

Vitamin C Can Alter Lead-Induced Passive Avoidance Learning Impairment in Rats

Iraj Salehi¹, Maryam Sahab Soleimani², Mona Pourjafar³, Fahimeh Ghasemi Moravej⁴, Sara Soleimani Asl^{1,4*}

1. Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, Iran.

2. Department of Physiology, School of Science, Hamedan Branch, Islamic Azad University, Hamedan, Iran.

3. Department of Molecular Medicine, School of Science, Hamedan University of Medical Sciences, Hamedan, Iran.

4. Department of Anatomy, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.



Sara Soliemani Asl is assistant professor of anatomical sciences in Department of Anatomy, School of Medicine, Hamadan University of Medical Sciences, Hamedan, Iran. She teaches goss anatomy, histology, and embryology and her research interests are drug addiction and memory.

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ABSTRACT

Introduction: Lead (Pb) is a neurotoxin that its different effects on the central nervous system are well-known. Previous studies have reported the potent effects of vitamin C on memory. The present study was undertaken to evaluate the protective effects of vitamin C against lead-induced amnesia.

Methods: Male Wistar rats were divided into 4 groups: the control (saline), negative control (lead), positive control (Vitamin C, 150 mg/kg), and experimental (Lead+Vitamin C). To induce lead toxicity, the rats received water containing 0.2% Pb instead of regular water for 1 month. Passive avoidance learning was assessed by Shuttle Box 2 months later. Retention was tested 24 hours after training.

Results: The results showed that lead causes impairment in acquisition and retrieval processes of passive avoidance learning and memory. However, vitamin C administration reinforced passive avoidance learning and memory. All results were significant ($P < 0.001$).

Conclusion: Vitamin C administration in rats counteracts the negative effect of lead on spatial learning and memory.

Key Words:

Lead, Vitamin C, Passive avoidance learning, Rats.

1. Introduction

Learning and memory are the highest levels of the central nervous system functions. Memory is a biological adaptation that allows the organisms to use past experience to adjust their behavior to environmental changes [1]. Memory includes many processes such as acquisition,

encoding, consolidation, restructuring, and performance. However, learning is a neurological phenomenon by which organisms change their behavior through practice [2].

Lead (Pb) with atomic number 82 is a heavy metal. It is everywhere and can cause many physiological, biochemical, and behavioral disorders in humans and animals [3, 4]. Direct neurotoxic effects of lead include apoptosis (pro-

* Corresponding Author:

Sara Soleimani Asl, PhD

Address: Department of Anatomy, School of Medicine, Hamedan University of Medical Sciences, Hamedan, Iran.

Tel: +98(81) 38380208

E-mail: s.soleimanasl@umsha.ac.ir

grammed cell death), increased irritability, disorder in the process of storage and release of neurotransmitters, mitochondrial dysfunction, second messenger dysfunction, damage to brain endothelial cells and glial cells [3, 5].

Effect on the brain's development is one of the most damaging effects of lead. In this regard, hippocampus is one of most sensitive and vulnerable areas to lead toxicity. Many psychologists and neurologists believe that the hippocampus plays an important role in the formation of new memories about the observed events. Long-term exposure to low levels of lead associates with behavioral and learning abnormalities in humans and animals [5, 6].

However, exogenous antioxidants can neutralize major cell damages caused by oxidative stress [7, 8]. Vitamins are non-enzymatic antioxidants. Vitamin C (ascorbic acid) is an essential supplement for normal metabolic reactions in humans and animals. It has antioxidant properties that plays an important role in cleaning up oxygen free radicals and stabilizing cell membranes as well as lowering the risk of several diseases such as cardiovascular disease and cancer [9-11]. Vitamin C is very effective in protecting brain cells. Oxidative damages during vitamin C deficiency increase in brain cells [12, 13]. Also, high dose administration of vitamin E and vitamin C may decrease the risk of Alzheimer disease [14] and improves learning and passive avoidance memory in diabetic rats [15].

In the present study, the effects of lead on learning and memory in male Wistar rats and the preventive effects of vitamin C on neurotoxicity of lead on the passive avoidance memory were examined.

