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ORIGINAL ARTICLE

Vitamin E attenuates liver injury induced by exposure to lead, mercury, cadmium and copper in albino mice

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Abstract Water pollution is the contamination of water resources by harmful wastes or toxins. Both community and private sources of drinking water are susceptible to a myriad of chemical contaminants. Heavy metals pollution of surface water can create health risks. The present study was aimed to investigate the effect of vitamin E supplementation on male mice exposed to a mixture of some heavy metals (lead, mercury, cadmium and copper) in their drinking water for seven weeks. Significant increases of blood alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) were detected in heavy metals-treated mice. Histopathologically, the liver sections from heavy metals-treated mice showed severe changes including disarrangement of hepatic strands, rupture in hepatocytes, advanced hepatocellular necrosis, dilation and congestion of blood vessels with hemorrhage, dense lymphocytic infiltration round the central vein and dark stained hepatocytic nuclei indicating cell pycnosis. Administration of vitamin E at a dose of 50 IU/kg body weight, five times weekly improved the observed biochemical and histopathological changes induced by these heavy metals intoxication. Hence, the results of this study suggest that vitamin E protects against these heavy metals-induced liver injury and the attenuating effect of vitamin E may be due to its antioxidant activity.

1. Introduction

Environmental pollution has many facets, and the resultant health risks include diseases in almost all organ systems. Humans whose position in the food chain is at the top were exposed to various types of environmental contaminants at different stages of life, majority of which are harmful. In the environmental community, the notation of heavy metals implies stable high-density metals. These elements are natural constituents of the earth's crust. As a result of anthropogenic activity, the input of heavy metal to the environment has increased sufficiently and resulted in the increase of their content in air, water, soil and tissues of living organisms. Heavy metals are mostly toxic but their exact toxicity varies depending on

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the chemical form of the metal, point of exposure and the degree of exposure to the metal (WHO, 1995). Metals when concentrated can be quite toxic and can result in death of organisms. Numerous hazardous heavy metals are inhaled and absorbed by humans and animals every day (Pentyala et al., 2010). The major concern with heavy metals is their ability to accumulate in the environment and thereby passing up to food chain (Leblanc et al., 2005). Heavy metals pollution is a serious environmental issue for not only advanced nations but also developing nations, because pollution has gradually been increasing, but decreasing pollutants is a difficult problem (Ieradi et al., 1996; Dey et al., 1999; da Silva et al., 2000; Swarup and Patra, 2005). Some of heavy metals such as the alkaline earth metals and particularly trace elements are essential for survival because they help build molecules that sustain life. Other metals such as lead (Pb), mercury (Hg) and cadmium (Cd), which are examples of heavy metals are very toxic at even minute quantities and serve no purpose of sustaining life (Cockerham and Shane, 1994). After the industrial revolution, the use of metals, especially heavy metals, increased dramatically and gradually accumulated in the biosphere. Heavy metals are widely used for metallic processed products, and are also used for medical supplies and catalysts. They are extensively used and therefore detected in housing complexes of heavy industries developed as residential areas (Burger, 1993; Burger and Eichhorst, 2005). In addition, the development of information technology has caused new metal pollution as rare elements are needed for integrated circuits (IC) chips. Pollution of food as well as groundwater is due to our wide usage of metals. Globally, the problem of water pollution is also growing at an alarming rate. Water pollution is any chemical, physical or biological change in the quality of water that has harmful effects on any living organism that uses it. When humans drink polluted water, it often has serious affects on their health. Water pollution can also make water unsuited for the desired use. A little negligence on the part of civic bodies can result in the spread of many diseases (Mukhi and Srivastava, 1987). Furthermore, it has been noted that the air and aquatic pollution does not only settle in one country. Therefore, it is important to know how heavy metal pollution is encroaching into urban areas. The most common heavy metals, implicated in acute and/or chronic intoxication can effect the development and the overall health causing depression, learning difficulties and neurological disorders. Furthermore, these metals may cause cell damage, impairment of enzymes, functions or alter genetic material (DNA), causing cancer or birth defects, when absorbed (David, 2001).

