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ORIGINAL ARTICLE

The effect of ellagic acid on the neurotoxicity of lead exposed rats

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Abstract

Objective Lead is a toxic metal that damages neural connections (especially in children) and causes blood and brain diseases. Ellagic acid is a natural phenolic compound that has antioxidant properties. The objective of this study was to evaluate the efficacy of ellagic acid in lead-induced toxicity.

Methods In this study, the effects of lead-induced toxicity on brain cells were investigated. The role of Ellagic acid in its antioxidant properties has also been studied. Levels of glutathione, nitric oxide, malondialdehyde, TNF-α, IL-1B, glutathione reductase, and catalase were evaluated. The amount of delay in rat fall and delay in dark box entry were also investigated.

Results The study found that lead reduced the delay in rat fall, decreased rat entry into the dark box, decreased glutathione, increased malondialdehyde, increased nitric oxide, decreased catalase, superoxide dismutase and glutathione peroxidase, and also increased TNF-α and IL-1B. In all cases, Ellagic acid had a therapeutic role and had a significantly different function to lead in all cases ($P < 0.05$).

Conclusion The results of a recent study have shown that lead is very harmful to humans and could endanger human life. It has also been extracted from this study that ellagic acid, as a natural compound, is very useful and can alleviate the damaging effects of heavy metals, especially lead, on the human body.

Keywords Ellagic acid · Lead · Neurotoxicity · Antioxidant

Introduction

Heavy metals such as mercury, lead and cadmium are not vital elements and have no beneficial effects on the life of living organisms, so their accumulation in the body of living organisms, especially mammals, can lead to dangerous diseases [1–3]. Routes of entry to mammals are typically through polluted air that enters the soil and groundwater in industrial areas after rainfall, as well as through the sea and oceans [3].

The results of many published studies have shown that lead is very harmful to humans and could endanger human life. It has also been extracted from this study that ellagic acid, as a natural compound, is very useful and can alleviate the damaging effects of heavy metals, especially lead, on the human body [1, 4, 5].

Lead poisoning has negative effects on the central nervous system and also affects the body's enzymes. In long-term exposure to low amounts, it has adverse effects on all functions and causes irreversible nephropathy [6], anemia, brain and nerve damage, and developmental delay in children [6, 7].

Lead builds up in bones and endangers your health in adulthood, poisoning the nervous system, affecting the heart, and increasing blood pressure [8]. Chronic poisoning can cause kidney and lung cancer because it is mutagenic and carcinogenic. It causes pain in the gastrointestinal tract and reduces in men the number and concentration of sperm in semen and interrupts the sperm production process [9]. In summary, lead, if introduced chronically, affects almost every system of the human body. Daily consumption of 5 µg of lead per kg body weight causes chronic poisoning as it affects the central and peripheral nervous system. Symptoms of toxicity include ataxia, distal skeletal muscle weakness, numbness of the hands and feet, and fatigue. In laboratory animals it produces

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neurological symptoms that in many ways are similar to the neurotoxicity that occurs in humans [10].

Given the importance of lead-induced neurotoxicity, several studies have been carried out to find the mechanisms involved in this toxicity, the decrease in glutathione content and increase in the peroxidation of lipids in the brain tissue, changes in the levels of proteins involved in apoptosis (Bax, Bcl-2 and Caspase3) in different parts of the brain, swelling and degeneration of the final axonal regions, including the mechanisms involved in their neurotoxicity. Since oxidative stress induction plays an important role in the neurotoxicity of lead, studies on antioxidant compounds appear necessary to inhibit the toxicity of this compound [11].

In recent years, it has been well demonstrated that consumption of fruits and vegetables containing natural antioxidants has prevented many diseases, including heart disease and even various cancers. Ellagic acid (EA) is a bioactive compound that has many pharmaceutical and industrial applications. EA is a polyphenolic acid found in fruits such as pomegranates, strawberries, raspberries and grapes. This molecule has various properties including antioxidant properties. Studies on EA in cancer cells have shown apoptosis induction and cell death and inhibition of continuous tumor growth [12–14].

