

Full Length Research Paper

Antioxidant effects of whole ginger (*Zingiber officinale* Roscoe) against lead acetate-induced hematotoxicity in rats

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Humans are exposed to a number of toxic elements in the environment. Lead, 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 lead-exposure related disease. The aim of the study was to investigate the effect of ginger on oxidative stress in rats exposed to lead. Ginger was administered orally (160 mg/kg b.w.). After 50 days, significant increases in sulfhemoglobin percent (SHb%), methemoglobin percent (metHb%), superoxide dismutase (SOD) activity, malondialdehyde (MDA) concentration and hemolysis test were observed in lead exposed rats compared to control group ($P < 0.05$). Glutathione peroxidase (GPx) activity significantly decreased in lead compared to control group ($P < 0.001$), while GSH concentration showed insignificant change. Ginger treatment of lead exposed rats significantly lowered SHb%, metHb%, carboxyhemoglobin percent (HbCO%), while it significantly increased oxyhemoglobin percent (HbO₂%) compared to lead alone group ($P < 0.05$). Also ginger treatment significantly increased GPx activity of lead exposed rats compared to lead alone group ($P < 0.05$). Ginger treatment of lead exposed rats lowered MDA concentration and hemolysis percent by 21.21 and 29.38, respectively. The findings of this study suggest that ginger elevated the GPx activity perturbed by exposure to lead and had ameliorative effect on lipid peroxidation and erythrocytes hemolysis. Moreover, the results of multi-component spectrophotometric analysis suggest that ginger treatment of lead exposed rats lowered the levels of inactive hemoglobins and elevated the level of active HbO₂. Ginger may exert its protective actions against lead-induced hematotoxicity in rats possibly through its antioxidant mechanisms and may have future therapeutic relevance.

Key words: Lead, ginger, oxidative stress, erythrocytes, Hb-derivatives, rats.

INTRODUCTION

Lead is an environmental contaminant due to its significant role in modern industry (Shalan et al., 2005). Both occupational and environmental exposures remain a serious problem in many developing and industrializing countries (Yücebilgic et al., 2003). Lead (Pb) is a toxic heavy metal and harmful even in small amounts (Gidlow,

2004). It has many undesired effects, especially hematological dysfunctions (Mousa et al., 2002; Sivaprasad et al., 2003). In addition to killing cells via cytotoxicity, lead causes toxic effects by oxidative stress either directly or by indirectly-produced lipid peroxidation. Lead alters lipid metabolism, enhances lipid peroxidation and decreases

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cell membrane fluidity of developing rats (Gurer and Ercal, 2000; Villeeda-Hernandez et al., 2001). In addition to membrane peroxidation, lead exposure causes hemoglobin oxidation, which can also cause erythrocytes hemolysis (Ribarov et al., 1981). The mechanism responsible for this reaction is lead-induced inhibition of delta aminolevulinic acid dehydratase (ALAD). As a result, elevated levels of the substrate ALA are found in the blood of lead-exposed subjects (Farant and Wigfield, 1982). Elevated levels of ALA generate hydrogen peroxide (H_2O_2) and superoxide radical ($O_2^{\cdot-}$), and also interact with oxyhemoglobin, resulting in the generation of hydroxyl radicals (OH \cdot), the most reactive of the free radicals (Bechara, 1996; Courtois et al., 2003). Oxidants and antioxidants have attracted widespread interest in nutrition research, biology and medicine. It has become clear that constant generation of pro-oxidants including oxygen free radicals, is an essential attribute of aerobic life (Sies, 1991). Reactive oxygen species (ROS) are very reactive molecules ranked as free radicals owing to the presence of one unpaired electron such as superoxide ion ($O_2^{\cdot-}$), nitrogen oxide (NO) and hydroxyl radical (OH \cdot). Even though naturally present in the organism, they are mainly confined to cell compartments and counterbalanced by natural antioxidant molecules, such as glutathione, glutathione peroxidase, superoxide dismutase, vitamin E and vitamin C, acting as free radical scavengers (Aruoma et al., 1994). Recent studies showed that lead inhibited the activities of the antioxidant defense system, including superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) (Ercal et al., 2000; Bolin et al., 2006). Lead also lowered the level of reduced glutathione (GSH) (Sandhir and Gill, 1995). 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). The alcohol ginger extract inhibited the hydroxyl radicals 79.6% at 37 °C and 74.8% at 80 °C, which showed a higher antioxidant activity than quercetin (Stoilova et al., 2007). The myriad beneficial effects of ginger are supposed to be due to the presence of bioactive phytochemicals like gingerols, shogaols, paradols, gingerdiols, and zingerone (Baliga et al., 2013). Zingerone scavenges superoxide anion. 6-gingerol and zingerone are reported to be good scavengers of peroxy radicals. 6-shogaol also inhibited the production of NO. 6-Gingerol is the major bioactive constituent responsible for the antiinflammatory, antitumour and antioxidant activities of ginger (Nagendra et al., 2013). Ginger and its constituents are stated to have antiemetic, antithrombotic, antihepatotoxic, anti-inflammatory stimulant, cholagogue, androgenic and antioxidant effects (Khaki et al., 2009). Ginger is a strong anti-oxidant substance and may either mitigate or prevent generation of free radicals. It is considered a safe herbal medicine with only few and insignificant adverse/side effects (Ali et al., 2008). Ginger extracts have been extensively studied for a broad range of

