active HbO₂. Curcumin may exert its protective actions against cadmium-induced hematotoxicity in rats possibly through its antioxidant mechanisms and may have future therapeutic relevance.

Key words: Cadmium, curcumin, oxidative stress, erythrocytes, hemolysis, Hb-derivatives, rats.

INTRODUCTION

Cadmium (Cd) is an important industrial and environmental pollutant that currently ranks seventh on the Agency, for Toxic Substances and Disease Registry (ATSDR)/EPA list of hazardous substances (ATSDR 2003,). Cd will invariably be present in our society, either in useful products in the form of nickel-cadmium batteries, dyes, plastics, electrochemistry, paint pigments or in controlled wastes as a major source of pollution, in water

*Corresponding author. E-mail: yousatef@yahoo.com Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> License 4.0 International License and as a constituent of food material (Jarup et al., 1998; Ikeda et al., 2000). Cd is an omnipresent heavy metal which enters the biological systems from natural sources, such as volcanic emissions, weathering of rocks, mining processes as well as from industrial applications, agricultural practices and human usages. Cd emissions into the environment are normally continuous between the three main environmental compartments, air, water and soil. The majority of Cd exposure arises from ingestion of food substances due to uptake of Cd by plants from fertilizers, sewage, sludge, manure and atmospheric deposition (Anderson et al., 1988; Hotz et al., 1989; Lauwerys et al., 1991; Iwata et al., 1992; Bernard et al., 1992; Ikeda et al., 2000). Human uptake of Cd is mainly through cigarette smoking, food and water intake. In vegetarian diet, mushrooms, cacao powder, potatoes, fruits, wheat, grains, bran, sugerbeet fiber, carrot, dried seaweeds, etc., are the source of Cd intake. Similarly, in non-vegetarian diets shellfish, mussel, meat and fish are rich in cadmium. These Cd rich foods can greatly increase cadmium concentration in the human body (Friberg et al., 1985; Vahter et al., 1996; WHO, 2000). Therefore, Cd is a wide-spread environmental pollutant, characterized by its toxicity to various organs, including kidney, liver, lung, testis, brain, bone, blood system (Gunnarsson et al., 2003; WHO, 1992). The molecular mechanisms of its toxicity are not yet well defined. Cd has been demonstrated to stimulate free radical production, resulting in oxidative deterioration of lipids, proteins and DNA, and initiating various pathological conditions in humans and animals (Manca et al., 1991; Shaikh et al., 1999). After the intake and resorption. Cd enters the blood where it binds to the red blood cell (RBC) membranes and plasma albumin (Bauman et al., 1993). In the blood and tissues, Cd stimulates the formation of metallothioneins (Simpkins et al., 1998) and reactive oxygen species (ROS), thus causing oxidative damage in RBCs and in various tissues, which result in a loss of membrane functions (Sarkar et al., 1995). Cd also induces the onset of decreases the RBC count, hemoglobin anemia, concentration and hematocrit value as well as producing reduced blood iron levels (Kostić et al., 1993). Moreover, a variety of accompanying changes in antioxidant defense enzymes were reported (Zikiċ et al., 1998; Kostiċ et al., 1993).

Antioxidants are the natural defense mechanism existing in our system and these are capable of scavenging the deleterious free radicals. 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. Thus, it is believed that antioxidant should be one of the important components

of an effective therapy of Cd poisoning. Curcumin, a vellow coloring ingredient of the spice turmeric obtained from the rhizomes of Curcuma longa Linn (Zingiberacea), a perennial herb cultivated in upper Egypt and distributed mainly throughout the tropical and subtropical regions of the world. Curcumin represents a class of antiinflammatory and antioxidants reported to be a potent inhibitor of ROS formation (Venkatesan et al., 2000; Biswas et al., 2005). Reddy and Lokesh (1994a) indicated that curcumin is a potent scavenger of a variety of ROS including superoxide anion radicals (O₂) and hydroxyl radicals (OH). Curcumin administration has been reported to prevent the arsenic, gentamicin and acetaminophen-induced oxidative stress in rats (Fatma et al., 2009; Farombi and Ektor, 2006; Cekmen et al., 2009). The protective effects of curcumin against chemicallyinduced hepatotoxicity are well documented and have been attributed to its intrinsic antioxidant properties (Nanji et al., 2003: Rukkumani et al., 2004), Curcumin, like many antioxidants, can be a "double-edge sword." whereby, in the test tube, carcinogenic and pro-oxidant effects may be seen in addition to anticancer and antioxidant effects (Kawanishi et al., 2005). However, a previous in vivo study reported that gavage administration of 200 or 600 mg/kg curcumin effectively suppressed diethylnitrosamine (DEN)-induced liver inflammation and hyperplasia in rats, as evidenced by histopathological examination (Chuang et al., 2000). The antioxidant effects of vitamin E and taurine 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 curcumin, 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 curcumin against cadmium chloride-induced perturbations in oxidative biomarkers of blood such as levels of Hb-derivatives, plasma MDA level and GSH concentration, SOD and activities in erythrocytes hemolysate GPx and erythrocytes hemolysis test, in rats.

