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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (16): 2704-2708, 2007
ISSN 1028-8880

Comparison of the Effects of Dose-dependent Zinc Chloride on Short-term and Long-term Memory in Young Male Rats

[1]A.A. Moazedi, [1]Z. Ghotbeddin and [2]G.H. Parham
[1]Department of Biology, School of Science
[2]Department of Statistic, School of Mathematic, Shahid Chamran University, Ahwaz, Iran

Abstract: The aim of the present study was to evaluate the effects of dose-dependent of zinc chloride on short-term and long-term memory in a shuttle box. Young Wistar rats (94±10 g) (age 27-30 days) consumed zinc chloride drinking water in five different doses (20, 30, 50, 70 and 100 mg $kg^{-1}day^{-1}$) for two weeks by gavage. After 14 days on experimental diets, a shuttle box used to test short- and long-term memory. Two criteria considering for behavioral test, including latency in entering dark chamber and time spent in the dark chamber. This experiment shows that after 2 weeks oral administration of $ZnCl_2$ with (20, 30 and 50 mg kg^{-1} day^{-1}) doses, the rat's working (short-term) has been improved ($p<0.05$). Whereas $ZnCl_2$ with 30 mg kg^{-1} day^{-1} dose has been more effected than other doses ($p<0.001$). But rat which received $ZnCl_2$ with 100 mg kg^{-1} day^{-1}, has been shown significant impairment in working memory ($p<0.05$) and there was no significant difference in reference (long-term) memory for any of groups. In general, this study has demonstrated that zinc chloride consumption with 30 mg kg^{-1} day^{-1} dose for two weeks was more effective than other doses on short-term memory. But consumption of $ZnCl_2$ with 100 mg kg^{-1} day^{-1} dose for two week had the negative effect on short-term memory. On the other hand, zinc supplementation did not have an effect on long-term memory.

Key words: Zinc chloride, short-term and long-term memory, rat

INTRODUCTION

The effect of zinc and their supplementation on learning and memory and cognitive behavior is controversial. So in this research we want to see the effects of different doses of zinc chloride on the short-term and long-term memory in young male rats. Several studies have suggested a functional role for zinc in the mental function. For instance, zinc supplementation improves cognitive behavior in school-age children (Penland *et al*., 1997; Maureen *et al*., 2004). Zinc has known to be an essential element for more than a hundred years. To date more than 300 zinc dependent enzymes have been identified in all the main biochemical pathways. It acts uniquely as a lewis acid catalyst (an electron acceptor) in all life processes. This metal has been an essential component of both DNA and RNA polymerases. It is also vital for normal brain development, particularly concerning the hippocampal function (Takeda *et al*., 2005, 2004, 2000). Furthermore, as zinc is the most important trace metal in subcellular DNA and RNA fractions, this will also explain its vital role in the neuronal maturation and proliferation (Takeda *et al*., 2005, 2004; Pfeiffer and Braveman, 2000). At the molecular level, zinc is unevenly distributed in the brain with the highest concentration in the olfactory bulb and hippocampus (Ono and Cherian, 1999). In the hippocampus region, zinc participates in neurotransmission. Glutamatergic vesicles in the mossy fiber region contain ionic zinc, which is coreleased with glutamate when the neurons are stimulated (Pang and Wang, 1999). The hippocampal mossy fiber system forms a dense plexus of terminal on apical dendrites of CA3 pyramidal neurons and is involved in the modulation of processes related to spatial learning and memory (Tommie, 2000). Zinc is present in most food, but meat and fish provide the best source, as bioavailability of zinc from animal products is considered to be far greater than from plant foods. It is because of plant foods contain high phytic acid and fiber content. Furthermore, such every day dietary stables as coffee and milk products have been shown to reduce the bioavailability of zinc in human, because both iron and calcium have been found to interfere with zinc absorption (Tuula, 1995). In vitro studies indicate that the GABA (γ-amino butyric acid) and NMDA (N-methyl-D-aspartate) receptor, which participate in memory formation, are modulated by zinc (Patenaude *et al*., 2002; Li *et al*., 2001; Chu *et al*., 2003). Many investigators have evaluated the association