biological activities, especially antioxidant activities (Miller et al., 1993). Ahmed et al. (2000) found that ginger significantly lowered lipid peroxidation by maintaining the activities of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase in rats. The antioxidant effects of methionine, α -lipoic acid, N-acetylcysteine, homocysteine and wormwood (*Artemisia absinthium*) extract against lead acetate-induced oxidative stress to erythrocytes and hematotoxicity in rats have been studied (Caylak et al., 2008; Kharoubi et al., 2008). However, effects of ginger, as a powerful antioxidant, on lead acetate-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 whole ginger against lead acetate-induced perturbations in oxidative bio-markers of blood such as levels of Hb-derivatives, plasma MDA level and GSH concentration, SOD and GPx activities in erythrocytes hemolysate and erythrocytes hemolysis test, in rats.

MATERIALS AND METHODS

Eighteen non-pregnant female albino rats weighing 110 to 140 g 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 rats were housed in stainless steel cages in a temperature-controlled room ($25\pm 2^\circ\text{C}$) with a 12 h light and 12 h dark exposure.

Grouping of animals and treatment

The animals were randomly divided into three groups of six animals each, control, lead acetate alone, and lead acetate with ginger. All groups were given only standard rat feed and water during the 1st week. After this period of adjustment to their environment, control was given a standard rat chow and water. Rats in lead alone and lead with ginger groups received lead acetate in their drinking water (500 ppm) for 50 days. Ginger, which is the underground stem or rhizome of the plant *Z. officinale Roscoe*, was purchased in a powder form from El Gabry Company for Medicinal Herbs, Giza, Egypt. The ginger treated group received once a day ginger at a dose of 160 mg/kg body weight for 50 days. Ginger was diluted with distilled water to the desired concentration (160 mg/kg body weight) and this solution was administered orally to rats with a mouth injector (0.5 ml/rat/day).

Animal sacrifice and collection of samples

The experiments lasted for 50 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 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 assay of GSH concentration and

Table 1. Effects of lead acetate and ginger on levels of inactive and active hemoglobins in blood of rats.

Parameter	Control	Lead acetate	Lead acetate + ginger
SHb%	0.122 ± 0.054	1.351± 0.676 ^a	0.291 ± 0.136 ^b
metHb%	1.823 ± 0.279	3.718± 1.034 ^a	2.011 ± 0.439 ^b
HbCO%	5.180 ± 0.213	5.750± 0.346	4.954 ± 0.245 ^b
HbO ₂ %	92.875±0.506	89.180±2.002 ^a	92.742±0.695 ^b

Data are presented as mean ± S.E. ^a significantly different from control- $P < 0.05$. ^b significantly different from lead alone treatment group- $P < 0.05$.

Table 2. Effects of lead acetate and ginger on concentrations of plasma MDA, GSH, SOD and GPx activities in erythrocyte hemolysate in rats.

Parameter	Control	Lead acetate	Lead acetate + ginger
MDA (nmol/ml)	4.758 ± 0.621	7.190 ± 0.937 ^a	5.665 ± 1.325 ^c
GSH (mg/dl)	6.799 ± 0.411	7.099 ± 0.358	6.588 ± 0.605
SOD (U/g b)	3775.2± 152.6	6591.45±1127 ^a	6406.3±666.61 ^a
GPx (mU/ml)	433.38±40.991	225.875±12.46 ^b	293.097±23.4 ^{b,c}

Data are presented as mean ± S.E. ^a significantly different from control- $P < 0.05$. ^b significantly different from control- $P < 0.001$. ^c significantly different from lead alone treatment group- $P < 0.05$.

antioxidant enzymes activities.

