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Protective Effect of Vitamin E Against Lead-induced Memory and Learning Impairment in Male Rats

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Background: Lead (Pb^{2+}) is a neurotoxin substance that has been known for its adverse effects on central nervous system and memory. Previous studies reported the potential effect of vitamin E as a memory enhancer.
Objectives: The purpose of the present study was to assess the protective effects of vitamin E against Pb-induced amnesia.
Materials and Methods: Forty-eight male Wistar rats (200-250 g) were divided equally into the saline, Pb, Pb + vitamin E, and vitamin E alone groups. To induce Pb toxicity, rats received water that contained 0.2% Pb instead of regular water for 1 month. Rats pretreated, treated or post treated with vitamin E (150 mg/kg) for 2 months. Passive avoidance learning was assessed using Shuttle-Box after two months. Retention was tested 24 and 48 hours after training.
Results: The results showed that Pb caused impairment in acquisition and retrieval processes in passive avoidance learning. Vitamin E reversed learning and memory deficits in pre, post or co- exposure with Pb ($P < 0.001$).
Conclusions: According to the results of this study, administration of vitamin E to rats counteracts the negative effects of Pb on learning and memory. To more precisely extrapolate these findings to humans, future clinical studies are warranted.

Keywords: Lead; Vitamin E; Avoidance Learning

1. Background

Lead (Pb) is a neurotoxic heavy metal, which plays a significant role in modern industry (1) and has a serious public health concern (2). The World Health Organization (WHO) estimates that 0.6% of global diseases and 600,000 causes of intellectual disability in children are caused by Pb poisoning (3). Lead is toxic to many organs of the body, causing a broad range of physiological, biochemical dysfunctions, behavior disorders, slowed growth, convulsions, and death (3, 4); It crosses the blood-brain barrier, aggregates in the brain (5) and affects neurogenesis in hippocampus as a (6) vital area of the brain associated with learning and memory (7). It has been shown that an exposure to Pb during gestation (8) or both gestation and lactation (9) can cause long-term cognitive deficits in adult offspring in rats. Pb-exposed animals during both gestation and lactation showed a significant behavioral dysfunction even when tested as adults (8, 9). Moreover, Pb is able to induce the production of reactive oxygen species that interact with nucleophilic sites in DNA and causing breaks and other DNA damages (2, 10, 11). Reactive oxygen species induces lipid peroxidation of polyunsaturated fatty acids and subsequently can cause cell membrane damages. The antioxidant defense against the adverse effects of free radicals indicates an important aspect of investigation in animals (10). Vitamin E or α-tocopherol as a low-molecular mass antioxidant, form a part of the internal antioxidant system. It has been reported that high plasma levels of vitamin E are associated with a reduced risk of Alzheimer's disease in older patients (12). The Pb administration results in toxicity of hippocampus and memory impairment (6). Oxidative stress has been suggested to be one of the important mechanisms of toxic effects of Pb (4).

2. Objectives

The present study aimed to assess the protective effects of vitamin E against Pb-induced learning and memory impairment in the passive avoidance task.

3. Materials and Methods

Lead and vitamin E were purchased from the (Sigma Aldrich, St. Louis, MO, USA). Other chemicals were purchased from commercial source.

3.1. Animal Classification

Forty-eight male Wistar rats (weighing 200-250 g) were purchased from Pasteur Institute of Iran (Karaj, Iran). The rats were kept in air-conditioned room, with standard conditions of 21°C ± 2°C (50% ± 10% humidity) and a natural light-dark cycle (12-12 hours). Animals were allowed standard rat chow diet and water ad libitum. Ethics Committee of Hamadan University of Medical Sciences approved the protocols for the experiment. After 2 weeks of acclimatization, the rats were randomly subdivided into 6 groups (n = 8 per each group). All animals were exposed to their special diet for 12 weeks by gavage as follows:

1) Control group received 2 mL saline.
2) The Pb groups were exposed to 0.2% lead acetate.
3) Vitamin E group received 150 mg/kg vitamin E for 2 months.
4) Treatment group received lead acetate and vitamin E synchronous.
5) Post-treatment group received 0.2% Pb acetate for 2 months and then vitamin E for 1 month.
6) Pre-treatment group received vitamin E for first 1 month, and lead acetate for 2 months.

The day after last administration, memory was evaluated using the inhibitory avoidance apparatus.

