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Full Length Research Paper

Antioxidant effects of ginger (*Zingiber officinale* Roscoe) against lead acetate-induced hepatotoxicity 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. Effects of aqueous solution of whole ginger on hepatic injury due to lead-induced oxidative stress in experimental rats have been investigated. Lead acetate (LA) at a dose of 500 ppm in drinking water was administered to rats for 50 days to induce hepatic injury. Freshly prepared aqueous whole ginger solution at a dose of 160 mg/kg body weight was administered orally to rats. Lead acetate treatment caused hepatic injury as evident from increased activities of plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST) atrnd alkaline phosphatase (ALP), depleted hepatic reduced glutathione (GSH) and elevated hepatic malondialdhyde (MDA) concentration. Lead-induced oxidative stress in liver was evident from increased levels of lipid peroxidation and reduced level of GSH. The decreased activity of glutathione peroxidase (GPx) indicates possible accumulation of ROS. The changes in AST and GPx activities and MDA concentration were found to be mitigated when the rats were treated with whole ginger. Also, ginger treatment of LA-exposed rats was found to enhance superoxide dismutase (SOD) activity. Results indicate that ginger has the potential to ameliorate lead-induced hepatic injury due to oxidative stress in rats. Ginger may exert its protective actions against lead-induced hepatotoxicity in rats possibly through its antioxidant mechanisms and may have future therapeutic relevance.

Key words: Lead, ginger, oxidative stress, antioxidants, hepatotoxicity, albino rats.

INTRODUCTON

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). The manifestations of lead poisoning in humans are nonspecific. They may include weight loss, anemia (Khalil-Manesh et al., 1994), nephropathy, infertility, liver, testis and heart damages (Patocka and Cerny, 2003; Gurer-Orhan et al., 2004), etc. Lead is known to produce oxidative damage in the liver tissues by enhancing peroxidation of membrane lipids (Chaurasia and Kar, 1997), a deleterious process solely carried out by free

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radicals (Halliwell and Gutteridge, 1990). 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 (Pande et al., 2001). Disruption of pro-oxidant/antioxidant balance might lead to tissue injury. It was reported that lead increased the level of lipid peroxides and altered the antioxidant defense system in the hepatic tissues (Sandhir and Gill, 1995). A previous study confirmed the possible involvement of reactive oxygen species (ROS) in lead-induced toxicity (Gurer and Ercal, 2000). Oxidants and antioxidants have attracted widespread interest in nutrition research, biology and medicine. It has become clear that constant generation of prooxidants including oxygen free radicals, is an essential attribute of aerobic life (Sies et al., 1991). 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). Based on the observation that free radicals were generated during the pathogenesis processes induced by lead exposure, it was presumed that supplementation of antioxidants could be an alternative method for chelation therapy (Flora et al., 2003). 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 (Masuda et al., 2004; Kikuzaki and Nakatani, 2006). 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 (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). Antioxidant activities of the bark extracts of *Garcinia hombroniana* and essential oils of the leaves and stem of *Tarchonanthus camphoratus* have been reported (Nargis et al., 2013; Nanyonga et al., 2013). 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 blood of rats. Effects of *Juniperus phoenicea* extract on the activity of antioxidant enzymes in liver of oxonate-treated rats have been studied (Gdoura et al., 2013). Also, the antioxidant effects of whole ginger against lead acetate-induced liver apoptosis in rats have been studied (Khaki and Khaki, 2010). However, effects of ginger, as a powerful antioxidant, on lead acetate-induced oxidative stress to liver tissue homogenate and liver injury 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 such as malondialdhyde (MDA) and glutathione (GSH) concentrations, superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in liver tissue homogenate and bio-markers of liver injury such as plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities in rats.

MATERIALS AND METHODS

Twenty four non-pregnant female albino rats weighing 110 to 140 g were obtained from the animal house, National Research Center, Cairo, Egypt. Female rats were selected because they are available and there was a deficiency in male rats at that time. 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 ± 2°C) with a 12 h light and 12 h dark exposure.

Grouping of animals and treatment

The animals were randomly divided into four groups of 6 animals each: control, ginger alone, 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, the control group 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 Elgabry company for medicinal herbs, Giza, Egypt. Ginger treated groups received once a day whole ginger at a dose of 160 mg/kg body weight for 50 days. Aqueous solution of whole ginger of the desired concentration (160 mg/kg body weight) was prepared and 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 various biochemical assays. Following the collection of blood samples, all animals were sacrificed, the liver from each rat was removed, washed using chilled saline solution. Tissue was minced and homogenized in appropriate buffer and then centrifuged, according to the instructions of the biochemical assay. The resulting clear supernatant was collected and stored at -80°C till further analysis and was used for various enzymatic and non-enzymatic biochemical assays. All the

biochemical assays. All the enzyme analyses were done within one week of collecting the samples.