MATERIALS AND METHODS

Eighteen male albino rats (age: 6 to 8 weeks and about 80 to 110 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% fibre (w/w) with adequate minerals and vitamin contents. The rats were housed in stainless steel cages in a temperature-controlled room $(25\pm2^{\circ}C)$ with a 12 h light and 12 h dark exposure.

The animals were randomly divided into three groups of 6 animals each, control, cadmium chloride alone, and cadmium chloride with curcumin. All groups were given a standard rat chow and water. Rats in cadmium alone and cadmium with curcumin groups were given treatments orally by gavage needle for 24 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 curcumin group received a daily dose of a solution containing 10 mg/kg body weight of monohydrated cadmium chloride and 600 mg/kg body weight of curcumin dissolved in 2 ml of distilled water orally. The dose of curcumin used in this study was selected on the basis of the previous study (Chuang et al., 2000). Curcumin, an active component of turmeric (C. longa Linn) and a yellow coloured phenolic pigment yield from the rhizome of this tumeric, was purchased in a powder form from Elgabry Company for medicinal herbs, Giza, Egypt.

Animal sacrifice and collection of samples

The experiments lasted for 24 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° C) was used for MDA determination. Erythrocytes were 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 assay of GSH concentration and antioxidant enzymes activities. According to the antioxidant assays, appropriate phosphate buffers of pH 7 for GPx and pH 8.5 for SOD were added to the hemolysate samples; therefore, the antioxidant enzymes do not loss their activities.

Biophysical assays

Levels of hemoglobin derivatives (sulfhemoglobin (SHb), methemoglobin (metHb), carboxyhemoglobin (HbCO), and oxyhemoglobin (HbO₂)) in blood of rats were determined by the multicomponent spectrophotometric method described previously (Attia et al., 2011a). Percentages of erythrocytes hemolysis were determined according to the method of Attia et al. (2011b).

Biochemical assays

For biochemical analysis specially manufactured kits were used. Reduced glutathione (GSH) concentration was determined spectrophotometrically by the method of Beutler et al. (1963). The method based on the reduction of 5.5 dithiobis (2-nitrobenzoic acid) (DTNB) with GSH to produce a yellow compound. The reduced chromogen directly proportional to GSH concentration and its absorbance can be measured at 405 nm. Plasma malondialdehyde (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. Superoxide dismutase (SOD) activity was determined spectrophotometrically by the method of Nishikimi et al. (1972). The principle of this assay relies on the ability of the enzyme to inhibit the phenazine methosulphatemediated reduction of nitroblue tetrazolium dye. The percent inhibition directly proportional to SOD activity was calculated,

depending on the increase in absorbance at 560 nm for control and sample, respectively. Gutathione peroxidase (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 (c-GPx). Oxidized glutathione (GSSG), produced upon reduction of an organic peroxide by c-GPx, is recycled to its reduced state by the enzyme glutathione reductase (GR). The oxidation of NADPH to NADP⁺ is accompanied by a decrease in absorbance at 340 nm (A₃₄₀) providing a spectrophotometric means for monitoring GPx enzyme activity. To assay c-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 A₃₄₀ is recorded. The rate of decrease in the A₃₄₀ is directly proportional to the GPx activity in the sample.

Statistical analysis

Data were presented as the mean \pm standard error (SE) values. One way analysis of variance (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 significant.

RESULTS

Blood hemoglobin derivatives

Table 1 shows the levels of inactive hemoglobins (SHb, metHb, HbCO) and active HbO_2 in all groups. After 24 days, significant increase in metHb% was observed in cadmium-exposed rats, compared to the control group (P < 0.05), while cadmium treatment had no significant effects on SHb% and HbCO%. Curcumin treatment of cadmium exposed rats significantly lowered metHb%, while significantly increased HbO₂%, compared to the cadmium alone group (P < 0.05).