Vitamins are ideal antioxidants to increase tissue protection from oxidative stress due to their easy, effective and safe dietary administration in a large range of concentrations (Cadenas and Cadenas, 2002; Kanter et al., 2005). One of the most important vitamins for the body is vitamin E. In nature, vitamin E comprises eight natural fat-soluble compounds, including 4 tocopherols [d-alpha-, d-beta-, d-gamma- and d-delta-tocopherol] and 4 tocotrienols [d-alpha-, d-beta-, d-gamma- and d-delta-tocotrienol] (Malafa et al., 2002; Songthaveesin et al., 2004). Vitamin E is an important antioxidant factor. It is known to possess various physiological functions. A major contributor to non-enzymatic protection against lipid peroxidation is vitamin E, a known free radical scavenger (Fraga et al., 1987; Rikans et al., 1991). Vitamin E as a lipid soluble, chain-breaking antioxidant (Halliwell, 1989, 1992; Kagan et al., 1992; Packer, 1992) plays a major protective role against oxidative stress (Fraga et al., 1987) and prevents the production of lipid peroxides by scavenging free radicals in biological membranes (Suga et al., 1984). Since the discovery of vitamin E in 1922 by H.M. Evans, when it was first described as an anti-sterility agent, many scientists and physicians have sought to elucidate its biochemistry, health benefits and clinical applications (Packer, 1992). The main objective of the present study was to evaluate the role of vitamin E on liver toxicity induced by selected heavy metals including lead (Pb), mercury (Hg), cadmium (Cd) and copper (Cu) in mice.

2. Materials and methods

2.1. Animals

A total of 40 adult healthy albino male mice of MF1 strain weighing 37.6–39.3 g were obtained from the Experimental Animal Unit of King Fahd Medical Research Center, King Abdul Aziz University, Jeddah, Saudi Arabia. Mice were left for one week before the start of experiments for acclimatization. They were caged in a quite temperature controlled room (24 ± 1 °C) and fed on balanced commercial diet with free access of food and water. All experimental procedures and animal maintenance were conducted in accordance with ethical guidelines of the Animal Care and Use Committee of King Abdul Aziz University.

2.2. Experimental treatments

For the experimental purpose, mice were randomized and divided into four groups (number of mice in each group, $n = 10$). Mice of group one served as control and received normal drinking water without any heavy metals. Mice of group two were exposed to the mixture of heavy metals (Pb, Hg, Cd and Cu) for seven weeks in their drinking water as follows: 30 ppm Pb, 10 ppm Hg, 30 ppm Cd and 30 ppm Cu. Animals of group three were exposed to the same drinking solution given to group two and intraperitoneally injected with vitamin E at a dose of 50 IU/kg body weight, BW, five times weekly for seven weeks. Mice of group four received normal drinking water and were subjected to vitamin E at the same doses given to group three.

2.3. Blood sampling

After seven weeks, blood samples were taken from orbital venous plexus under total anesthesia with diethyl ether. These blood samples were collected in the lithium heparin coated tubes. Plasma specimens were obtained and used for determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) using an automatic analyzer (Reflotron® Plus System, Roche, Germany).

2.4. Histopathological examinations

Liver tissues excised from each mouse were fixed in 10% formalin, dehydrated and embedded in paraffin. Sections 4 μm thin from blocks were stained with hematoxylin and eosin (H&E). Qualitative examinations of prepared tissues and the

obtaining of their photos were carried out using Motic digital microscope, DM-B1 series, Motic Company.

2.5. Statistical analysis

The obtained data were expressed as mean ± standard deviation (SD). All data were analyzed statistically using one-way analysis of variance (ANOVA) followed by Student's *t*-test. Statistical significance was considered at $P < 0.05$. Statistical Package for Social Sciences (SPSS) for Windows version 12.0 software was used for this analysis.

