

Full Length Research Paper

Antioxidant effect of ginger to prevents lead-induced liver tissue apoptosis in rat

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Accepted 30 June, 2010

Lead acetate is an example of heavy metals that for decades being known for its adverse effects on various body organs and systems such that their functions are compromised. In the present study, the ability of lead to adversely affect the liver tissue apoptosis was investigated. Ginger is source of antioxidants was administered orally to prevent the adverse effects of lead acetate. Thirty Wistar rats, randomised into 3 groups (n = 10), were used for this study. Animals in group (A) served as the control and were drinking distilled water. Animals in groups (B) and (C) were drinking 1% lead acetate. Group (C) animals were, in addition to drinking lead acetate, treated with 100 mg/kg/rat of ginger. All treatments were for 10 weeks. The obtained results showed that lead acetate caused a significant reduction in the liver weight, plasma superoxide dismutase and catalase activity, but a significant increase in plasma malondialdehyde concentration, using ginger cause to modified these harmful effects. These findings lead to the conclusion that ginger significantly decreased the adverse harmful effects of lead acetate exposure on the liver as well as lead acetate -induced oxidative stress.

Key words: Apoptosis, ginger, lead acetate, liver, reactive oxygen species.

INTRODUCTION

Lead (Pb) is a dangerous heavy metal and harmful even in small amounts. Nevertheless, humans get exposed to Pb through their environment and diet (Gidlow, 2004). The manifestations of Pb poisoning in humans are nonspecific. They may include weight loss, anemia, (Khalil-Manesh et al., 1994; Waldron, 1966) memory loss, (Hopkins, 1970) nephropathy, infertility, liver, testis and heart damages' (Patocka et al., 2003; Gurer-Orhan et al., 2004) etc. *Zingiber officinale* R., family: Zingiberaceae. (Ginger), and its constituents are stated to have antiemetic, anti-thrombotic, anti-hepatotoxic, anti-inflammatory, stimulant, cholagogue, androgenic and

antioxidant (khaki et al., 2009; Lisa, 2002). The main pharmacological actions of ginger and compounds isolated there from include immuno-modulatory, anti-tumorigenic, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-lipidemic and anti-emetic actions. 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). 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). A disturbance in the pro-oxidant/antioxidant system has been defined as oxidative stress. Reactive oxygen species (ROS) are very reactive molecules ranked as free radicals owing to the presence

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of one unpaired electron such as a 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). Ginger extracts have been extensively studied for a broad range of biological activities, especially antioxidant activities (Miller et al., 1991; Ahmed et al., 2000) found that ginger significantly lowered lipid per oxidation by maintaining the activities of the antioxidant enzymes such as super oxide dismutase, catalase and glutathione peroxidase in rats. This research focuses on whether oral administration of ginger prevents lead acetate induced liver toxicity and apoptosis or not.

MATERIALS AND METHODS

Thirty adult male Wistar rats (200 ± 10 g) were used for this study. They were obtained from animal facility of pasture institute of Iran rats were housed in temperature controlled rooms (25°C) with constant humidity (40 - 70%) and 12/12 h light/ dark cycle prior to use in experimental protocols. All animals were treated in accordance to the principles of laboratory animal care. The experimental protocol was approved by the animal ethical committee in accordance with the guide for the care and use of laboratory animals prepared by Tabriz medical university. All rats were fed a standard diet and water. The daily intake of animal water was monitored at least one week prior to start of treatments in order to determine the amount of water needed per experimental animal.

Grouping of animals and treatment

The rats were grouped into 3 groups (groups A, B, and C, $n = 10$). Animals in group A served as the control group and were drinking distilled water. Animals in groups B and C were drinking 1% lead acetate (LA). Group C animals were, in addition to drinking LA, treated with 100 mg/kg/rat of ginger. All treatments were for 10 weeks.

Animal sacrifice and collection of samples

Ten weeks after the last treatment, each animal was sacrificed and blood samples were collected via heart puncture. Blood sample obtained from each rat was divided into 2: One half in a plain bottle and the other half in an ethylenediaminetetraacetic acid bottle. Liver was excised from each rat and fixed in % 10 formalin buffer and prepared for tunel assay.

The samples were prepared for measurements of plasma superoxidodismutase (SOD), catalase (CAT) and malondialdehyde (MDA) were determined using the method described by (Khaki et al., 2009).

