

## Protective effect of vitamin C, vitamin B12 and omega-3 on lead-induced memory impairment in rat

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### Abstract

Lead belongs to the heavy metal group and is considered as an environmental contaminant. Acute or chronic contact to lead can change the physiological function of human organs. One of the most important disorders following the lead exposure is neurotoxicity. Lead neurotoxicity consists of the neurobehavioral disturbances like cognitive impairment. The aim of the current study is to evaluate the possible protective effect of vitamin C (Vit C), vitamin B12 (Vit B12), omega 3 ( $\omega$ -3), or their combination on the lead-induced memory disorder. Adult wistar rats were orally administered Vit C (120 mg/kg/day) or Vit B12 (1 mg/kg/day) or  $\omega$ -3 (1000 mg/kg/day) or their combination for 3 weeks in groups of 7 animals each. Then lead acetate (15 mg/kg/day) was injected intraperitoneally for one week to all pretreated animals. The control group received normal saline as a vehicle while the positive control for cognitive impairment received just lead acetate. At the end of treatments animal memories were evaluated in Object Recognition Task. The results showed, although 15 mg/kg lead acetate significantly declines the memory-evaluating parameters, pretreatment with Vit C, Vit B12,  $\omega$ -3, or their combination considerably inverted the lead induced reduction in discrimination (d2) index ( $P < 0.001$ ) and recognition (R) index ( $P < 0.001$ ,  $P < 0.05$ ,  $P < 0.05$ , and  $P < 0.001$ , respectively). Our findings indicate while lead acetate impairs spatial memory in rat, administration of Vit C, Vit B12,  $\omega$ -3, or their combination prior to the lead exposure inhibits the lead induced cognitive loss. There was no remarkable difference in this effect between the used supplements.

**Keywords:** Vitamin C; Vitamin B12; Omega-3; Lead; Memory impairment; Object Recognition Task

### INTRODUCTION

Lead is a highly toxic heavy metal and a major environmental pollution. The lead contamination can occur from different sources such as paint, glazed ceramics, water, food containers, cigarettes, and cosmetics. It is well documented that chronic exposure to lead can cause its accumulation in the bone, muscle, liver, kidneys, hematopoietic system and the brain followed by the toxicity events. Neurotoxicity is a popular disorder from the lead exposure which involves both central and peripheral nervous system. Lead-induced neurotoxicity triggers a wide range of structural and behavioral changes in the nervous system (1,2). Cognitive impairment is

one of the most common deterioration occurs from the lead neurotoxicity (3).

Although the alteration of vital enzyme activity, receptors like glutamate, neurotransmitters like acetylcholine, calcium homeostasis, and DNA damage are considered as the mechanisms of lead toxicity (2), increase in generation of reactive oxygen species (ROS) and suppression of cell antioxidant capacity play a key role in the lead-induced structural and behavioral neurotoxicity. Suppression of cell antioxidant capacity can permanently impair the integrity of cell membrane, DNA and other macromolecules, finally causes cell apoptosis

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or cell death known as neurodegenerative effects (4). Therefore, the antioxidant agents could potentially protect the neuronal damage against to the lead toxicity (5).

There are several agents possess antioxidant property or scavenging effect of ROS. Some of the well-known antioxidant that is freely accessible for human is ascorbic acid (Vit C) and the fish oil ( $\omega$ -3). Also the vitamin B12 (Vit B12, cobalamin) is one of the most important supplements need for the normal function of neurons in the nervous system (6).

Vit C, a six carbon lactate, is a water soluble vitamin found in the dietary sources such as citrus fruits, grape fruits, berries, cabbage, tomatoes, pepper, and leafy vegetables (7).

The  $\omega$ -3 polyunsaturated fatty acid, consist of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), is mainly present in fish, shell fish, and sea mammals and also finds in land animals and plants (8,9).

Vit B12, a water soluble vitamin, exists in the egg, milk, meat, and fish. It plays a regulatory role in the CNS enzymes activity as a co-factor and causes their correct metabolic function (10).

The aim of the current study was firstly to identify the cognitive impairment of repeated lead exposure and secondly to evaluate the possible protective effect of Vit C, Vit B12,  $\omega$ -3 or their combination on the lead-induced memory disorder using Object Recognition Task (ORT).

## MATERIALS AND METHODS

### *Animals*

The experiments were carried out on male rats ( $220 \pm 20$  g) bred in School of Pharmacy animal house, Isfahan, Iran. The animals had free access to food and drinking water during the experiment and were kept at a constant room temperature ( $25 \pm 5$  °C), under a 12 h light/dark cycle.