3. Results

As seen in Fig. 1A–D, the levels of plasma ALT (+100.5%), AST (+149.0%), ALP (+85.5%) and GGT (+261.0%) were significantly increased in mice treated with the mixture of heavy metals compared with control, heavy metals plus vitamin E and vitamin E treated groups. Furthermore, the levels of plasma ALT (+35.4%), AST (+66.5%), ALP (+14.0%) and GGT (+79.8%) were statistically increased in mice treated with the mixture of heavy metals plus vitamin E compared with control and vitamin E treated groups. Insignificant alterations in the levels of these enzymes were observed in mice treated with only vitamin E. The liver sections from control mice showed normal structure characterized by a radial arrangement of hepatocytes around the central vein along with Kupffer cells (Fig. 2A). The liver sections from heavy metals-treated mice showed severe changes when compared with those from the control mice. These changes include disarrangement of hepatic strands, rupture in hepatocytes, advanced hepatocellular necrosis, dilation and congestion of blood vessels with hemorrhage, dense lymphocytic infiltration round the central vein and dark stained hepatocytic nuclei indicating cell pycnosis (Fig. 2B–F). In mice treated with heavy metals plus vitamin E, the microscopic examination of liver sections showed several alterations including mild disarrangement of hepatic strands, rupture in hepatocytes and dilation of blood vessels (Fig. 2G and H). Moreover, the liver sections from mice treated with heavy metals plus vitamin E showed an absence of hemorrhage, dense lymphocytic infiltration round the central vein and pycnosis. In comparison with control group, the liver sections from mice treated with only vitamin E showed normal structure (Fig. 2I).

4. Discussion

Water pollution has become a global problem. Heavy metals have long been recognized as serious pollutants of the aquatic environment. Health problems have been widely reported due long-term ingestion of contaminated drinking water with heavy metals. Pollution of water bodies with heavy metals from variety of sources is becoming a matter of global concern (Dike et al., 2004). Though effects of chemical contamination of drinking water are not felt on short-term basis (except nitrate), their accumulation over a long period in the body has significant health effects (Musa et al., 2004). Safe drinking water is a human birthright – as much a birthright as clean air. As a matter of fact, in most of the African and Asian countries, even in relatively advanced countries; safe drinking water is not easily available. Of the 6 billion people on earth, more than one billion lack accesses to safe drinking water and, about 2.5 billion do not have access to adequate sanitation services (TWAS, 2002). In addition to these

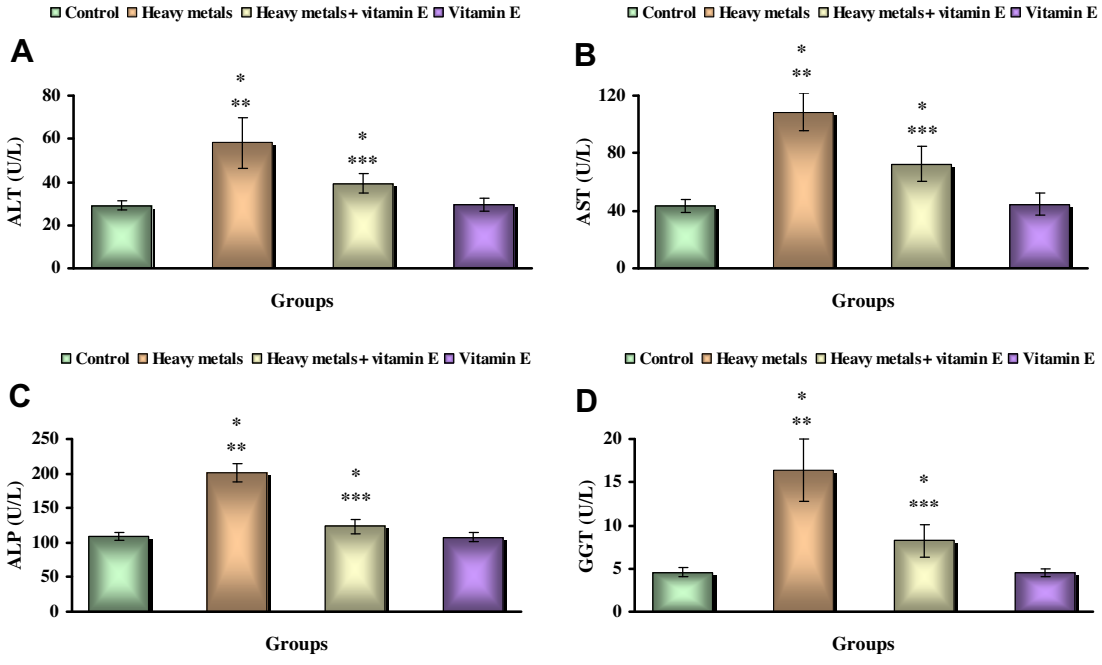

Figure 1 (A–D) Levels of plasma ALT (A), AST (B), ALP (C) and GGT (D) of control, heavy metals, heavy metals plus vitamin E and vitamin E treated mice. $^{*}P < 0.05$: Student's *t*-test, significant levels shown for difference between control and treated groups. $^{**}P < 0.05$: Student's *t*-test, significant levels shown for difference between mice treated with heavy metals and heavy metals plus vitamin E or vitamin E. $^{***}P < 0.05$: Student's *t*-test, significant levels shown for difference between mice treated with heavy metals plus vitamin E and vitamin E.