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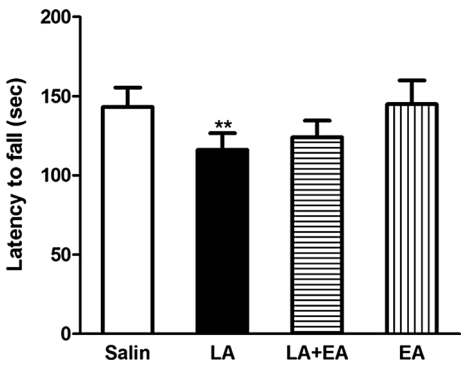

Fig. 1 Effect of EA on delay time in falling lead-exposed rats

Results

Rotarod performance in different experimental groups are presented in Fig. 1 and revealed that that in rat exposed to lead; they were falling faster than others and had significant differences to the negative control group ($P < 0.01$).

According to Fig. 2, the early latency was not significantly different between groups. The retention latency in lead-treated group was shorter than that of the negative group ($P < 0.001$). The retention latency in lead and EA-treated group was shorter than that of the negative group ($P < 0.001$) but was longer than lead group ($P < 0.05$).

Based on Fig. 3, the glutathione levels in brain tissue in rats treated with lead were significantly lower than other groups ($P < 0.001$). Also, the glutathione levels in rats treated with lead and EA had significant differences to the lead-exposed rats ($P < 0.05$).

Malondialdehyde in rats treated with lead was significantly higher than control group ($P < 0.001$) and in rats treated with lead and EA the amount of malondialdehyde had significant difference to the lead-exposed rats ($P < 0.01$) (Fig. 4).

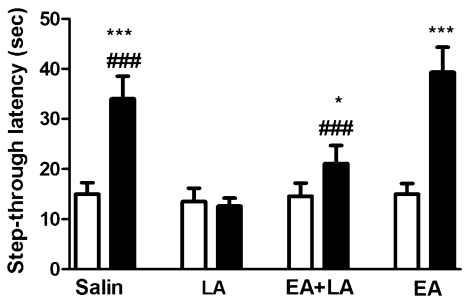

Fig. 2 Effect of EA on delayed entry into the dark box of lead-exposed rats (White bar: early latency, Black bars: retention latency)

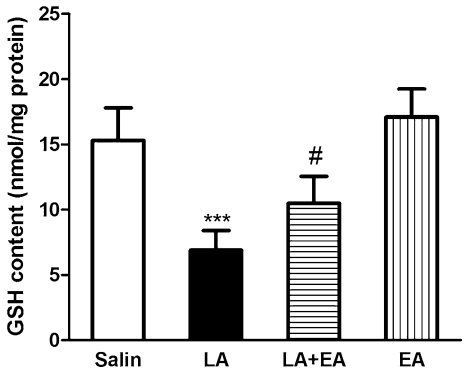

Fig. 3 Effect of EA on glutathione levels in brain tissue of lead-exposed rats

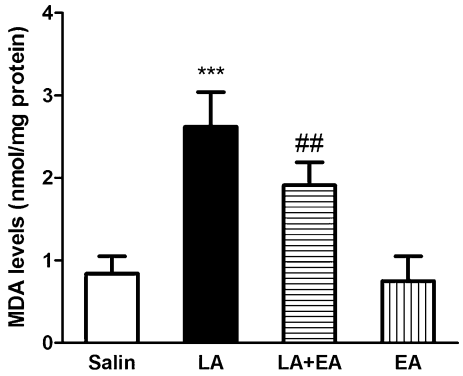

Fig. 4 Effect of EA on the amount of malondialdehyde in the brain tissue of lead-exposed rats

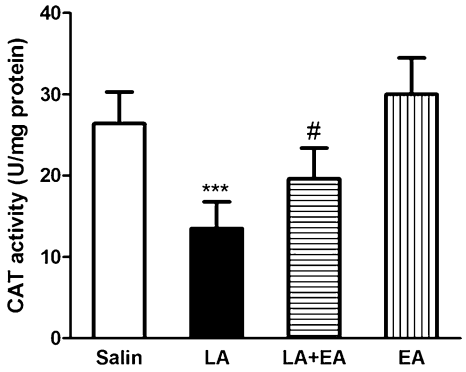

Fig. 6 Effect of EA on the amount of catalase enzyme in the brain tissue of lead-exposed rats