All experimental procedures were conducted in the light phase of the cycle. All animal experiments were approved by the Ethics Committee of Isfahan University of Medical Science and performed in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals.

### *Drugs*

Lead acetate was purchased from Sigma-Aldrich Co. (USA). Vit C, Vit B12, and  $\omega$ -3 were gifts from Daroupakhsh Co. (Iran). The solutions of drugs were prepared freshly every day.

### *Apparatus and objects*

The ORT apparatus consisted of a circular arena, 83 cm in diameter and 40 cm high wall was made of white polyvinyl chloride. Two different sets of objects consist of a massive aluminum cube ( $10.0 \times 5.0 \times 7.5$  cm) and a massive aluminum cube with a tapering top ( $13.0 \times 8.0 \times 8.0$  cm) were used. Each object was available in triplicate. The objects could not be displaced by rats.

### *Drug administration*

The animals were divided in different groups each 7 rats. The following schedules were used to treat the animals: Control group: Animals were daily gavaged 3 mL distilled water for 3 weeks. Afterwards they received normal saline intraperitoneally (i.p) for 1 week. Lead acetate group: Animals were gavaged daily 3 mL distilled water for 3 weeks. Then 15 mg/kg (1) lead acetate was injected i.p for 1 week. Vit C, Vit B12, or  $\omega$ -3 groups: Animals in these groups were fed 120 mg/kg Vit C (7), 1 mg/kg Vit B12 (11), or 1000 mg/kg  $\omega$ -3 (12) by gavage for 3 weeks. After the administration of the last dose of the supplement each group was injected 15 mg/kg lead acetate i.p for 1 week. Combination group: Animals in this group received a combination of 120 mg/kg Vit C, 1 mg/kg Vit B12, and 1000 mg/kg  $\omega$ -3 orally. They were then injected i.p with lead acetate at 15 mg/kg for 1 week.

### *Experimental procedure*

During the lead administration period, the animals were transferred to the arena for procedure adaptation. For this purpose they were allowed to explore the apparatus (without any objects) twice daily for 5 min with 1 h intervals. Object recognition consisted of three clearly defined phases: a training session or first trial ( $T_1$ ), a 1-h training test interval, and a test session or second trial ( $T_2$ ). Each trial

lasted for 5 min. The experimental procedure details and the calculation of important factors of memory assessment like d2, R index and the frequency of exploration have been previously explained in details (13).

**Statistical analysis**

Data are expressed as mean ± SEM. The values of memory evaluation factors were analyzed using one way analysis of variance (ANOVA). For multiple comparisons, the Tukey’s post hoc tests were used. The Graph pad prism 4 Software was employed for statistical analysis. P-values less than 0.05 were considered statistically significant.

**RESULTS**

**Effect of lead acetate on memory indices**

The daily i.p administration of lead acetate at 15 mg/kg for one week significantly decreased both d2 (Fig. 1,  $P < 0.001$ ) and R (Fig. 2,  $P < 0.05$ ) indices in trial T2. Also treatment with lead caused a mild decrease in frequency of exploration for the new object compared with familiar one in trial T2 (Fig 3).

**Effects of Vit C, Vit B12, ω-3 or their combination on d2 indices**

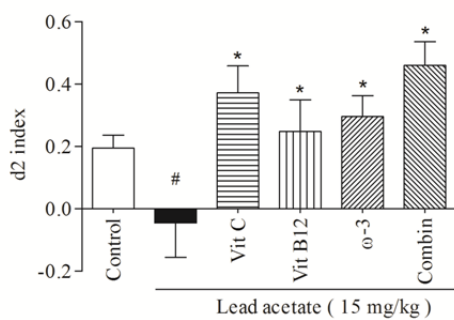
Pre-treatment with 120 mg/kg Vit C for 3 weeks before lead administration could reverse the effect of lead element on d2 indices ( $P < 0.001$ ). Administration of Vit B12 at 1 mg/kg

for 3 weeks caused a significant increase ( $P < 0.001$ ) in lead-induced d2 diminution. Also the group received ω-3 at 1000 mg/kg for 3 weeks showed significant improvement ( $P < 0.001$ ) in d2 indices compared to the lead-administered group in trial T2 (Fig. 1).