Biochemical assays

All kits used for biochemical analyses were purchased from the Biodiagnostic Company, Cairo, Egypt. Plasma ALT and AST activities were determined spectrophotometrically by the method of Reitman and Frankel (1957). The pyruvate or oxaloacetate formed by transaminases is measured in its derivatives form 2, 4-dinitrophenylhydrazine. The absorbance of the colored product was measured at 505 nm. Plasma ALP activity was determined spectrophotometrically by the method of Belfield and Goldberg (1971). The principle of this method relies on conversion of phenyl phosphate by ALP into phenol and phosphate. The liberated phenol was measured in the presence of 4- aminophenazone and potassium ferricyanide at 510 nm. Hepatic reduced GSH concentration was determined spectrophotometrically by the method of Beutler et al. (1963). The method was 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. Hepatic 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 TBA reactive product. The absorbance of the resulting pink product can be measured at 534 nm. Hepatic 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 methosulphate-mediated 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. Hepatic 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 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 (A_{340}) providing a spectrophotometric means for monitoring GPx enzyme activity. To assay c-GPx, tissue homogenate is added to a solution containing GSH, GSH 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.

Statistical analysis

Data were presented as the mean ± 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 tests using a statistical package program (SPSS version 14). $P < 0.05$ was considered as statistically significant.

RESULTS

Plasma enzymes

The results of plasma ALT, AST and ALP activities of all groups are shown in Table 1. There was a significant hepatotoxicity with lead acetate in drinking water for 50 days as evidenced by a significant increase ($P < 0.05$) in plasma ALT and AST levels and a significant increase in ALP level ($P < 0.005$), compared to control rats. Treatment of lead-exposed rats with whole ginger significantly reduced ($P < 0.05$) plasma AST activity compared to lead alone group. Treatment of lead-exposed rats with ginger did not change ALT and ALP activities as compared to lead alone group. Treatment with ginger alone did not change the activities of ALT, AST and ALP as compared to control rats.

Hepatic lipid peroxidation and GSH concentration

The results of MDA and reduced GSH concentrations in liver tissue homogenate of all groups are shown in Table 2. The concentration of hepatic MDA, which is an indicator of lipid peroxidation, markedly increased ($P < 0.05$) and the antioxidant GSH concentration significantly decreased ($P < 0.001$) in the lead alone group as compared to control group. Treatment with whole ginger of the lead-exposed rats significantly reduced MDA concentration as compared to lead alone group ($P < 0.001$). Treatment of lead-exposed rats with whole ginger did not change GSH concentration as compared to lead alone group. Treatment with ginger alone did not change hepatic MDA and GSH concentrations as compared to the control rats.

Hepatic antioxidant enzyme activities

The results of antioxidant enzymes SOD and GPx activities in liver tissue homogenate of all groups are shown in Table 2. Treatment with lead alone significantly reduced liver GPx activity ($P < 0.002$), while it has no effect on SOD activity as compared to control rats. Treatment with whole ginger of lead-exposed rats significantly increased SOD and GPx activities ($P < 0.05$, +14 and +22.58%, respectively) as compared to lead alone group. However, GPx and SOD activities of ginger + lead group are significantly different ($P < 0.002$ and $P < 0.02$, respectively) from controls. Treatment with ginger alone significantly increased SOD activity ($P < 0.002$), while it did not significantly change GPx activity as compared to the control rats.

DISCUSSION

In this study, lead acetate treatment caused hepatic injury as evident from increased activities of plasma ALT, AST and ALP and elevated hepatic MDA concentration. Lead-induced oxidative stress in liver was evident from increased levels of lipid peroxidation and reduced level of GSH. The decreased activity of GPx indicates possible

Table 1. Effects of lead acetate and ginger on plasma enzyme levels in rats.

Group	ALT (U/ml)	AST (U/ml)	ALP (IU/L)
Control	42.333 ± 4.079	68.340 ± 5.861	196.777 ± 21.180
Lead acetate	54.000 ± 4.099[a]	116.906 ± 38.482[a]	368.010 ± 42.214[c]
Lead acetate + ginger	59.500 ± 5.965[b]	59.070 ± 4.431[d]	348.723 ± 33.404[c]
Ginger	46.833 ± 4.339[d]	63.540 ± 5.590[d]	206.406 ± 39.752[d]

Data are presented as mean ± SE. [a]Significantly different from control ($P < 0.05$); [b]Significantly different from control ($P < 0.02$); [c]Significantly different from control ($P < 0.005$); [d]Significantly different from lead alone treatment group ($P < 0.05$).