Plasma MDA concentration

Table 2 shows the concentration of MDA in plasma of all groups. After 24 days, significant increase in MDA concentration was observed in cadmium exposed rats as compared to control group (P < 0.005). Curcumin treatment of cadmium exposed rats lowered MDA concentration (P < 0.05, -10%) compared to cadmium alone group.

Erythrocyte antioxidant enzyme activities and GSH concentration

Table 2 shows the concentration of GSH as well as the activities of SOD and GPx in erythrocytes of all groups. Superoxide dismutase (SOD) and GPx activity significantly increased (P < 0.05) in cadmium compared to the control group, while GSH concentration showed insignificant change. Curcumin treatment significantly increased

Parameter	Control	Cadmium chloride	Cadmium chloride + curcumin
SHb%	0.942 ± 0.241	0.993 ± 0.178	0.758 ± 0.127
metHb%	1.215 ± 0.112	1.680 ± 0.327 ^a	0.961 ± 0.402^{b}
HbCO%	3.203 ± 0.257	2.901 ± 0.270	3.121 ± 0.260
HbO ₂ %	94.634 ± 0.159	94.425 ± 0.530	95.201 ± 0.345^{b}

Table 1. Effects of cadmium chloride and curcumin on levels of inactive and active hemoglobins in blood of rats.

Data are presented as mean \pm standard error (SE). ^aSignificantly different from control P < 0.05. ^bSignificantly different from cadmium alone treatment group P < 0.05.

 Table 2. Effects of cadmium chloride and curcumin on concentrations of plasma MDA, GSH,

 SOD and GPx activities in erythrocyte hemolysate in rats.

Parameter	Control	Cadmium chloride	Cadmium + curcumin
MDA (nmol/ml)	12.359 ± 0.957	17.844 ± 0.487 ^b	16.06 ± 1.275 ^{a,c}
GSH (mg/dl)	4.018 ± 0.301	4.246 ± 0.366	4.445 ± 0.391
SOD (U/g Hb)	6396.134 ± 873.252	9512.439 ± 703.613 ^a	10356.180 ± 954.806 ^b
GPx (mU/ml)	145.90 ± 5.978	170.757 ± 12.714 ^a	190.211 ± 7.971 ^{b,c}

Data are presented as mean \pm standard error (SE). ^aSignificantly different from control P < 0.05. ^bSignificantly different from control- *P* < 0.005. c significantly different from cadmium alone treatment group P < 0.05.

increased GPx activity of cadmium exposed rats as compared to cadmium alone group (P < 0.05), while it has no effect on SOD activity and GSH concentration. However, SOD and GPx activity of cadmium+curcumin group is significantly higher (P < 0.005) than controls.

Percentages of erythrocytes hemolysis

Figure 1 shows the hemolysis percent 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 curcumin, it decreases by 9%.

DISCUSSION

Cd is a toxic metal that is widely used in different industries. The Agency for Toxic Substances and Disease Registry (ATSDR, 1989) in Atlanta, Georgia has listed Cd as a number 7 in its top 20 list of hazardous substances. It 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 24 days of Cd treatment. This finding is in agreement with several reports demonstrating that Cd induces oxidative stress in tissues by increasing lipid peroxidation and altering the antioxidant status in several tissues (Ognjanović et al., 2003; Sinha et al., 2008; Kanter et al., 2009; Onwuka et al., 2011; Tarasub et al., 2011, 2012; El-Sokkary et al., 2009).

This study showed that erythrocytes hemolysis in Cd treated animals is higher than controls consistent with a previous study (Kanter et al., 2009). This high rate of Cd-induced hemolysis decreases by 9% after curcumin treatment. Curcumin represents a class of antioxidants reported to be a potent inhibitor of ROS formation (Venkatesan et al., 2000; Biswas et al., 2005). Reddy and Lokesh (1994b) indicated that curcumin is a potent scavenger of a variety of ROS including superoxide anion radicals (O_2) and hydroxyl radicals (OH). This high antioxidant activity of curcumin can account for the decrease in lipid peroxidation and erythrocyte hemolysis after curcumin treatment of Cd-exposed rats, observed in this study.

The inactive components of Hb (SHb, metHb and HbCO) are unable to transport oxygen, while HbO₂ is the active Hb. 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 inactive 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 HbO₂-autoxidation reactions (Waltkins et al., 1985) and its improvement after treatment with curcumin can account for the decrease in metHb% and



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.