Biochemical assays

All kits used for biochemical analyses were purchased from the Biodiagnostic Company, Cairo, Egypt. Reduced glutathione and MDA were determined spectrophotometrically according to Beutler et al. (1963) and Satoh (1978) methods, respectively. Activities of superoxide dismutase and glutathione peroxidase were determined spectrophotometrically according to Nishikimi et al. (1972) and Paglia and Valentine (1967) methods, respectively.

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., 2011). Percentages of erythrocytes hemolysis were determined according to the method of Attia et al. (2011).

Statistical analysis

Data were presented as the mean±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 significant.

RESULTS

Blood hemoglobin derivatives

Table 1 shows the levels of inactive hemoglobins (SHb,

metHb, HbCO) and active HbO₂ in all groups. After 50 days, significant increases in SHb%, and metHb% were observed in lead-exposed rats, compared to the control group ($P < 0.05$). Ginger treatment of lead exposed rats significantly lowered SHb%, metHb%, HbCO%, while it significantly increased HbO₂%, compared to the lead alone group ($P < 0.05$).

Plasma MDA concentration

Table 2 shows the concentration of MDA in plasma of all groups. After 50 days, significant increase in MDA concentration was observed in lead exposed rats compared to control group ($P < 0.05$). Ginger treatment of lead exposed rats lowered MDA concentration ($P < 0.05$, -21.21%) compared to lead 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) activity significantly increased ($P < 0.05$) and GPx activity significantly decreased ($P < 0.001$) in lead compared to control group, while GSH concentration showed insignificant change. Ginger treatment significantly increased GPx activity of lead exposed rats compared to lead alone group ($P < 0.05$), while it has no effect on SOD activity and GSH concentration. However, GPx activity of ginger + Lead group is significantly lower ($P < 0.001$) than controls.

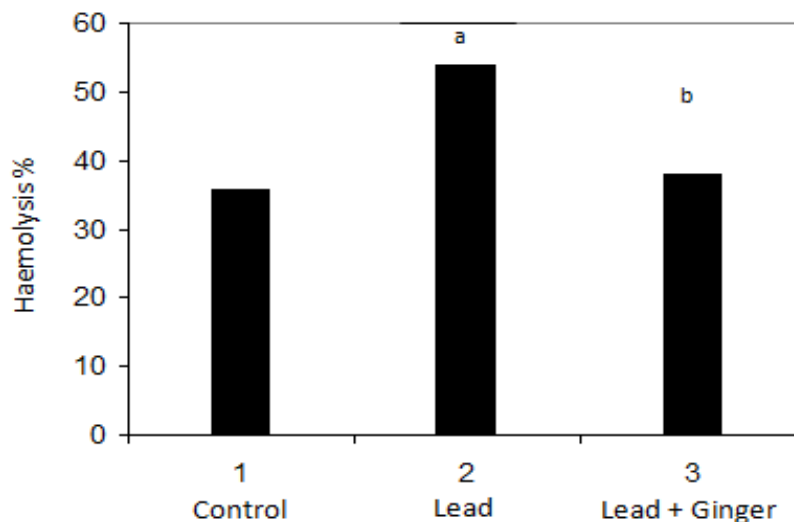


Figure 1. Percentages of erythrocytes hemolysis in all groups. Hemolysis test was carried out after incubation of erythrocytes in phosphate buffered saline (PBS), pH 7.4, for 3 days at room temperature. ^a significantly different from control- $P < 0.05$, ^b significantly different from lead alone treatment group- $P < 0.05$.

Percentages of erythrocytes hemolysis

Figure 1 shows the hemolysis percent of erythrocytes in all groups. The hemolysis test indicates that intoxication by lead significantly increases the hemolytic effect ($P < 0.05$), whereas after treatment with ginger, it decreases by 29.38%.