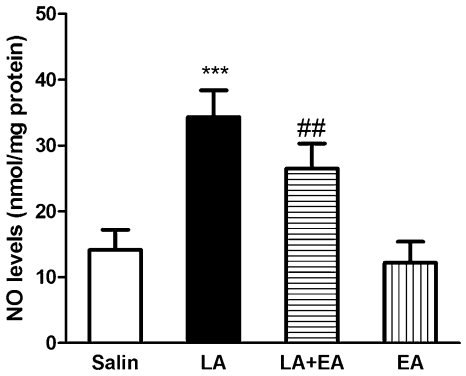

Fig. 5 Effect of EA on the amount of Nitric oxide in the brain tissue of lead-exposed rats

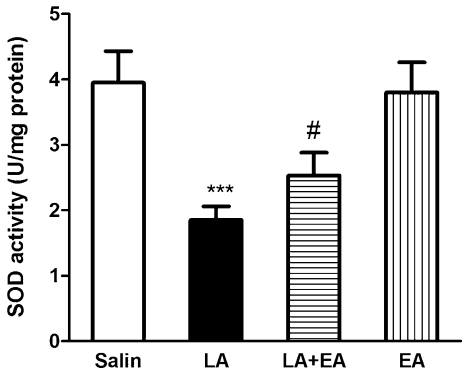

Fig. 7 Effect of EA on the amount of Superoxide dismutase enzyme in the brain tissue of lead-exposed rats

As shown in Fig. 5, the amount of Nitric oxide in lead and EA-lead groups were significantly higher than EA and control groups with P value less than 0.001 and 0.1.

The amount of catalase enzyme in lead-exposed rats was significantly lower than control group ($P < 0.001$). Also in EA-lead groups the amount of catalase enzyme is significantly higher than lead-treated group ($P < 0.05$) and lower than control group ($P < 0.001$) (Fig. 6).

According to Fig. 7, the amount of Superoxide dismutase enzyme in rats treated with lead was lower than other groups ($P < 0.001$) and in rats treated with EA and lead was significantly lower than negative control group ($P < 0.05$).

The amount of Glutathione peroxidase enzyme in rats treated with lead was lower than other groups ($P < 0.001$) and in rats treated with EA and lead was significantly lower than negative control group ($P < 0.05$) (Fig. 8).

Based on Fig. 9, the amount of TNF-α in the brain tissue in lead-exposed rats was significantly higher than negative control group ($P < 0.001$). In rats treated with EA and lead the amount of TNF-α in the brain tissue was lower than

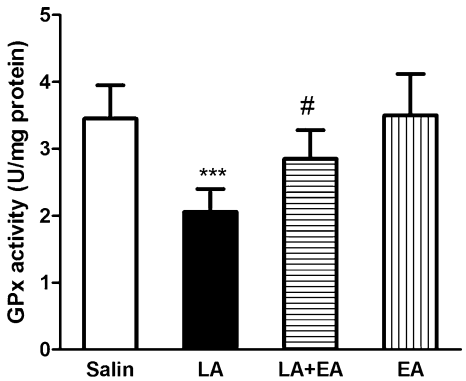

Fig. 8 Effect of EA on the amount of Glutathione peroxidase enzyme in the brain tissue of lead-exposed rats

lead-treated group and higher than negative control group ($P < 0.05$) (Fig. 9a).