2. Materials and Methods

Lead and vitamin C were purchased from the Sigma Aldrich (St. Louis, MO, USA).

Animal treatment and experimental procedures

In this experiment, 48 male Wistar rats, weighing 180 ± 20 g were used. They kept under proper conditions of 12:12 h light/dark cycle at a temperature of $21 \pm 2^\circ\text{C}$. All rats were fed with chow and water. This study was approved by Ethics Committee of Hamadan University of Medical Sciences. After 15 days of acclimatization, the rats were randomly divided into 6 groups ($n=8$ per each group):

1. Control group received 2 mL saline.
2. Negative control group (Pb group) received water containing 0.2% lead [16].

3. Vitamin C group (positive control) received 150 mg/kg vitamin C (Sigma Aldrich, St. Louis, MO, USA) for 2 months.

4. Treatment group received lead acetate and vitamin C simultaneously.

5. Posttreatment group received 0.2% Pb acetate for 2 months and then vitamin C for 1 month.

6. Pretreatment group received vitamin C for the first 1 month, and lead acetate for 2 months. Passive avoidance learning was assessed by Shuttle-Box 2 months later. Retention was tested 24 hours after training.

Passive avoidance learning test

Passive avoidance learning (PAL) is a 2-part Plexiglas box that has bright and dark parts. The size of the 2 parts is equal and it has an 8×8 cm valve. At the bottom of 2 parts, the stainless steel bars are located at a distance of 1 cm. A 100W lamp is switched 40 cm over the device. An electrical circuit is connected to the bottom of the dark part. The test follows 3 steps [17]:

Adoption

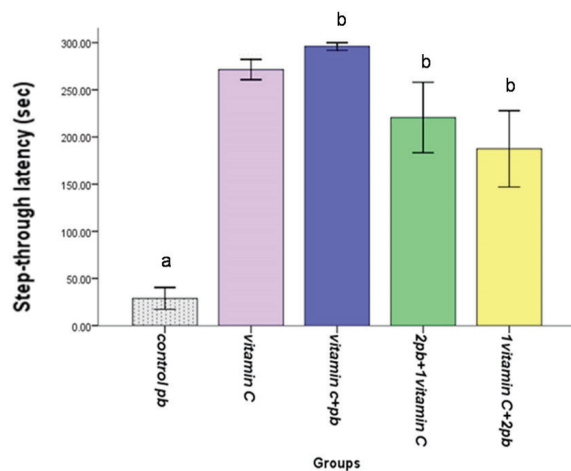
At First, all experimental groups became accustomed to the device. Thirty minutes after placing the animals in the light, the valve was opened. Immediately after the arrival of the animals in the dark part the door was closed. Animals were taken from the dark side and returned to the cage. This procedure was repeated 30 minutes later.

2. Acquisition

Thirty minutes after the second time adaptation, the acquisition step was trained. Immediately after the entry of the rats in the dark part, the door was closed and electric shock (50 Hz and 0.8 mA) applied to the rats for 2 seconds. After 30 seconds, the rats were taken from the dark part and returned to the cage. Two minutes later, the behaviors of rats were examined as in the earlier experiment. Refusing to enter into the dark part within 120 seconds was considered as the successful acquisition of light and dark parts. Otherwise, the rats received another shock upon entrance to the dark part for the second time.

Retention

Two days after training, the rats were placed in the light part and 5 seconds later the door was opened between the light and dark parts. The time it took for the animal to enter the dark part (step-through latency, STL) and how



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Figure 1. The mean of the STL in the passive avoidance task. The values were shown as mean±S.E.M. (a $P<0.001$ vs. control group, b $P<0.001$ vs. lead group).

long it spend there (TDC), and the number of entries into the dark room for 10 minutes were recorded.

Statistical analysis

The values are presented as mean±SEM. All results of this study were analyzed by ANOVA (1-way analysis of variance). Post hoc comparisons were performed using Tukey test. $P<0.05$ was considered as statistically significant of differences.