Corresponding Author: Moazedi Ahmad Ali, Department of Biology, School of Science, Shahid Chamran University, Postal Code 65355-141, Ahvaz, Iran Tel: 0098-611-3363340 Fax: 0098-611-3331045

between fetal zinc nutritious and brain development in early life and established a negative effect of prenatal zinc deficiency on the brain function of experimental animals. Adverse consequences include reduced activity and responsiveness; impaired learning ability, attention and memory (Tuula, 1995).

Because of zinc is an essential dietary element associated with cognition and deficiency of this vital element is able to impair seriously in brain size, total brain count and learning and memory function, so the present study was conducted to test the effects of different doses of zinc supplementation on short-term and long-term memory in young male rats and find out the effective and adverse dose of this vital element on cognitive behaviors.

MATERIALS AND METHODS

Animals: Were 60 male Wistar rats (94±10), 27-31 days old, were individually housed in stainless cages at a temperature of 23±2°C and a 12/12 h cycle: 7:00 am light on, 7:00 pm light off. All animals were provided from Ahvaz University of Medical Science animal house. Then they were divided into six groups. One group was control group with free access to food and water and five groups drank zinc chloride in different doses (20, 30, 50, 70, 100 mg kg^{-1} day^{-1}) by gavage methods for two weeks. The amount of time required for this study was 6 months. All experiments were performed in the laboratory of learning and memory in biology department of Shahid Chamran University in Iran.

Apparatus: The apparatus used for passive avoidance response training was shuttle box, which consisted of two adjacent Plexiglas compartments of identical dimensions (27×14.5×14) cm. Two compartments separated by a sliding door in the middle part of this apparatus. Of the two compartments, one is illuminated and the other is dark. A sliding door separated the two compartments and could be lowered to form a 2.5 cm hurdle. The floor consisted of 6 mm diameter stainless-steel rods spaced 1.7 cm between centers. The rods were connected to shock generator which could deliver to either compartment a scrambled foot shock, a flashing light (7.5 W) was fixed to the outside wall of the white chamber.

Procedure: The first day (Acquisition) rats had free access to either the light or dark compartment of the box, on the second day (Training) rat was placed in the illuminated compartment and 30 sec later the sliding door was raised. Upon entering the dark compartment the door was closed and a 1.5 mA constant-current shock was applied for 2 sec after 20 sec the rat was removed from the dark compartment and placed into home cage. For testing short-term and long-term memory, 48 h (two days) and one month (30 days) after passive avoidance response training, the rat was placed in illuminated chamber and 30 sec later the sliding door was raised and the latency of entering the dark compartment (step-through latency) and the time spent there during 5 min was recorded because the maximum time that considered for this procedure were 300 sec (5 min). (Arlene *et al*., 1997; Takeda *et al*., 2005).

Data analysis: Statistical analysis of data using one way analysis of variance (ANOVA). Post hoc analysis using the Least Significant Difference (LSD). The decision criterion for statistical tests of significance was $p<0.05$.

RESULTS

Our data shows that in step-through latencies 48 hour after training were significant difference between rats received 20 mg kg^{-1} day^{-1} $ZnCl_2$ and control group ($p<0.05$). Furthermore in this step there was significant difference between rats received (30, 50 mg kg^{-1} day^{-1}) $ZnCl_2$ with control group as respectively shown ($p<0.001$; $p<0.01$). There were no significant differences between control group and rats which received (70, 100 mg kg^{-1} day^{-1}) (Fig. 1). But 30 days after training there was no significant difference between control group and any of groups which received zinc chloride in different doses (Fig. 2).