DISCUSSION

Lead, a common environmental occupational toxic heavy metal, is known to have indirect oxidative effects on biological systems and cells. Lead-induced oxidative stress in blood and other soft tissues has been postulated to be one of the possible mechanisms of lead-induced toxic effects (Waters et al., 2008; Auman et al., 2007; Pande et al., 2002). Lead exposure induces severe oxidative damage in erythrocytes by inhibiting heme synthesis and changing erythrocyte morphology and survival (Leggett, 1993). Oxidative stress also leads to lipid peroxidation in erythrocyte membranes, autoxidation of hemoglobin and limited repair processes, leading to decreased survival (Rice-Evans and Baysal, 1987).

This study showed that erythrocytes hemolysis in lead treated animals is higher than controls consistent with a previous study (Kharoubi et al., 2008). This high rate of lead-induced hemolysis decreases by 29.38% after ginger treatment. Ginger 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 (Masuda et al., 2004; Kikuzaki and

Nakatani, 2006). Among them, 12 compounds exhibited higher antioxidant activity than α -tocopherol. This high antioxidant activity of ginger can account for the decrease in lipid peroxidation and erythrocyte hemolysis after ginger treatment of lead exposed rats, observed in this study.

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 lead treatment of rats, with decrease in their levels after ginger treatment.

Since the inactive components of Hb (SHb, metHb and HbCO) are unable to transport oxygen, the net level of active functional Hb (HbO₂) is an indicator of the actual degree of anemia. Increase in SHb%, metHb% and HbCO% in lead exposed rats may explain the decrease in HbO₂% in this group. While their decrease with ginger treatment may explain the increase in HbO₂% observed after ginger treatment.

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 lead exposed animals can account for the increase in metHb% produced through HbO₂-autoxidation reactions (Walkins et al., 1985) and its improvement after treatment with ginger can account

for the decrease in metHb% and increase in HbO₂% observed in the present study.

Previous investigations showed that chronic treatment with lead 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 (Gurer et al., 1998; Hiraku and Kawanishi, 1996; Costa et al., 1997; Sugawara et al., 1991).

The results obtained in our present study show that treatment with lead induces an increase of the level of lipid peroxidation product, MDA, in the blood of rats, which were accompanied by increased formation of ROS (Gurer and Ercal, 2000). 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 (Hiraku and Kawanishi, 1996). Treatment with ginger was effective in decreasing oxidative damage induced by lead which resulted in markedly lower MDA concentration. Ginger was capable of inhibiting formation of ROS which caused hemolysis, through its high antioxidant activity (Masuda et al., 2004; Kikuzaki and Nakatani, 2006; Miller et al., 1993; Ahmed et al., 2000).

In animals exposed to lead, the activity of SOD was significantly increased (Table 2). These results are consistent with previous studies (Soltaninejad et al., 2003; Abdel-Kader et al., 2011). It is known that lead induces the formation of superoxide anion radicals in erythrocytes and it is reasonable to expect an increased activity (Costa et al., 1997; Ye et al., 1999). The mechanism of Hb oxidation to metHb and superoxide, followed by SOD catalyzed dismutation of superoxide to H₂O₂, has long been known, and it is proposed that high metHb values found in lead-exposed workers reflect both lead-induced direct HbO₂ oxidation and co-oxidation with aminolevulinic acid (ALA) (Monteiro et al., 1986).

In the present study, lead induced a decrease in GPx activity, in contrast to a previous study which showed an increase in GPx activity with lead treatment (Chiba et al., 1996). Other studies showed a decrease in GPx activity with lead treatment (Sivaprasad et al., 2004; Abdel-Kader et al., 2011; Haleagrahara et al., 2010; Anuradha et al., 2011; Wang et al., 2012). Lead also binds to enzymes that have functional sulfhydryl groups, rendering them non-functional and further contributing to impairment in oxidative balance. The treatment with ginger after lead administration increased GPx activity, indicating that this substance eliminates the toxic effects of lead on the activity of this enzyme. At the same time, erythrocyte GSH concentration remain at the level of control values which confirm the protective role of ginger. Reduced levels of GPx were all found to correlate with reduced GSH levels in occupationally-exposed workers (Sandhir and Gill, 1995). The treatment with antioxidants helped to elevate the GPx activity and erythrocyte GSH content (Flora et al., 2003; 2004).

Conclusion

Results of the study thus revealed that lead 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 levels of inactive hemoglobins (SHb and metHb), concomitant with a significant decrease in HbO₂% after lead treatment of rats. Ginger expressed protective role against toxic influence of lead on all analyzed parameters in rats. Ginger may exert its protective actions against lead-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 lead occupationally or environmentally.

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