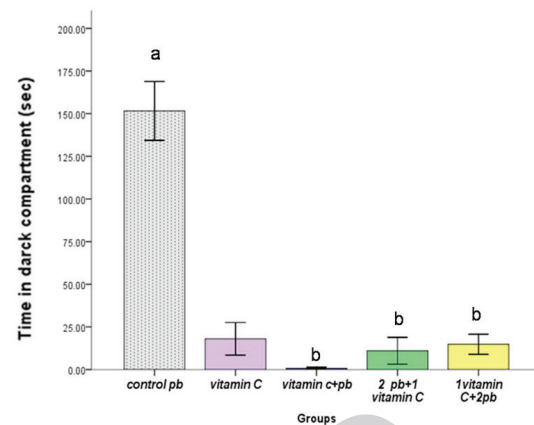
3. Results

As shown in Figure 1, lead treatment caused impairment in acquisition and retrieval processes of PAL and administration of vitamin C reversed learning and memory deficits in pre-, post-, or co-exposure with lead-treated rats. Our results showed that STL decreased in the lead group in comparison to control group ($P<0.001$) and vitamin C increased that in retention trial compared to lead group ($P<0.001$).

Furthermore, our results showed that lead-treated group spent more time in the dark compartment (TDC) compared to control group ($P<0.001$, Figure 2). The TDC significantly decreased in vitamin C treated groups compared to lead group ($P<0.001$).

4. Discussion

Lead is one of most poisonous chemicals in the environment that can induce acute or chronic diseases. The toxic effects of lead have been discovered in organs like liver, kidney, and brain. Lead can influence hippocampus, cor-



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Figure 2. The mean of the time in the dark compartment in the passive avoidance task. The values were shown as mean±S.E.M. (a $P<0.001$ vs. control group, b $P<0.001$ vs. lead group).

tex, and cerebellum. Since this system plays an important role in learning and memory formation, the defect in the communication of this system through lead exposure even at low concentrations, impairs memory and learning [5].

Increase in STL and decrease in TDC during the retention test have positive effects on memory retention [18]. In our study, STL decreased and TDC increased, which demonstrates memory retention deficits induced by lead. Furthermore, the toxic effects of lead on learning and memory after 2 months confirms the results of earlier studies.

The findings of this study showed that treatment with vitamin C for 2 months significantly improved PAL and memory of control group rats. Pretreatment with vitamin C may help to neutralize the toxic effects of lead on learning and memory. According to our results, treatment with vitamin C could improve memory after chronic exposure (co-exposure) to lead in rats. Also, posttreatment with vitamin C for 2 months alleviated the negative influence of lead on learning and memory. In this regard, the number of trials in acquisition step decreased in lead group treated with vitamin C. Likewise, with regard to the retention step, increased STL and decreased TDC were observed in diabetic rats. Clinical studies have reported that vitamin C improves cognitive functions in mice [19]. Vitamin C prevents memory loss caused by aging in rats too [20]. It has also been shown to improve memory in diabetic rats and combination of vitamin C and E showed a significant improvement in learning and memory in control rats [21].

Vitamins like antioxidants protect against memory impairment [22]. Lead causes serious damages to the mem-

ory. Neuronal death is caused by free radical production and the balance between oxidants and antioxidants [5, 23]. Therefore antioxidant properties of vitamin C can protect against the toxic effects of lead.

In conclusion, vitamin C administration counteracts the negative influence of lead on spatial learning and memory in rats. Future clinical studies are warranted to more precisely extrapolate these findings to humans.

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Conflict of Interests

None of the authors had any financial interest to report.