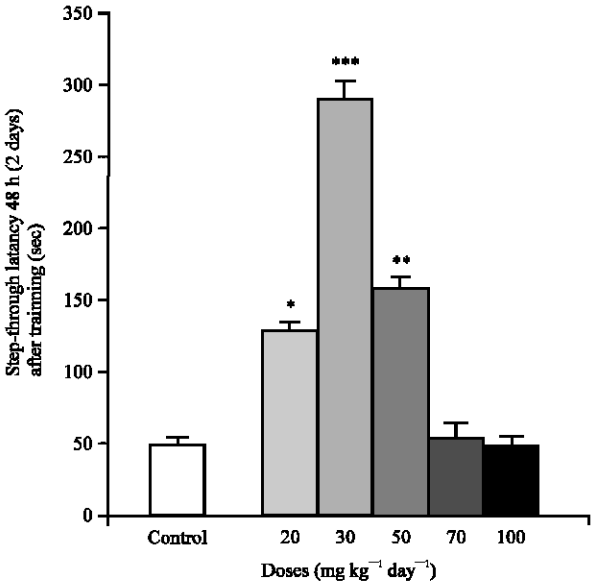

Fig. 1: Effect of zinc chloride on step-through latency 48 h (2 days) after training, *$p<0.05$, n = 10, **$p<0.01$, ***$p<0.001$

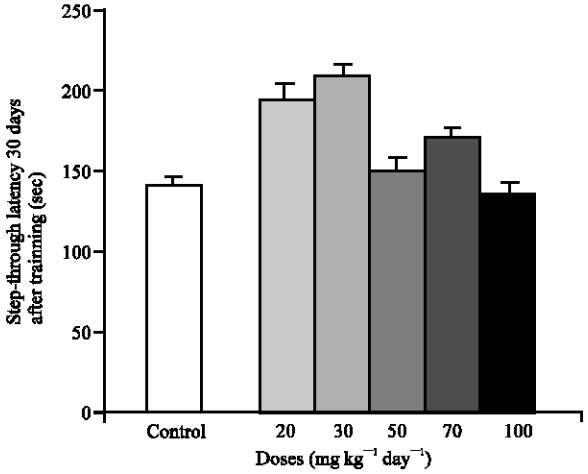

Fig. 2: Effect of zinc chloride on step-through latency one month (30 days) after training, n = 10

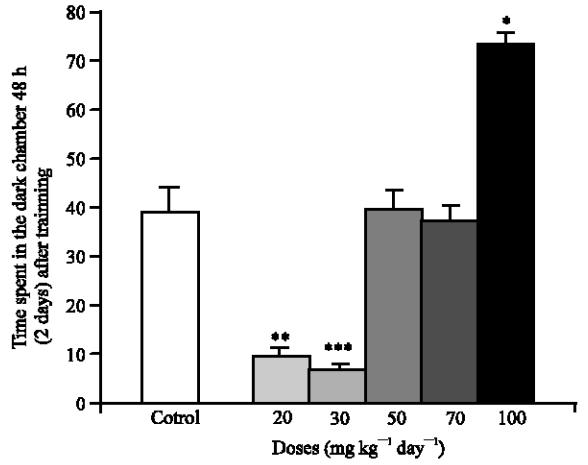

Fig. 3: Effect of zinc chloride on time spent in the dark chamber 48 h (2 day) after training, *p<0.05, n = 10, **p<0.01, ***p<0.001

On the other hand, statistical analysis of data in the time spent in the dark chamber 48 h after training, showed significant difference between rats received 20 mg kg^{-1} day^{-1} $ZnCl_2$ and control group ($p<0.01$) and rats received 30 mg kg^{-1} day^{-1} Zn Cl_2, showed significant difference with control group ($p<0.001$). But in this step, there were no significant difference between control group and rats which received (50, 70 mg kg^{-1} day^{-1}) and significant difference between rats received 100 mg kg^{-1} day^{-1} $ZnCl_2$ and control group ($p<0.05$) (Fig. 3). But 30 days after training, there was no significant difference between control group and any of groups which received zinc chloride in different doses (Fig. 4).