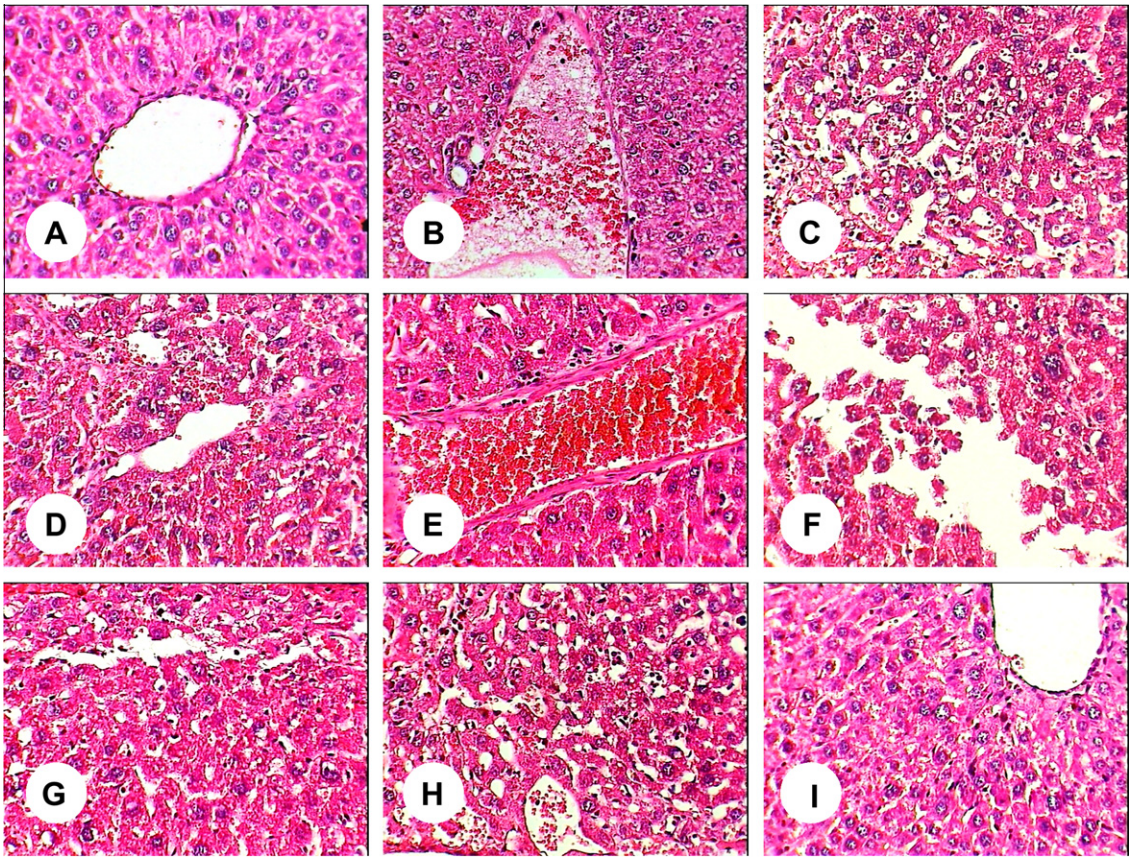

Figure 2 (A–I) Liver micrographs of control (A), heavy metals (B–F), heavy metals plus vitamin E (G and H) and vitamin E (I) treated mice. Original magnification ×400.