Collection of data and statistical analysis

Livers from each rat were homogenized for tissue superoxide

dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) were determined using the method described by Khaki et al. (2009).

Tunel analysis of apoptosis

The *in-situ* DNA fragmentation was visualized by Tunel method (khaki et al., 2008). Briefly, dewaxed liver tissues sections were predigested with 20 mg/ml proteinase K for 20 min and incubated in phosphate buffered saline solution (PBS) containing 3% H_2O_2 for 10 min to block the endogenous peroxidase activity. The sections were incubated with the Tunel reaction mixture, fluorescein-dUTP (*in situ* cell death detection, POD kit, Roche, Germany), for 60 min at 37°C . The slides were then rinsed 3 times with PBS and incubated with secondary antifluorescein-POD-conjugate for 30 min. After washing 3 times in PBS, diaminobenzidine H_2O_2 (DAB, Roche, Germany) chromogenic reaction was added on sections and counterstained with hematoxylin. As a control for method specificity, the step using the Tunel reaction mixture was omitted in negative control serial sections, and nucleotide mixture in reaction buffer was used instead. Apoptotic hepatocytes were quantified by counting the number of Tunel stained nuclei per cross section. Cross sections of 150 liver tissue samples per specimen were assessed and the mean number of Tunel positive cells (dark brown cells) per tubule cross- section was calculated.

Statistical analysis

All values were expressed as mean \pm SE. Differences in mean values were compared using SPSS 11.0 by one-way ANOVA test. $P < 0.05$ was considered as statistically significant.

RESULTS

The following results were obtained and are presented as mean \pm SEM. Level of significance is taken at "P-value < 0.05 " (*).

Plasma SOD activity

Group B showed a significant (P -value < 0.05) decrease in plasma SOD activity. Group C was, however, not significantly (P - value > 0.05) different from the control in terms of the plasma SOD activity (Table 1).

Plasma CAT activity

Group B showed a significant (P -value < 0.05) decrease in the plasma CAT activity. However, group C showed no significant (P -value > 0.05) difference in the CAT activity from the control (Table 1).

Plasma MDA concentration

Group B showed a significant (P -value < 0.05) increase in

Table 1. Showed result of plasma SOD, CAT, MDA, positive tunel hepatocytes in whole control and experimental groups.

Groups(n = 10)	Plasma SOD	Plasma CAT	Plasma MDA (mmol/l)	Positive tunel hepatocytes (%)
(A) Control	1.958 ± 0.05	0.3874 ± 0.03	0.25 ± 0.04	8.1 ± 0.02
(B) 1% Lead acetate	1.124 ± 0.05*	0.2440 ± 0.02*	4.1 ± 0.06*	20.1 ± 0.02*
(C) 1% Lead acetate + ginger (100 mg/kg/rat/daily)	1.783 ± 0.06*	0.3692 ± 0.01	2.2 ± 0.06*	6.1 ± 0.02

Data are presented as mean ± SE. *Significant different at $p < 0.05$ level, (compared with the control and ginger treated group).

the plasma MDA concentration whereas Group C showed no significant (P -value > 0.05) difference from the control (Table 1).

Positives tunel hepatocyte

Group B showed a significant (P -value < 0.05) increased in the apoptotic cells. However, Group C showed no significant (P -value > 0.05) difference in the apoptotic cells from the control (Table 1).

DISCUSSION

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). Disruption of pro-oxidant/antioxidant balance might lead to the tissue injury. It was reported that lead increased the level of lipid peroxidation (Upasani et al., 2001) and brain thiobarbituric acid-reactive substances and altered the antioxidant defense system (Adanaylo et al., 1999). Similar effects were also reported in the hepatic tissues (Sandhir et al., 1995). A number of recent studies confirmed the possible involvement of reactive oxygen species (ROS) in lead-induced toxicity (Gurer et al., 2000). Several antioxidant enzymes and molecules have been used to evaluate lead-induced oxidative damage in animal and human studies. Reduced glutathione (GSH) and glutathione disulfide (GSSG) concentrations, as well as modifications in superoxide dismutase (SOD) activity are the most frequently used markers in tissues or in blood (Khaki et al., 2010). Based on the observation that free radical was generated during the pathogenesis processes induced by lead exposure, it was presumed that supplementation of antioxidants could be an alternative method for chelation therapy (Khaki., 2010; Flora et al., 1986).