Table 2. Effects of lead acetate and ginger on liver oxidative parameters in rats.

Parameter	Control	Lead acetate	Lead acetate + ginger	Ginger
MDA (nmol/g tissue)	1267.97± 40.52	1380.62±76.49[a]	1001.19±95.08[af]	1170.46±33.72[e]
GSH (mg/g tissue)	212.867± 51.56	21.064±3.681[d]	46.662±10.458[d]	127.32±36.014[e]
SOD (U/g tissue)	2863.85± 154.5	3190.79±265.2	3638.15±11[ce]	3648.02±12.91[ce]
GPx (U/g tissue)	62.683± 8.964	33.502±5.138[c]	41.068±3.617[be]	59.56±2.899[f]

Data are presented as mean ± SE. [a]Significantly different from control ($P < 0.01$); [b] Significantly different from control ($P < 0.02$); [c]Significantly different from control ($P < 0.002$); [d]Significantly different from control ($P < 0.001$); [e]Significantly different from lead alone treatment group ($P < 0.05$); [f]Significantly different from lead alone treatment group ($P < 0.001$).

accumulation of ROS. The changes in AST and GPx activities and MDA concentration were found to be mitigated when the rats were treated with whole ginger. Also, ginger treatment of LA-exposed rats was found to enhance SOD activity. Furthermore, treatment of rats with ginger only has no effect on plasma ALT, AST and ALP activities, hepatic MDA and GSH concentrations.

Liver injury following lead exposure is well characterized by elevated levels of plasma hepatic marker enzymes which indicate cellular leakage and loss of functional integrity of hepatic membrane architecture. High levels of aminotransaminases (ALT and AST) and ALP are crucial parameters in detecting liver damage. In this study, a significant increase in the plasma levels of ALT, AST and ALP was observed in lead-treated rats when compared with the control rats (Table 1). Haleagrahara et al. (2010) reported increased activities of ALT, AST and ALP in rats treated with lead acetate (LA). Sivaprasad et al. (2004), Wang et al. (2012) and El-Nekeety et al. (2009) also reported increased activities of ALT and AST in rats during lead poisoning. These findings are consistent with our results which showed increased activities of ALT, AST and ALP in plasma of lead-treated rats. Changes in the activities of these enzymes are liver specific and are considered a tool used to study varying cell viability and cell membrane permeability (Dasgupta et al., 1996). The administration of whole ginger (160 mg/kg body weight) markedly attenuated lead-induced hepatotoxicity in rats, as indicated by the significant decrease in AST activity of lead-exposed rats after ginger treatment. The aforementioned effects clearly indicate that ginger may offer protection by stabilizing the cell membrane in lead-induced hepatic damage. Furthermore, treatment of rats with ginger only has no effect on plasma ALT, AST and ALP activities, indicating clearly that ginger by itself does not cause any adverse effect on the hepatic tissue.

That the tissue injury observed following treatment of rats with lead acetate was brought about due to oxidative stress is evident from a marked increase in the level of lipid peroxidation products (MDA) and also of reduced GSH level of hepatic tissue. These two parameters are considered as the primary bio-markers of oxidative stress. Lead exposure can generate free radicals which results in the elevation of lipid peroxidation (MDA concentration) (Ademuyiwa et al., 2009). Lipid peroxides inactivate cell constituents through oxidative stress by undergoing a radical chain reaction ultimately leading to loss of membrane integrity or oxidation (Maiti et al., 1995; Abdel-Wahhab and Aly, 2005). GSH is a sulfhydryl peptide widely found in all biological systems. It forms the first line of defense against oxidative insult by acting as a nonenzymatic antioxidant. Its sulfhydryl (SH) group can directly interact with ROS or it can be involved in the enzymatic detoxification reaction of ROS as a cofactor or a coenzyme (Sivaprasad et al., 2002, 2004). In this study, a significant increase in lipid peroxidation as indicated by a marked increase in MDA was observed and significant decrease in GSH concentration in lead-treated rat liver as compared to controls, which is in agreement with previous studies (Wang et al., 2007; El-Nekeety et al., 2009;