^aSignificantly different from control P < 0.05, ^bSignificantly different from cadmium alone treatment group P < 0.05.

increase in HbO₂% observed in the present study.

Previous investigations showed that chronic treatment with Cd induced oxidative damage in erythrocytes of rats, causing destruction of red cell membranes and increased lipid peroxidation, as well as alteration of the antioxidant defense system, energy metabolism and appearance of anemia (Kostič et al., 1993; Kanter et al., 2005; Ognjanovič et al., 2000; Zikič et al., 1997, 2001; Pavlovic et al., 2001).

The results obtained in our present study show that treat-ment with Cd induces an increase of the 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 curcumin was effective in decreasing oxidative damage induced by Cd which resulted in markedly lower MDA concentration. Curcumin was capable of inhibiting formation of ROS which caused hemolysis. through its high antioxidant activity (Venkatesan et al., 2000; Biswas et al., 2005; Reddy and Lokesh, 1994a).

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), curcumin as antioxidants may be important components of an effective Cd intoxication treatment. The inhibitory effect of turmeric on Cd induced lipid peroxidation in blood was in parallel with Lalitha and Selvam (1999) who suggested that, curcumin provided a protection against lipid peroxidation and hemolysis of RBCs induced by H_2O_2 .

In animals exposed to Cd, the activities of SOD and GPx in RBC were significantly increased (Table 2). These results are consistent with previous studies (Zikić et al., 2001; Ognjanović et al., 2003; Kanter et al., 2005, 2009). 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. Cd 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 curcumin of Cd exposed rats increased GPx activity, indicating that this substance eliminates the toxic effects of Cd on the activity of this enzyme. At the same time, erythrocyte GSH concentration remains at the level of control values which confirm the protective role of curcumin. Moreover, curcumin treatment enhances the activity of SOD as compared to controls. The indirect antioxidant capacity of curcumin is defined by its ability to induce the expression of antioxidant enzymes such as SOD (Panchal et al., 2008) and GPx (Yarru et al., 2009). The antioxidant enzymes induced by curcumin are regulated by the nuclear factor erythroid-derived 2 (Nrf2) (Cuadrado et al., 2009; Rojo et al., 2012), which in turns is also activated by curcumin (Calabrese et al., 2008; Eggler et al., 2008).

The antioxidant mechanism of curcumin was attributed

to its conjugated structure which includes two methoxylated phenols and an enol form of β -diketone. The structure showed a typical radical trapping ability as a chain breaking antioxidant (Masuda et al., 2001). Curcumin exhibit a differential antioxidant activity in several in vitro and in vivo models, for example, preventing lipid peroxidation in a variety of cells such as erythrocytes and rat liver microsomes, where peroxidation is induced by Fenton's reagent, as well as for metals and hydrogen peroxide (H_2O_2) (Reddy and Aggarwal, 1994b). Furthermore, it has been reported that curcumin is a bifunctional antioxidant (Dinkova-Kostova and Talalay, 2008), because of its ability to react directly with reactive species and to induce an up-regulation of various cytoprotective and antioxidant proteins. Curcumin is able to scavenge superoxide anion (O2) (Ak and Gülcin, 2008; Sreejayan and Rao, 1996), hydroxyl radicals (OH) (Barzegar and Moosavi-Movahedi, 2011), singlet oxygen (Das and Das. 2002), nitric oxide (Sreejayan, 1997; Sumanont et al., 2004), peroxynitrite (Kim et al., 2003) and peroxyl radicals (ROO) (Barzegar Moosavi-Movahedi. 2011). Together. and these mechanisms might explain, at least in part, some of the cytoprotective effects of this compound. Features as the presence of phenolic groups in the structure of curcumin explains its ability to react with reactive oxygen species (ROS) and reactive nitrogen species (RNS) and might probably be one of the mechanisms through which curcumin treatment protects erythrocytes from oxidative damage.

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 antioxidant enzymes. Moreover, the results of multi-component spectrophotometric analysis showed an increase in the level of inactive methemoglobin (metHb). Curcumin expressed protective role against toxic influence of cadmium on all affected parameters in rats. Curcumin may exert its protective actions against cadmium-induced hematotoxicity in rats possibly through its antioxidant mechanisms. The results raise the possibility of curcumin 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 Interests

The author(s) have not declared any conflict of interests.

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