Specifically, ascorbic acid, the known chelating agent

with antioxidant features, was widely reported with the capability of protecting cells from oxidative stress (Patra et al., 2004). More importantly, due to the presence of health-protective antioxidants such as lycopene, vitamin C, and vitamin A in TP (Patra et al., 2004) despite its relatively low caloric value (21 kcal/100 g) and low protein content (0.85% by weight) (Upasani et al., 2001). There was no significant (P -value > 0.05) difference in the SOD activity of the plasma of the control and that of the animals treated with tomato along with Pb. But, there was a significant (P -value < 0.05) decrease in the plasma SOD activity in animals treated with Pb only compared with the control. This finding is in agreement with (Pinon-Lataillade et al., 1995). There was a significant (P -value < 0.05) decrease in plasma CAT activity of animals treated with Pb only relative to the control. There was, however no significant (P -value > 0.05) difference between the control and the animals treated with ginger along with Pb in this respect.

This further establishes that ginger must have reduced the oxidative stress that Pb could cause. Finally, there was no significant (P -value > 0.05) difference in both the plasma and the tissue MDA concentration of the control and those of the animals treated with ginger along with Pb, whereas animals treated with Pb only showed a significant (P -value < 0.05) increase in plasma MDA concentration. This confirms that it was ginger, the source of antioxidants, (Lisa, 2002; khaki et al., 2009) that reduced the oxidative stress that Pb exposure could have caused in the ginger -treated animals. Free radical-induced oxidative damage has been implicated in the pathogenesis of a number of injury and disease states. We have previously found that ROS played a pivotal role in apoptosis of testis cells in lead-exposed mice (Wang et al., 2006). In the present study, it was exhibited that a significant increase in DNA damage and apoptosis in liver cells occurred via ROS as it played a very important role in apoptosis induction under both physiological and pathological conditions. Interestingly, mitochondria were both the source and target of ROS. ROS, which was predominantly produced in the mitochondria, led to the

free radical attack of membrane phospholipids and loss of mitochondrial membrane potential, which caused the intermembrane proteins, such as cytochrome c, to be released out of the mitochondria and ultimately triggered caspase-3 activation. Caspase-3 activation led to DNA breakage, nuclear chromatin condensation and cell apoptosis (Wang et al., 2006; Li et al., 2006; khaki et al., 2008). In summary, ginger can decreased the damage to liver cells from oxidative damage induces by lead, and it is dependent on their antioxidant effects.