3.2. Inhibitory Avoidance Apparatus (Shuttle Box)

We used Shuttle box to evaluate the inhibitory avoidance apparatus. The apparatus was consisted of two boxes of 20 × 20 × 30 cm size with a guillotine door in the middle of a separating wall. The on chamber was consisted of white opaque resin and the other one was dark. Intermittent electric shocks (50 Hz, 2 s and 0.8 mA intensity) were delivered to the grid floor by an isolate stimulator in the dark compartment. After accustoming in the experimental room for at least 30 minutes, each rat was placed in the white compartment, the guillotine door was opened and the animal was permitted to enter the dark chamber. Excluding criterion for this phase is animals, which waited more than 120 seconds to enter the dark chamber. When the animal entered to the dark chamber the door was closed and the rat was immediately removed from the chamber. This test was repeated after 30 minutes. In the acquisition trial, an electric shock (50 Hz, 2 s and 0.8 mA intensity) was immediately delivered to the grid floor, when the animal entered the shock chamber. The trials repeated in the same way until the rat avoids entering the dark compartment during 120 seconds. The interval between trials was two minutes. If the rat entered the dark compartment within 120 seconds, the door was closed and the animal received another shock again. Forty-eight hours later, the retention trial was done and the rat was placed in the light chamber and after 5 seconds, the door was opened and the latency which the animal entered the dark chamber (step through latency, STL), total time spent in dark compartment (TDC) and the times of entering in a duration of 10 minutes were recorded in the absence of electric shocks, as indicators of inhibitory avoidance behavior.

3.3. Statistical Analysis

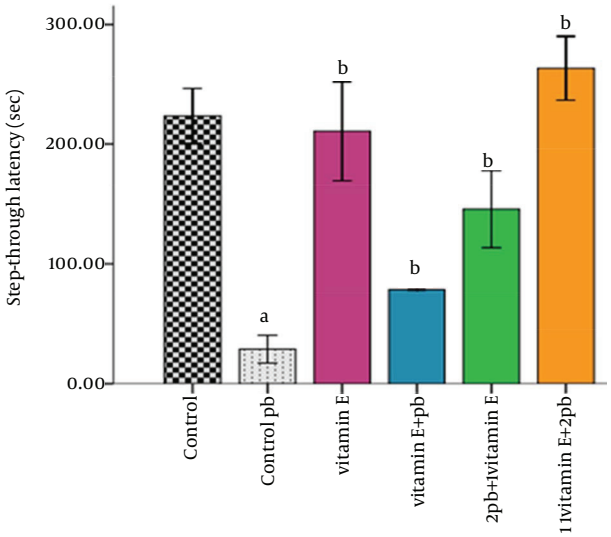
The values are presented as the mean ± SEM and analyzed by SPSS 16 software (SPSS INC., Chicago, ILL, USA). Data analysis and evaluation of statistical significance between the groups were performed using one-way analysis of variance (ANOVA). Post-hoc comparisons were performed using Tukey's test. P values less than 0.05 were considered statistically significant.

4. Results

4.1. Vitamin E Improved Lead-Induced Step-Through Latency Impairment in the Passive Avoidance Learning

Our results showed that Pb caused impairment in acquisition and retrieval processes of passive avoidance learning (PAL) and memory and cotreatment of vitamin E reversed learning and memory deficits in pre, post or co-exposure with Pb-treated rats. As shown in Figure 1, Pb reduced the step-through latency (STL) compared to the control group (P < 0.001) and vitamin E increased STL in retention trial compared to the Pb group (P < 0.001).

Figure 1. The Mean of the Step-Through Latency in the Passive Avoidance Task



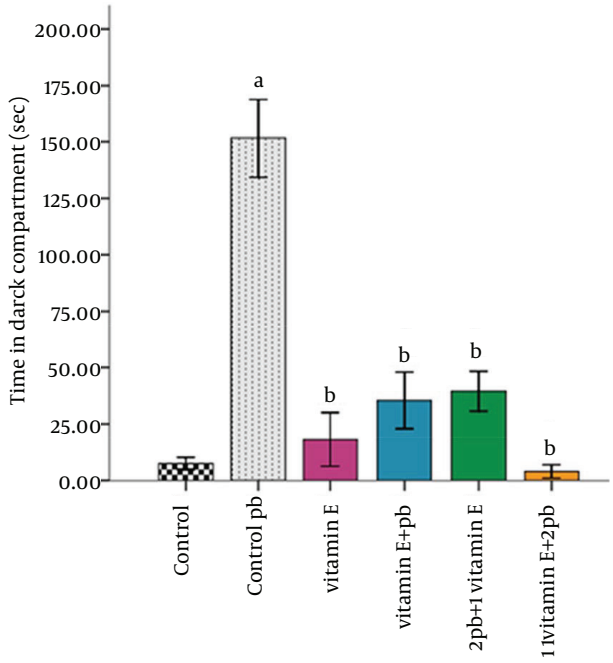
The values were reported as mean ± SEM (a, P < 0.001 vs. control group; b, P < 0.001 vs. Pb group).