IL-1B in lead-exposed rats was higher than negative control group and in rats treated with lead and EA was lower

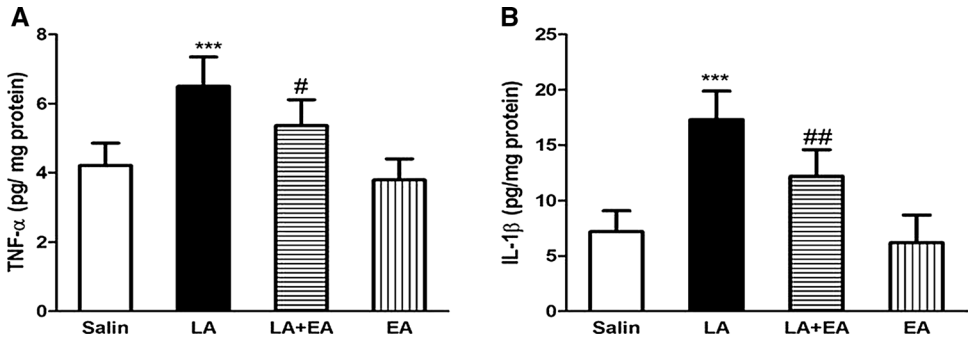

Fig. 9 **a** Effect of EA on the amount of TNF-α in the brain tissue of lead-exposed rats. **b** effect of EA on the amount of IL-1B in the brain tissue of lead-exposed rats

than lead-treated group and higher than negative control group ($P < 0.001$) (Fig. 9b).

Administration of lead acetate alone significantly decreased the expression level of glutathione peroxidase in brain tissue compared to normal saline group and Glutathione peroxidase enzyme gene expression in brain tissue was significantly different in lead acetate and EA groups ($P < 0.05$). But the level of glutathione peroxidase enzyme in brain tissue group was similar to normal saline group and there was no significant difference ($P > 0.05$) (Fig. 10a).

Lead acetate alone decreased the expression of SOD gene in brain tissue compared to normal saline group and expression of SOD gene expression in brain tissue was significantly different in lead acetate group and EA group in lead acetate group ($P < 0.05$). Expression of superoxide dismutase gene expression in brain tissue was not similar in the group receiving normal saline alone and with no significant difference ($P > 0.05$) (Fig. 10b).

Discussion

The results of this study showed that lead consumption caused behavioral impairments so that passive avoidance learning and pain threshold decreased significantly after lead consumption. His delay time to dark room after shock induction in the lead group showed a significant decrease compared to the control group. The results of this study also showed that administration of different doses of EA in ischemic groups increased learning, memory and pain threshold. Rotarod performance in different experimental groups revealed that that in rat exposed to lead were falling faster than others and had significant differences to the negative control group. The delaying in entry into the dark box in the early latency was not significantly different between groups but; in retention latency, in lead-treated group was shorter than that of the negative group. The results of the delay time of falling and the delay in entering the dark box of lead-exposed rats showed that lead consumption in rats accelerated the fall and increased the rate of delayed entry into the dark box. In both trials, ellagic acid has been shown to have positive therapeutic effects for treating neurological disorders.

EA is a natural agent derived from nuts and fruits [15] which is not toxic in dose of up to 50 mg/day for 45 days in rats. Anticancer, antidiabetic, anti-inflammatory, antiviral and antioxidant activities of EA are reported by Garcia-Nino et al. [14]. Also, potent protective effects of EA were reported in lung, liver and kidney organs [14]. EA exhibited anti-inflammatory effects by affection on cycloxygenase enzyme, IL-1β, TNF-α and IL-6 modulates production [16] and it will reduce inflammation and damage cells in the brain [17, 18]. Ghasemzadeh Dehkordi et al. [19] evaluated the effect of EA on memory and pain in rats with brain ischemia and revealed that EA in concentration of 10, 25 and 50 mg/kg could development the memory and decline the pain [19].

