Attenuated Lead Induced Apoptosis in Rat Hepatocytes in the Presence of Lycopersicon Esculentum

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Abstract- Lead (Pb), has, for decades, being known for its adverse effects on various body organs and systems. In the present study, the damage of Pb on the Liver tissue apoptosis was investigated, and Lycopersicon esculentum as an antioxidants source was administered orally to prevent the adverse effects of Pb. Eighteen Wistar rats, randomized into three groups (n=6), 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 (LA). Group C animals were, in addition to drinking LA, treated with 1.5 ml/day of Lycopersicon esculentum. Treatments were for three months. The obtained results showed that lead acetate caused significant reductions in the liver weight, plasma and tissue superoxide dismutase and catalase activity, but a significant increase in plasma and tissue malondialdehyde concentration but Lycopersicon esculentum have an inhibitory effect on LA liver adverse effect. So, it can be concluded that Lycopersicon esculentum have a significant protective effect on liver lead acetate adverse effects as well as, lead acetate -induced oxidative stress.

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Keywords: Lead; Apoptosis; Hepatocytes; Lycopersicon

Introduction

1. Lead acetate, a dangerous heavy metal, is harmful even in small amounts. Nevertheless, humans get exposed to Pb through their environment and diet (1). The manifestations of Pb poisoning in humans are nonspecific. They may include weight loss, anemia (2,3), memory loss, (4) nephropathy, infertility, liver, testis, heart damages, *etc.* (5). However, oxidation accompanies lead toxicity (6). Lycopersicon esculentum (tomato), on the contrary, is a source of antioxidants (7,8) and is made up of components very appropriate for detoxification, illnesses prevention (9), attaining growth (10), helping the immune system (11), maintaining blood in good state (2), etc. This research, therefore, was focused on whether oral administration of Lycopersicon esculentum prevents lead acetate induced liver toxicity or not.

Materials and Methods

2. Eighteen 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 12h/12h light/ dark cycle prior to use in experimental protocols. All animals were treated in accordance with 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 the start

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of treatments to determine the amount of water needed per experimental animal.

2.1. Preparation of tomato paste. Tomato paste (TP) was prepared by grinding tomatoes and heating in a water bath for 30 min at 81°C.

2.2. Grouping of animals and treatment. The rats were grouped into three groups (Groups A, B, and C, n=6). 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 1.5 ml/day of TP. All treatments were for three months.

2.3. Animal sacrifice and collection of samples. 48 hours after the last treatment, each animal was sacrificed, and blood samples were collected via heart puncture. A blood sample obtained from each rat was divided into two: One-half in a plain bottle and the other half in an ethylene EDI ammine tetraacetic acid bottle. The liver was excised from each rat. Plasma superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) were determined using the method described by Fridovich (12).

2.4. TUNEL analysis of apoptosis. The in-situ DNA fragmentation was visualized by TUNEL method (27). Briefly, dewaxed tissue sections were predigested with 20 mg/ml proteinase K for 20 min and incubated in phosphate-buffered saline solution (PBS) containing 3 % H2O2 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 three times with PBS and incubated with secondary anti fluorescein-POD-conjugate for 30 min. After washing three times in PBS, diaminobenzidine-H2O2 (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 tissue samples per specimen were assessed, and the mean number of TUNEL positive cells per cross- section was calculated (13).

2.5. Statistical analysis. All values were expressed as mean ± 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

3.The following results were obtained and were presented as mean ± SE. Level of significance is taken at "$P<0.05$" (*) and/or "$P<0.01$" (**).

3.1. Plasma Albumin: Group B showed a significant ($P<0.01$) decrease in plasma Albumin. Group C was, however, not significantly ($P>0.05$) different from the control in terms of the plasma Albumin activity (Table 1).

3.2. Plasma SOD activity. Group B showed a significant ($P<0.01$) decrease in plasma SOD activity. Group C was, however, not significantly ($P>0.05$) different from the control in terms of the plasma SOD activity (Table 1).

3.3. Plasma CAT activity. Group B showed a significant ($P<0.01$) decrease in the plasma CAT activity. However, Group C showed no significant ($P>0.05$) difference in the CAT activity from the control (Table 1).

3.4. Plasma MDA concentration. Group B showed a significant ($P<0.05$) increase in the plasma MDA concentration whereas Group C showed no significant ($P>0.05$) difference from the control (Table 1).

3.5. Positives Tunnel hepatocyte. Group B showed a significant ($P<0.01$) increased in the Apoptotic cells. However, Group C showed no significant ($P>0.05$) difference in the Apoptotic cells from the control (Table 1).