shortcomings, various types of waterborne diseases kill on an average more than 6 million children each year i.e., about 20,000 children a day (TWAS, 2002). The liver is the first organ to encounter ingested nutrients, drugs and environmental toxicants that enter the hepatic portal vein from the digestive system, and liver function can be detrimentally altered by injury resulting from acute or chronic exposure to toxicants. The present study showed that the mixture of heavy metals (Pb, Hg, Cd, and Cu) induced significant increases of plasma ALT, AST, ALP and GGT. Similar observations were reported in many experimental investigations on animals exposed to Pb (Bersényi et al., 2003; Shalan et al., 2005; Garg et al., 2007; Liu et al., 2010), Hg (Bersényi et al., 2003; Agarwal et al., 2010; Bashandy et al., 2011), Cd (Bersényi et al., 2003; Erdogan et al., 2005; Haouem et al., 2007; Bashandy and Alhazza, 2008; Fouad et al., 2009; Hamden et al., 2009; Kumar et al., 2010; Renugadevi and Prabu, 2010; Swapna and Reddy, 2011) and Cu (Fuentealba et al., 2000; Zia-Ur-Rahman et al., 2001; Li et al., 2008). The marked elevation of these enzymes indicates impaired liver function which confirmed by severe histopathological alterations. Moreover, many scientific researches showed that intoxication with Pb, Hg, Cd and Cu caused several hepatic damages in experimental animals (Aburto et al., 2001; Jihen et al., 2008; Li et al., 2008; Cavusoglu et al., 2009; Fouad et al., 2009; Agarwal et al., 2010; Haleagrahara et al., 2010; Liu et al., 2010; Oguz et al., 2010; Renugadevi and Prabu, 2010; Jabeen and Chaudhry, 2011).

The present investigation showed that vitamin E reversed Pb, Hg, Cd and Cu-induced liver injury. Beytut et al. (2003) investigated the role of vitamin E on Cd-induced oxidative damage in New Zealand White rabbit's blood, liver and kidneys. They reported that vitamin reduced oxidative stress in Cd-treated rabbits and suggested that the reductions in increased thiobarbituric acid reactive substances (TBARS) due to Cd toxicity may be an important factor in the action of vitamin E. Ahmadizadeh and Baghpa (2008) studied the preventive effect of vitamin E on cadmium-induced toxicity in NMARI male rat liver and kidney. They showed that the levels of blood ALT, AST, ALP, urea nitrogen (BUN) and creatinine increased in Cd treated rats with several damages of liver and kidney tissues structure. Additionally, they concluded that vitamin E has potential to protect rat liver and kidney tissues against Cd toxicity. Osfor et al. (2010) showed that vitamin E could improve daily food intake, body weight gain and feed efficiency ratio; reduced Pb and Cu levels in serum and tissues of liver and kidney as well as diminished ALT, AST, urea and creatinine levels in Pb and Cu intoxicated male rats. Sajitha et al. (2010) reported that administration of vitamin E declined the histopathological and biochemical alterations induced by Pb intoxication in female Sprague-Dawley albino rats. Concerning the influences of vitamin E supplementation on toxicity of other metals, Rao et al. (2006) investigated the ameliorative role of vitamin E on disturbances of liver antioxidant system induced by chromium (Cr) and nickel (Ni) in male albino mice of Swiss strain. They found that Cr