References

- [1] Rashidy-Pour A, Motamedi F, Motahed-Larijani Z. Effects of reversible inactivations of the medial septal area on reference and working memory versions of the Morris water maze. *Brain Research*. 1996; 709(1):131-40.
- [2] Parle M, Singh N, Vasudevan M. Regular rehearsal helps in consolidation of long term memory. *Journal of Sports Science & Medicine*. 2006; 5(1):80.
- [3] Marchetti C. Molecular targets of lead in brain neurotoxicity. *Neurotoxicity Research*. 2003; 5(3):221-35.
- [4] Tuomaa TE. The adverse effects of lead. *Journal of Nutritional and Environmental Medicine*. 1994; 4(4):449-61.
- [5] Lidsky TI, Schneider JS. Lead neurotoxicity in children: Basic mechanisms and clinical correlates. *Brain*. 2003; 126(1):5-19.
- [6] NourEddine D, Miloud S, Abdelkader A. Effect of lead exposure on dopaminergic transmission in the rat brain. *Toxicology*. 2005; 207(3):363-8.
- [7] Gurer-Orhan H, Sabır HU, Özgüneş H. Correlation between clinical indicators of lead poisoning and oxidative stress parameters in controls and lead-exposed workers. *Toxicology*. 2004; 195(2):147-54.
- [8] Ercal N, Gurer-Orhan H, Aykin-Burns N. Toxic metals and oxidative stress part I: Mechanisms involved in metal-induced oxidative damage. *Current Topics in Medicinal Chemistry*. 2001; 1(6):529-39.
- [9] Podmore ID, Griffiths HR, Herbert KE, Mistry N, Mistry P, Lunec J. Vitamin C exhibits pro-oxidant properties. *Nature*. 1998; 392(6676):559.
- [10] Carr AC, Frei B. Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. *American Journal of Clinical Nutrition*. 1999; 69(6):1086-107.
- [11] Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee JH, et al. Vitamin C as an antioxidant: evaluation of its role in disease prevention. *Journal of the American College of Nutrition*. 2003; 22(1):18-35.
- [12] Harrison FE, May JM. Vitamin C function in the brain: Vital role of the ascorbate transporter SVCT2. *Free Radical Biology and Medicine*. 2009; 46(6):719-30.
- [13] Tveden-Nyborg P, Johansen LK, Raida Z, Villumsen CK, Larsen JO, Lykkesfeldt J. Vitamin C deficiency in early postnatal life impairs spatial memory and reduces the number of hippocampal neurons in guinea pigs. *American Journal of Clinical Nutrition*. 2009; 90(3):540-6.
- [14] Parle M, Dhingra D. Ascorbic acid: A promising memory-enhancer in mice. *Journal of Pharmacological Sciences*. 2003; 93(2):129-35.
- [15] Reis EA, Zugno AI, Franzon R, Tagliari B, Matté C, Lambers ML, et al. Pretreatment with vitamins E and C prevent the impairment of memory caused by homocysteine administration in rats. *Metabolic Brain Disease*. 2002; 17(3):211-7.
- [16] Salehi I, Saheb Soleimani M, Poorhamze M, Ghasemi Moravej F, Komaki A, Soleimani Asl S. Protective Effect of Vitamin E Against Lead-induced Memory and Learning Impairment in Male Rats. *Avicenna Journal of Neuro Psych Physiology*. 2015; 2(1):e27267.
- [17] Shahidi S, Komaki A, Mahmoodi M, Atrvash N, Ghodrati M. Ascorbic acid supplementation could affect passive avoidance learning and memory in rat. *Brain Research Bulletin*. 2008; 76(1):109-13.
- [18] Baydas G, Nedzvetskii VS, Nerush PA, Kirichenko SV, Yoldas T. Altered expression of NCAM in hippocampus and cortex may underlie memory and learning deficits in rats with streptozotocin-induced diabetes mellitus. *Life Sciences*. 2003; 73(15):1907-16.
- [19] Arzi A, Hemmati A, Razian A. Effect of vitamins C and E on cognitive function in mouse. *Pharmacological Research*. 2004; 49(3):249-52.
- [20] Harrison F, Hosseini A, McDonald M, May J. Vitamin C reduces spatial learning deficits in middle-aged and very old APP/PSEN1 transgenic and wild-type mice. *Pharmacology Biochemistry and Behavior*. 2009; 93(4):443-50.
- [21] Hasanein P, Shahidi S. Effects of combined treatment with vitamins C and E on passive avoidance learning and memory in diabetic rats. *Neurobiology of Learning and Memory*. 2010; 93(4):472-8.
- [22] Kato J, Ruram AA, Singh SS, Devi SB, Devi TI, Singh WG. Lipid peroxidation and antioxidant vitamins in urolithiasis. *Indian Journal of Clinical Biochemistry*. 2007; 22(1):128-30.
- [23] Adonaylo V, Oteiza P. Pb 2+ promotes lipid oxidation and alterations in membrane physical properties. *Toxicology*. 1999; 132(1):19-32.