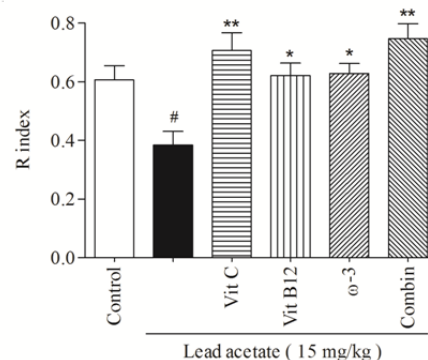
Combination of Vit C (120 mg/kg), Vit B12 (1 mg/kg), and ω-3 (1000 mg/kg) orally administered for 3 weeks led to a remarkable increase ( $P < 0.001$ ) in d2 indices compared to lead-treated animals (Fig. 1). Although different treatment, either alone or in combination, increased d2 indices in trial T2, but significant differences were not observed amongst treatments ( $P > 0.05$ ) (Fig. 1).

**Effects of Vit C, Vit B12, or ω-3 or their combination on R indices**

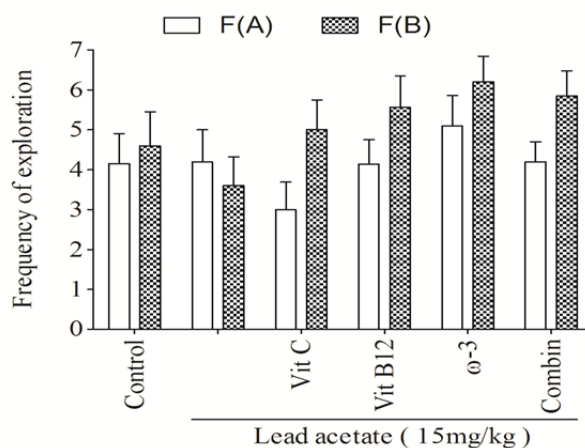
Administration of 120 mg/kg Vit C for 3 weeks before lead administration showed considerable improvement ( $P < 0.001$ ) in R indices of lead-treated animals. Pretreatment with Vit B12 at 1 mg/kg or ω-3 at 1000 mg/kg for 3 weeks showed remarkable increase ( $P < 0.05$ ) in R index compared to those of lead-administered group in trial T2. The combination of tested supplements also significantly ( $P < 0.001$ ) prevented the diminishing effect of lead on the R index (Fig. 2). Vit C, vit B12, ω-3, or their combination elevated the lead-induced reduction in R indices in trial T2; however, no significant differences were observed amongst (Fig. 2).



**Fig. 1.** The comparative effects of vit C, vit B12, or ω-3 and their combination on the d2 indices in trial T2. Data are presented as Mean ± SEM. (# $P < 0.001$ , compared to control group; \* $P < 0.001$ , compared to lead acetate group). (d2) discrimination, (Vit C) vitamin C, (Vit B12) vitamin B12, (ω-3) omega-3, (Combin) combination therapy.



**Fig. 2.** The comparative effects of vit C, vit B12, or ω-3 and their combination on the lead acetate decreased R indices in trial T2. Data are presented as Mean ± SEM. (# $P < 0.05$ , compared to control group; \* $P < 0.05$ , \*\* $P < 0.001$ , compared to lead acetate group). (R) recognition, (Vit C) vitamin C, (Vit B12) vitamin B12, (ω-3) omega-3, (Combin) combination therapy.



**Fig. 3.** The effects of Vit C, Vit B12, or  $\omega$ -3 and their combination on the frequency of exploration for old and new object in trial T2. (F(A)) Frequency of exploration for familiar object, (F(B)) Frequency of exploration for new object, (Vit C) vitamin C, (Vit B12) vitamin B12, ( $\omega$ -3) omega-3, (Combin) combination therapy.

### ***Effects of Vit C, Vit B12, or $\omega$ -3 or their combination on exploration frequencies***

Although none of the treatments affected the frequency of old or new objects exploration, but the supplements showed a different pattern in the exploration frequency between the old and new objects. Lead acetate decreased the exploration frequency of new object but all supplements, alone or in combination, increased the exploration frequency of new object compared to the familiar one (Fig. 3).

## **DISCUSSION**

During the development of industries in the world, exposure to lead element and consequently its toxicity has been increased worldwide. Central nervous system is the most important target for the lead toxicity. Lead-induced neurotoxicities include behavioral, morphological, and electrophysiological disruptions (1,14). In the present study, we evaluated the possible protective effects of Vit C, Vit B12, and  $\omega$ -3 alone or in combination, on the lead-induced memory impairment in a rat model.