3.6. Photomicrograph of liver tissue (hepatocyte) H&E staining X320 and Tunel staining, X320, in control group, in presence of 1% lead acetate, in presence of 1% lead acetate and 1.5ml of potato is observed in figure 1.

Table 1. Comparison the results among groups

group	Plasma SOD	Plasma CAT	Plasma Albumin (g/dl)	Plasma MDA(mmol/l)	Positive Tunel hepatocytes
Control	1.958 ± 0.05	0.3874 ± 0.03	8.1 ± 0.01	0.25 ± 0.04	8.1 ± 0.02
1% Lead acetate	1.124 ± 0.05*	0.2440 ± 0.02*	5.1 ± 0.01*	4.1 ± 0.06*	20.1 ± 0.02*
1% Lead acetate+1.5 ml of Tomato	1.883 ± 0.06	0.3752 ± 0.01	7.1 ± 0.01	2.1 ± 0.06	5.1 ± 0.02

*: P value≤ 0.05

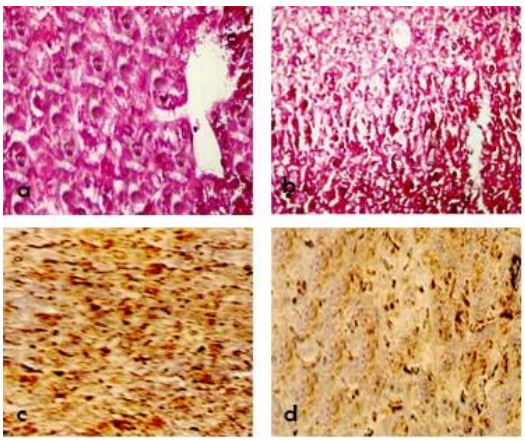

Figure 1. Photomicrograph (a): liver tissue (haptocyte) is normal (arrow) in control group, H&E staining,X320.Photomicrograph (b): liver tissue (haptocyte) showed necrosis (arrow) 1% Lead acetate Group, H&E staining, X320.Photomicrograph (c): liver tissue (haptocyte) showed apoptosis (dark brown) (arrow) in 1% Lead acetate Group, Tunel staining, X320.Photomicrograph (d): liver tissue (haptocyte) showed apoptosis (dark brown) (arrow) in 1% Lead acetate+1.5 ml of Tomato, Tunel staining, X320.

Discussion

The liver is considered as one of the vital organs in the body. It has several major functions including the production of bile to break down fat, glycogen storage, decomposition of red blood cells, production of cholesterol, plasma protein synthesis, and drug metabolism just to name a few. The latter takes place by a host of specialized detoxification enzymes and pathways that biochemically modify or metabolize xenobiotics to harmless metabolites and other byproducts for clearance from the body (14). Microarray gene expression analysis has been used to study the effects of toxicants and other environmental stressors on biological systems (15,16). 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 (17). Disruption of pro-oxidant/antioxidant balance might lead to the tissue injury. It was reported that lead increased the level of lipid peroxidation (18) and brain thiobarbituric acid-reactive substances and altered the antioxidant defense system (19). Similar effects were also reported in the hepatic tissues (20). A number of recent studies confirmed the possible involvement of reactive oxygen species (ROS) in lead-induced toxicity (21). 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. 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 chelating therapy (22). Specifically, ascorbic acid, the known chelating agent with antioxidant features, was widely reported with the capability of protecting cells from oxidative stress (23). More importantly, due to the presence of health-protective antioxidants such as lycopene, vitamin C, and vitamin A in TP (8), despite its relatively low caloric value (21 Kcal/100 g) and low protein content (0.85% by weight) (19). 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 significant (*P*-value <0.01) decrease in the plasma SOD activity in animals treated with Pb. only compared with the control. This finding is in agreement with that of Ping-Chi and Yueliang (24) and is at the same time in support of Lycopersicon esculentum (tomato) as an antioxidant. There was a significant decrease in plasma CAT activity of animals treated with Pb. only relative to the control. There was, however, no significant difference between the control and the animals treated with tomato along with Pb in this respect. This further establishes that TP must have reduced the oxidative stress that Pb. could cause. Finally, there was no significant difference in both the plasma and the testicular MDA concentration of the control and those of the animals treated with tomato along with Pb, whereas animals treated with Pb. only showed a significant increase in plasma MDA concentration. This confirms that it was TP, the source

of antioxidants (7,8), that reduced the oxidative stress that Pb. exposure could have caused in the tomato-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 play a pivotal role in apoptosis of testis cells in lead-exposed mice (25). In the present study, 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 (25,26). In summary, Lycopersicon esculentum can lessen the damage to liver cells from oxidative damage induce by lead, but the antioxidant effects are dependent on their concentrations.

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