Wang et al., 2012). The stimulation of lipid peroxidation as a result of lead treatment could be due to the formation of free radicals (El-Nekeety et al., 2009; Abdel-Wahhab and Aly, 2005) through an exhaustion of antioxidants, leading to oxidative stress and consequently lipid peroxidation (El-Nekeety et al., 2009). The decrease in GSH concentration may be due to the ability of lead to bind with the SH group which decreases the GSH levels, thereby interfering with the antioxidant activity (Sivaprasad et al., 2004). Ginger, which behaves as a powerful antioxidant and free radical scavenger (Masuda et al., 2004; Kikuzaki and Nakatani, 2006; Miller et al., 1993; Ahmed et al., 2000), can decrease MDA level perturbed by lead acetate in rat liver, as observed in this study. The findings of this study suggest that ginger could attenuate oxidative stress by decreasing the lipid peroxidation (MDA level) in lead-treated liver. Treatment of rats with whole ginger at a dose of 160 mg/kg body weight prevented the levels of lipid peroxidation to rise when the animals were challenged with lead acetate, indicating that this dose of ginger is fully capable of mitigating the oxidative stress induced following treatment of the animals with lead acetate. It is also interesting to note that ginger when given alone to another group of rats has almost no effect on these classical bio-markers of oxidative stress. This indicates that ginger may be considered safe for future human consumption, as evident at least from the results of our experiments.

Lead administration caused a significant ($P < 0.002$) decrease in the activity of the antioxidant enzyme GPx, while it did not change SOD activity (Table 2). This decreased GPx activity with lead treatment is in agreement with previous studies (Haleagrahara et al., 2010; Anuradha and Krishnamoorthy, 2011; Wang et al., 2012). SOD and GPx are important antioxidant enzymes (Boots et al., 2008). They constitute a mutually supportive defense mechanism against ROS. SOD decomposes superoxide radicals ($O_2^{\cdot-}$) to produce H_2O_2. GPx is a selenoenzyme which plays a major role in the reduction of H_2O_2 and hydroperoxide to produce nontoxic products (Renugadevi and Prabu, 2010). Therefore, the activities of these enzymes have been used to assess oxidative stress in cells. Many studies have shown that lead has high affinity for SH groups in several enzymes such as SOD and GPx, thus it can alter antioxidant activities by inhibiting functional SH groups in these enzymes (Hsu and Guo, 2002; Chiba et al., 1996). SOD and GPx are potential targets of lead toxicity as they depend on various trace elements for their correct molecular function and activity (Hsu and Guo, 2002). Moreover, if the balance between ROS production and antioxidant defense is broken, the enzyme may be exhausted and its concentration depleted (Liu et al., 2010). In the present study, the activity of GPx in rat liver was dramatically decreased by lead treatment. This suggested that lead exposure induced oxidative stress by inhibiting the activity of this antioxidant enzyme. Interestingly, the administration of whole ginger increased the activities of SOD and GPx (by +14 and +22.58%, respectively) in the liver of lead-treated rats, which might be due to the ability of ginger to reduce the accumulation of free radicals. This high antioxidant activity of ginger against lipid peroxidation in blood of rats was reported previously (Ahmed et al., 2000). The high antioxidant activity of ginger was attributed to the high content of polyphenol compounds (6-gingerol and its derivatives) and diarylheptanoids, which have a high antioxidant activity (Chen et al., 1986; Herrman, 1994; Masuda et al., 2004; Kikuzaki and Nakatani, 2006).

Ginger and the obtained extracts contain 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). The isolated antioxidants 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, 2006). 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, responsible to the pungent flavor of ginger, varies from 4.0 to 7.5% and also possesses substantial antioxidant activity (Balachandran et al., 2006). Ginger and its constituents are stated to have antiemetic, antithrombotic, antihepatotoxic, anti-inflammatory stimulant, cholagogue, androgenic and antioxidant effects *in vivo* (Khaki et al., 2009; Miller et al., 1993). The main pharmacological actions of ginger and compounds isolated from it include immuno-modulatory, anti-tumorigenic, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-lipidemic and anti-emetic actions (Ali et al., 2008). Ginger is a strong anti-oxidant substance and may either mitigate or prevent generation of free radicals. Ahmed et al. (2000) found that ginger significantly lowered lipid peroxidation by maintaining the activities of antioxidant enzymes such as SOD, catalase and glutathione peroxidase in rats.

Results of this study indicate that treatment of rats with the present dose of lead acetate brings about oxidative stress-induced hepatic tissue injury due to alterations in the balance between antioxidant/pro-oxidant systems and affecting the antioxidant enzyme (GPx). Ginger may exert its protective actions against lead-induced hepatic injury 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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