and/or Ni treatments to mice revealed a significant decline in the levels of hepatic glutathione (GSH), total sulfhydryl (–SH) group, total ascorbic acid, superoxide dismutase (SOD) and catalase. Concomitantly, a significant increase in lipid peroxidation (LPO) was noted. Supplementation of vitamin E prevents LPO and an antioxidant system in hepatic tissues of mice. Kutlubay et al. (2007) investigated the effects of vitamin E on aluminum (Al)-induced liver damage in male rats. They stated that vitamin E was demonstrated to serve as an antioxidant, and to prevent the degenerative effects of Al on the microscopic morphology of rat liver tissue. The dilatations in the sinusoids were significantly reduced, and the hepatocyte columns maintained a normal structure in the Al plus vitamin E co-exposed rats. In addition to this, the cytoplasmic spacing around the nuclei was no longer seen, and the hepatocyte nuclei had a normal appearance. In addition, they concluded that there was an apparent protective effect of vitamin E on parenteral Al exposure. In the other studies focused on the effects of vitamin E treatments against other liver chemical toxicants, Zaki and Eid (2009) examined the influence of vitamin E on amiodarone (a class III antiarrhythmic drug)-induced liver damage in male rats. They found that the electron microscopy examination of liver sections from the rat receiving amiodarone showed disrupted hepatocytes with increased vacuolations. Degenerated organelles and disrupted nuclei were observed. The microvilli of bile canaliculi were disrupted and the hepatocytes showed increased lipid contents. Both endothelial cells and Kupffer cells were damaged. Phospholipids inside the mitochondria showed a loss of cristae. Additionally, the sections from the liver of rats received amiodarone and vitamin E showed lesser effects, especially in depositions of phospholipids in the mitochondria and the whole organelles and the nucleus showed minor damage in comparison to amiodarone-treated rats. They concluded that the milder hepatotoxic effects are seen in rats administered amiodarone and vitamin E simultaneously suggesting that vitamin E may play a role in amelioration of the effects of amiodarone. Bharrhan et al. (2010) studied the effect of vitamin E supplementation on lipopolysaccharide (LPS)-induced liver damage in rats. They reported that the challenge with LPS resulted in a significant increase in the activities of serum ALT, AST and ALP along with histological alterations in the liver. These responses were associated with elevated levels of malondialdehyde (MDA) and reduced levels of GSH, SOD and catalase along with increased levels of tumor necrosis factor alpha (TNF-α) in the liver homogenates. Administration of vitamin before LPS challenge resulted in a significant reduction in the serum levels of ALT, AST and ALP. Supplementation with vitamin E decreased the incidence and severity of LPS-related histological changes of liver. Furthermore, vitamin E supplementation attenuated the oxidative stress by reducing the levels of MDA, restoring the levels of GSH, SOD and catalase, and decreasing the levels of TNF-α. Decreased TNF-α levels after vitamin E supplementation might have resulted into the modulation of above mentioned biochemical changes resulting into amelioration of hepatic architecture. Moreover, a variety of experimental researches on animal species showed that vitamin E treatments ameliorated liver injury induced by exposure to carbon tetrachloride (Liu et al., 1995; Naziroğlu et al., 1999; Pawlowska-Góral et al., 2007; Khalaf et al., 2009), doxorubicin, anthracycline antibiotic (Gokcimen et al., 2007), halothane (Beştaş et al., 2008), azathioprine (Amouoghli-Tabrizi et al., 2009) gasoline (Uboh et al., 2009), alcohol (Das et al., 2010; Sajitha et al., 2010) and pesticides such as diazinon, ethion, malathion and dimethoate (Kalender et al., 2005; Bhatti et al., 2010; Kalender et al., 2010; Ben Amara et al., 2011). Additionally, Al-Attar (2011) evaluated the antioxidant and protective role of vitamin E on a mixture of some heavy metals (Pb, Hg, Cd and Cu)-induced oxidative stress, and renal and testicular injuries in male mice. Exposure of mice to these heavy metals in drinking water for seven weeks resulted in statistically increases of plasma creatinine, urea and uric acid concentrations. The levels of GSH and SOD in kidney and testis tissues were significantly declined. Moreover, the histopathological examination of kidney and testis showed severe alterations in mice subjected to these heavy metals. Administration of vitamin E protected the kidney and testis of mice treated with heavy metals as evidenced by an appearance of normal histological structures, insignificant changes in the values of plasma creatinine, urea and uric acid, and the levels of kidney GSH and SOD, while the levels of testis GSH and SOD were statistically declined. Furthermore, Al-Attar (2011) suggested that vitamin E might be a useful preventive agent against the effect of the studied heavy metals at least partly due to its antioxidant properties. Based on the results obtained in the present investigation and above-mentioned previous studies, it can be suggested that administration of vitamin E might alleviate the liver biochemical disturbances and histopathological alterations from the oxidative stress produced by exposure to heavy metals. Collectively, this study demonstrated that the exposure to the mixture of heavy metals has a potential hepatotoxic influence in male mice. The biochemical and histopathological changes induced by administration of these heavy metals were improved under the treatment effect of vitamin E. The hepatoprotective effect of vitamin E may be due to its antioxidant activity. To strengthen these findings, further studies are required to clarify the mechanism actions of vitamin E as a therapeutic agent against the hepatotoxic influence of these heavy metals.

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