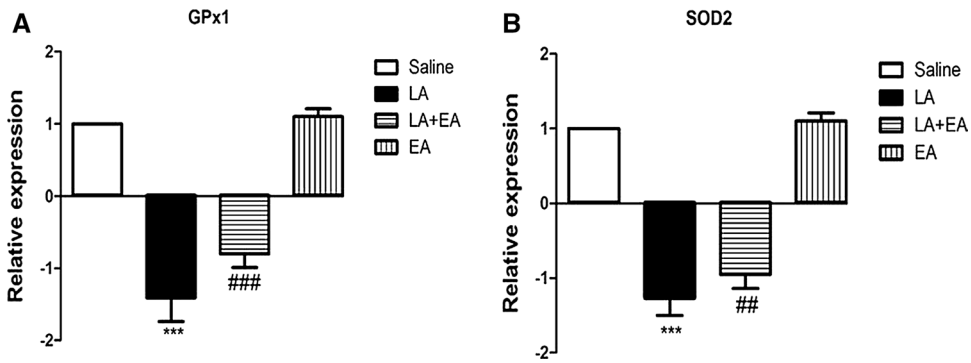

Fig. 10 **a** Effect of EA on the expression level of glutathione peroxidase enzyme in brain tissue of lead-exposed rats. **b** Effect of EA on the expression of superoxide dismutase gene in brain tissue of lead-exposed rats

Glutathione (GSH) is a peptide composed of three essential amino acids that plays an important role in the body. Researchers believe that this substance is very important for the health of the body. Glutathione interacts with drugs to digest them, is a co-factor for some important enzymes including Glutathione peroxidase and protects the body from oxidative damage, reduces peroxide, improves cancer apoptosis, plays an important role in immune function, prevent drug resistance, protects the body against environmental toxins, and also fights cancer [20, 21]. Researchers in different fields are looking for a way to increase glutathione in the body, which has been shown in this study by EA to compensate for glutathione depletion and to significantly increase this compound [14].

Malondialdehyde and nitric oxide in rats treated with lead were significantly higher than control group and in rats treated with lead and EA the amount of them was significantly decreased. Various studies have shown that many herbs and compounds, especially ascorbic acid, have very strong antioxidant capacity, and this study has shown that EA is a potent antioxidant in reducing malondialdehyde and nitric oxide [22]. The presence of oxidant compounds in the body is very dangerous, and since oxidant compounds such as lead exist in nature and are unfortunately in contact with humans, the presence of antioxidant compounds is crucial [23].

The use of lead in the current study increases the number of inflammatory mediators such as TNF-α and IL-1B, it is clear that these inflammatory cytokines could induce apoptosis in the nervous system of neurons, and the results of this study showed that ellagic acid can reduce inflammatory cytokines. In a similar study, Goudarzi and his colleagues (2018) investigated the role of EA in reducing acrylamide-induced neurotoxicity and showed that in addition to the antioxidant effect, EA can reduce levels of TNF-α and IL-1B in rat [22].

Many natural compounds are used today to reduce the level of oxidants and reduce the toxicity of chemicals. Mehri et al. (2009) investigated the role of *Silybum marianum* plant in reducing acrylamide-induced neurotoxicity. As the acrylamide concentration increased, the cell viability was decreased for 5 h. *S. marianum* extract at all concentrations decreased acrylamide-induced toxicity after 5 h of exposure to cells. Administration of acrylamide caused significant motor abnormalities in animals. Administration of ethanolic extract of *S. marianum* (400 mg/kg) improved animal movements compared to acrylamide group [24].

In addition to enzymatic methods, molecular methods have been used to confirm the results of enzymatic tests. The results of molecular methods showed that SOD and GPX genes expression was decreased at the time of administration of EA and EA-Lead acetate and was significant in comparison with control groups.

Materials and methods

Study design and groups

In this experimental study, six rats were randomly assigned to different groups. The dosage and method of administration of acetate and lead acetate (LA) are selected based on reference [25]. We divided the animals into four equal groups of six, including: Group 1: as negative control; received only phosphate buffered saline (PBS), Group 2: as positive control; received lead, Group 3: received lead and EA, and Group 4: Only received EA. Passive avoidance test and rotarod test were performed 24 h after the last dose.

Passive avoidance test

In this method, the training device consists of two boxes (light and dark) separated by a guillotine blade. The bottom of the dark box is covered with stainless steel shock bars. Each mouse is placed in a light box during testing, 60 s after the separator blade is adapted, and the delay time until the mouse enters the dark box is recorded. Immediately after the mouse enters the blade dark box, the shock is given an equivalent shock of 75 V, 0.2 mA, and 50 Hz for 3 s. After 5 s, the mouse is removed from the box and returned to its cage. This test was repeated 24 h later, but not the shock. Delay memory is calculated for a maximum of 600 s [22].