4.2. Vitamin E Decreased Time in the Dark Compartment in the Passive Avoidance Learning

The variance analysis of our results showed that the Pb-treated group spent more time in the dark compartment (TDC) compared to the control group (P < 0.001, Figure

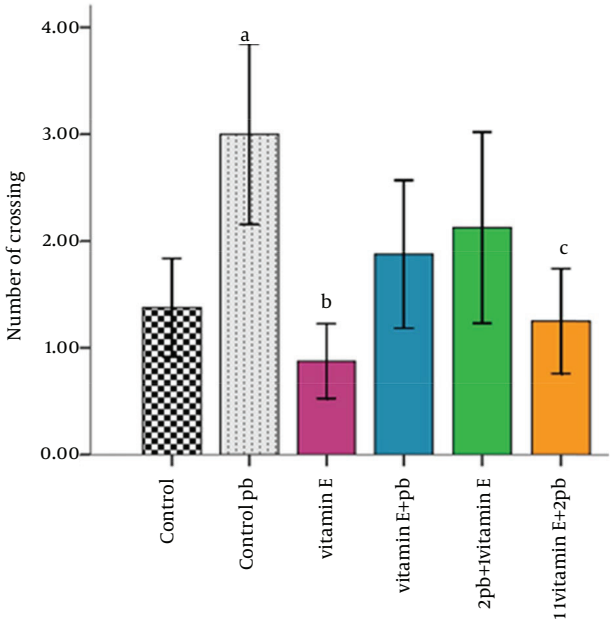
2). Moreover, the TDC significantly decreased in vitamin E groups compared to the Pb group (P < 0.001).

Figure 2. The Mean of the Time in the Dark Compartment in the Passive Avoidance Task



Vertical bars show SEM (a, P < 0.001 vs. control group; b, P < 0.001 vs. Pb group).

Figure 3. The Mean of the Number of Crossing in the Passive Avoidance Task



Vertical bars show SEM (a, P < 0.05 vs. control group; b, P < 0.01 and c, P < 0.05 vs. Pb group).

4.3. Vitamin E Improved Lead-Induced Number of Crossing Impairment in the Passive Avoidance Learning

As shown in Figure 3, there was a significant difference in crossing number between control and Pb groups (P < 0.05). Furthermore, the frequency of going to dark compartment in pre-treated rats was fewer compared to the Pb group (P < 0.05, respectively, Figure 3), while co-treatment and post-treatment with vitamin E for one month showed no significant difference compared to Pb group.

5. Discussion

A wide range of population is at risk of exposure to Pb and its compounds because of its important role in modern industry (13). The presence of Pb in the human body even in small amounts can induce harmful neurotoxic effects (14, 15). Several studies give evidence that the Pb plays critical role in oxidative damage and lipid proxidaion of cellular membranes (11, 16). The results of this study showed that Pb administration for two months can have negative effects on learning and memory in passive avoidance task and treatment with vitamin E counteracts the negative effect of Pb on learning and memory. Moreover, our results showed that administration of vitamin E at the same time as Pb, can improve memory and learning. Also, post-treatment with vitamin E showed the same result. Consistent to our results, Nagata et al. have reported that vitamin E slows or prevents memory impairments (17). Hasanein and Shahidi have shown that combined vitamins C and E administration to rats for 30 days from onset of diabetes decreased the negative influence of diabetes on learning and memory (18). It has been demonstrated that administration of vitamin E was important in avoiding hippocampal oxidative stress, and memory deficits induced by sleep deprivation (19, 20). In the study by Alzoubi et al. antioxidant contained vitamin E administration also prevents long-term memory impairments induced by consumption of high-fat high-carbohydrate diet (21). Result of a clinical study has suggested that long-term cumulative Pb exposure may be weakly associated with cognitive decline in community-dwelling women (22). Ebuehi et al. have reported oral administration of vitamin C and vitamin E in Pb exposed rats ameliorates the hepatic damage and significantly reduces the oxidative stress in the brain (23). It has been shown that Pb causes neuronal death in the hippocampus (16). Administration of vitamin C and E can decrease blood Pb level, alleviate damage of lipid peroxidation in hippocampus by Pb toxicity and significantly increase the concentrations of superoxide dismutase and can also reduce glutathione, nitric oxide and NOS levels in rats with Pb poisoning (24). In conclusion, we demonstrated that the Pb induced learning deficits in passive avoidance task and vitamin E administration improved STL and TDC in passive avoidance tasks. Therefore, it seems that

vitamin E may be useful to treat patients with impaired memory function.

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Authors' Contributions

Iraj Salehi and Maryam Sahab Soleimani contributed in study design and did the experiments. Mahsa Poorhamze and Fahimeh Ghasemi Moravej contributed in manuscript writing, Alireza Komaki contributed in data analysis and Sara Soleimani Asl edited the manuscript.

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