REFERENCES

- Adanaylo V, Oteiza P (1999). Lead intoxication: antioxidant defence and oxidative stress in rat brain. *Toxicology*, 135: 77-85.
- Ahmed RS, Seth 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.
- Ali BH, Blunden G, Tanira MO, Nemmar A (2008). Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): a review of recent research. *Food Chem. Toxicol.* 46: 409-420.
- Auman JT, Chou J, Gerrish K, Huang Q, Jayadev S, Blanchard K, Paules RS (2007). Identification of genes implicated in methapyriline-induced hepatotoxicity by comparing differential gene expression in target and nontarget tissue. *Environ. Health Perspect.*, 115: 572-578.
- Aruoma A, Bolognini L, Macciantelli D, Carratelli M (1994). The radical Cation of N, N Diethyl-para-phenilendiamine : a possible indicator of oxidative stress in biological samples. *Res. Chem. Intermed.*, 26: 253-267.
- Flora S, Tandon S (1986). Preventive and therapeutic effects of thiamine, ascorbic acid and their combination in lead intoxication. *Acta Pharmacol. Toxicol.*, 58: 374-378.
- Gidlow DA (2004). Lead toxicity. *Occup. Med. (Lond)*. 54: 76-81.
- Gurer-Orhan H, Sabir HU, Ozgüneş H (2004). Correlation between clinical indicators of lead poisoning and oxidative stress parameters in controls and lead-exposed workers. *Toxicology*, (195): 147-154.
- Gurer H, Ercal N (2000). Can antioxidant be beneficial in the treatment of lead poisoning? *Free Radic. Biol. Med.* 29: 927-945.
- Hopkins A (1970). Experimental lead poisoning in the baboon. *Br. J. Ind. Med.*, 27: 130-140.
- Khaki A, Heidari M, Ghaffari Novin M, Khaki AA (2008). Adverse effects of ciprofloxacin on testis apoptosis and sperm parameters in rats. *Iranian J. Reprod. Med.*, 6(2): 71-76.
- Khaki A, Fathiazad F, Nouri M Khaki AA, Chelar c, ozanci, Ghafari-Novin M, Hamadeh M (2009). The Effects of Ginger on Spermatogenesis and Sperm parameters of Rat. *Iranian J. Reprod. Med.*, 7: 7-12.
- Khaki A, Fathiazad F, Nouri M, Khaki AA, Jabbari -kh H, Hamadeh M (2009). Evaluation of Androgenic Activity of *Allium cepa* on Spermatogenesis in Rat. *Folia Morphologica*, 68(1);1: 45-51.
- Khaki A (2010). Protective effect of quercetin against necrosis and apoptosis induced by experimental ischemia and reperfusion in rat liver. *AJPP*, 4(1): 022-026.
- Khaki A, Fathiazad F, Nouri M, Khaki AA, Abassi Maleki N, Ahmadi P, Jabbari-kh H (2010). Beneficial Effects of Quercetin on sperm parameters in streptozotocin -induced diabetic male rats. *Phytother. Res. J. Vol.* (24), in press.
- Khalil-Manesh F, Tartaglia-Erler J, Gonick HC (1994). Experimental model of lead nephropathy. Correlation between renal functional changes and hematological indices of lead toxicity. *J. Trace Elem. Electrolytes Health Dis.* 8: 13-19.
- Li J, Tang Q, Li Y (2006). Role of oxidative stress in the apoptosis of hepatocellular carcinoma induced by combination of arsenic trioxide and ascorbic acid. *Acta Pharmacol. Sin.* 27: 1078-1084.
- Lisa T (2002). The Top 10 Antioxidant Foods. *Better Nutrition*. Available from: [last accessed on 2008 Dec].
- Miller JK, Brzezinska-Slebodzinska E, Madsen FC (1993). Oxidative stress, antioxidant and animals function. *J. Dairy Sci.*, 76: 2812-2823.
- Pande M, Flora S (2002). Lead induced oxidative damage and its response to combined administration of lipoic acid and succimers in rats. *Toxicology*, 177: 187-196.
- Pinon-Lataillade G, Thoreux-Manlay A, Coffigny H, Masse R, Soufi JC (1995). Reproductive toxicity of chronic lead exposure in male and female mice. *Hum. Exp. Toxicol. Toxicol.*, 14: 872-878.
- Patocka J, Cerný K (2003). Inorganic lead toxicology *Acta Medica (Hradec Kralove)*, 46: 65-72.
- Patra R, Swarup D (2004). Effect of antioxidant ascorbic acid, L-methionine on tocopherol alone or along with chelator on cardiac tissue of lead-treated rats. *Veterinarski Arch.* 74: 235-244.
- Sandhir R, Gill K (1995). Effect of lead on lipid peroxidation in liver of rats. *Biol. Trace Elem. Res.* 8: 91-97.
- Sies H (ed.) (1991). Oxidants and antioxidants. In: *Oxidative Stress*. Academic Press, London. pp. 15-22.
- Upasani C, Khara A, Balaraman R (2001). Effect of lead with Vitamins E, C, or spirulina on malondialdehyde: conjugated dienes and hydroperoxides in rats. *Indian J. Exp. Biol.*, 39:- 70
- Wang C, Zhang Y, Liang J (2006). Impacts of ascorbic acid and thiamine supplementation at different concentrations on lead toxicity in testis. *Clin. Chim. Acta.* 370: 82-88.
- Waldron HA (1966). The Anaemia of Lead Poisoning: A Review. *Br. J. Ind. Med.* 23: 83-100.
- Waters M, Stasiewicz S, Merrick BA, Tomer K, Bushel P, Paules R, Stegman N, Nehls G, Yost KJ, Johnson CH (2008). CEBS – Chemical Effects in Biological Systems: a public data repository integrating study design and toxicity data with microarray and proteomics data. *Nucleic acids research*, pp. 892-900.