Following repeated doses of lead animals showed a reduction in d2 and R indices compared to that of the control animals. d2 index indicates the differences between new and old object exploration time while the R index indicates the exploration time that animals especially spend around the new

object. This is a marker related to the memory of animal to the old object. The results showed that lead-treated group spent less time for exploration of the novel object than the familiar one in comparison with the control group. The lead also diminished the difference between exploration frequencies of the novel and old objects seen in the control group. Normally, the animals showed more exploratory behavioral in time and frequency to new object than the old one because they had a memory to the old object in trial T2. The memory destructive agent impaired the memory to old object and caused a reduction in differences between old and new object exploration. These data demonstrate that repeated exposure to lead causes destructive effects on the memory in rat.

The current results are in agreement with the large volume of experimental and clinical studies which demonstrated lead exposure impairs cognitive functions and destroys the memory and learning processes in human and animals (3,15,16). Several mechanisms have been proposed to explain the neurotoxicity of  $Pb^{2+}$ . One of the most important mechanisms in lead-induced neurotoxicity is the triggering of lipid peroxidation and oxidative stress processes by accumulation of reactive oxygen species (16-18). In addition, lead binds to the thiol groups of biologic macromolecules like glutathione and decreases their reductive potencies and antioxidant activities (19). It has been reported that the initiating of apoptosis

cascade, increasing inflammatory mediators, alteration in glutamate-induced neuroplasticity and changes in calcium homeostasis are some of other mechanisms involved in lead neurotoxicity (20-24).

The current study provided evidences that administration of 120 mg/kg Vit C, 1 mg/kg Vit B12, and 1000 mg/kg  $\omega$ -3 for three weeks before lead exposure could clearly prevent the lead-induced memory impairments. Vit C, Vit B12, and  $\omega$ -3 showed similar beneficial effects when administered in combination. Although each supplement improves the memory in the experimental groups, significant differences amongst treated groups were not observed.

The protective effect of Vit C on the lead-induced memory impairment is in agreement with the studies indicated that the Vit C could improve dementia induced by aging or anticholinergic agents in animals (7,25). It is well known that Vit C is highly concentrated in the central nervous system and possess the antioxidant properties. Indeed, Vit C scavenges the  $Pb^{2+}$  generated ROS (25-27).

There are several evidences representing Vit C anti-inflammatory effects by inhibiting the NF- $\kappa$ B, TNF- $\alpha$ , IL-1, and IL-6 (28), as well as its anti-apoptotic effect (29). With aforementioned mechanisms, Vit C protects the key pathways in the memory formation including the serotonergic, dopaminergic, and glutamatergic neurons against the lead-induced damages (30,31).

Vit B12 is actually a nerve supplement easily passing the blood brain barrier. Many studies have demonstrated that Vit B12 mediates recovery process in the nervous system and plays a protective role in the neurodegenerative disorders such as Parkinson's, multiple sclerosis, and Alzheimer's disease. Vit B12 possesses the antioxidant activity and is involved in the synthesis of phospholipids and myelin. It also shows anti-inflammatory, anti-apoptotic, and anti-necrotic effects (32-35). These mechanisms could explain the observed beneficial effects of Vit B12 in the present study.

Omega-3 fatty acids contain the most abundant polyunsaturated fatty acids that in the brain cause neuronal differentiation,

neurite growth, synapses formation, and receptor biogenesis (36). The polyunsaturated fatty acids could play the pro-oxidant role in the oxidative tensions and encounter the lipid peroxidation of neuronal cells (37). It can also decrease the inflammatory factors, suppress neuronal apoptosis as well as enhance nitric oxide generation causing improvement in cerebrovascular endothelial function (38,39). It is well-known that the polyunsaturated fatty acids modulate neural functions including neurotransmitters especially acetylcholine, membrane fluidity, ion channel, enzyme regulation, and neurotrophin gene expression (35,40,41). There are some studies consistent with our results that evidenced the preventive or ameliorating effects of polyunsaturated fatty acids in the memory impairment induced by stress, aging or different neurotoxic agents (8,40-42).

## CONCLUSION

In summary, the present study provided evidences that different neuro-influence agents, Vit C, Vit B12, and  $\omega$ -3 prevent the memory impairment induced by lead element. The antioxidant, anti-inflammatory, and anti-neurodegeneration properties of these supplements could be supposed as their main mechanism of action in the reversing of lead-induced memory losses.

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