Rotarod test

The Rotarod Behavioral Test is a validated test for assessing nerve damage to muscle strength, coordination of anterior and posterior organs, and balance. The measurement was based on when the animal was able to stay on the rotating machine and not fall. Once this test was done, the rotation speed was set at 5 rpm for 3 min and then gradually increased to 40 rpm over 12 min and remained constant until the end of the test. The maximum test time of each animal was 30 min. Each mouse was tested 3 times at 45-min intervals and the mean duration of tolerance on the spinning rod or the time of falling from it was recorded in seconds [22].

Sample preparations

The animals were then anesthetized with ketamine/xylazine and blood was taken directly from the animal's heart into the test tube (without heparin) and serum was separated to measure malondialdehyde and glutathione. After removal of brain tissue, it was weighed and placed in a 10% formalin solution for histological and pathological examination of the cortex. Other brain tissue was stored at − 70 °C to measure

some factors such as malondialdehyde, glutathione, nitric oxide, TNF-α, IL-1B, activity of catalase, glutathione peroxidase and superoxide dismutase and glutathione peroxide gene expression.

Protein levels measurement

0.5 g of brain tissue was homogenized in 0.1 M phosphate buffer with pH = 7.4 at a 10% concentration.vol/wt using a homogenizer. Protein levels were measured using Bradford's method. The Bradford reagent was prepared with Komassi blue (10 mg), 85% phosphoric acid, 96% ethanol and distilled water (1/10). Optical absorption was then measured using a spectrophotometer at 595 nm and a calibration curve was elaborated with specified BSA concentrations of 62.5, 125, 250, 500 µg/ml BSA (as standard) and the protein concentration was measured.

Evaluation of tissue lipid peroxidation

Satho method was used to determine the amount of malondialdehyde. 1.5 ml of 10% trichloroacetic acid solution was added to 0.5 ml of homogenized tissue and then centrifuged at 4000 g for 10 min. 2 ml of 0.67% thiobarbituric acid solution was added to 1.5 ml of the supernatant, and then incubate in boiling water bath for 30 min. Then, the absorbance was read at 532 nm. Concentration of malondialdehyde was determined using tetraethoxy propane as standard.

Tissue glutathione content

Kidney tissue glutathione content was identified and measured by the reaction of glutathione (GSH) with elemental reagent (5,5′-dithiobis-(2-nitrobenzoic acid), DTNB) and the generation of TNB (yellow). To 40 µL of homogenous tissue, 2 ml of phosphate buffer was added and then 40 µL of 10 mM ellman reagent was added and optical absorption at 412 nm was read.

Tissue nitric oxide (NO) measurement

Tissue nitric oxide content was determined by a grease reagent. Briefly, brain homogenate tissue (in phosphate buffer) was deprecated using zinc sulfate and then passed through cadmium powder to convert nitrate to nitrite. Then, the nitrite was read from a grease reagent at a wavelength of 540 nm.

Catalase, glutathione peroxidase and superoxide dismutase activity in brain tissue

Measured using ZellBio commercial kits and ELISA reader according to kit instructions.

Tumor necrosis factor (TNF)-α, Interleukin (IL)-1B in brain tissue

Measured using IBL commercial kits and ELISA reader according to kit instructions.

Statistical analysis

Data were analyzed using ANOVA, t-Test and Tukey's post hoc tests and SPSS-21 and the *P* value less than 0.05 was considered significant.

Conclusion

Due to the increase in oxidant compounds and inflammatory cytokines from lead consumption, it has been shown to be a highly dangerous compound for human health. EA as a natural phenolic compound derived from plant sources has been shown to be a potent antioxidant and may even protect the human body from possible damage caused by inflammatory cytokines. However, the information obtained in this study should be confirmed by further testing in the in vivo and human phases.

Acknowledgements This project did not receive any specific grant from funding agencies.

Compliance with ethical standards

Conflict of interest Ali amirahmadi, Javad babaei, Ramin Abrishami, Mehdi Goudarzi, Mojtaba kalantar declare that we have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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