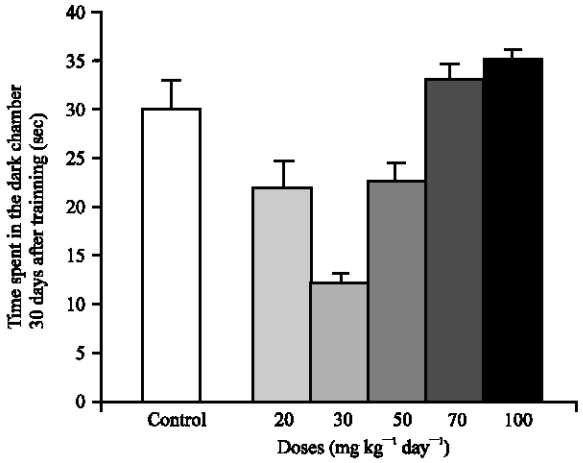

Fig. 4: Effect of zinc chloride on time spent in the dark chamber one month (30 days) after training

DISCUSSION

The effect of zinc chloride on learning task and memory is controversial. Furthermore, there are different reports about the effect of this essential nutrient, on cognitive behavior and memory, which pointed to some of them. In the present study, young rats (aged 27-30 days) consumed zinc chloride drinking water in five different doses (20, 30, 50, 70 and 100 mg kg^{-1} day^{-1}) for two weeks by gavage. After two weeks of oral administration, the passive avoidance learning tested by shuttle box. The result of this research shows, zinc consumption 30 mg kg^{-1} day^{-1} for two weeks, could have be effective on working memory in rats than other doses (Fig. 1-3) ($p<0.001$). This result is as similar as the result of many other investigators. For instance, as shown by Takeda *et al.* (2000), a deficiency of this nutrient in animals resulted in malformations and abnormal development and functioning of the central nervous system of the offspring. In another article, this scientist reported that zinc defiance in both humans and animals lead to impairment on passive avoidance learning, this result confirmed present results in this experiment. Furthermore, Kelleher *et al.* (2005) had shown, regulation of NMDA receptor (which is an important receptor in learning tasks and that named learning channel) was controlled by zinc and zinc deficiency can impair learning and memory later in life may be by reducing NMDA receptors; however, effects of zinc deficiency on the regulation of NMDA receptor activity are not well understood. As reported from Eugenio and other scientist, zinc is transported from extral cellular compartments into the neuronal and glial cells mainly via zinc transporters and transferred to various cellular

components to regulate some biological functions including the activity of transcriptional factors involved in the in the oxidative stress response and DNA repair. In glutamatergic neurons, zinc can also be accumulated into synaptic vesicles and released; into order to modulate directly NMDA and GABA receptors (Eugenio *et al*., 2005; Brown and Dyck, 2005; Li *et al*., 2004), both of these receptors are essential for memory functions. On the other hand, an intriguing aspect related to intracellular zinc ion availability in the Long-Term Potentiation (LTP) is a form of synaptic plasticity, which is implicated as a cellular mechanism subservient learning and memory (Baskys *et al*., 2000). The LTP induction, at the mossy fiber-CA3 synapses, is regulated by the release of zinc and by the subsequent entry of zinc into postsynaptic neurons (Li *et al*., 2001; Eugenio *et al*., 2005). Zinc deficiency may damage the learning-memory ability of the rats; the effects might be related to the low of CCK and NOS positive neurons in hippocampal CA1 and CA3 area in zinc deficiency rats (Li *et al*., 2004). Oxidative stress is associated with the development and progression of several different neuropathologies, including Alzheimer's disease and Parkinson's disease. Zinc is maintaining the integrity of the Blood Brain Barrier (BBB) by excluding toxic agents such as aluminum and other foreign compounds. Alterations or dysfunction of the BBB have been observed in many brain disorders. Zinc protects the BBB against oxidative stress through its antioxidant properties and in so doing, helps to maintain homeostasis within the brain and prevent the development of neurological disorders (Karen and Julie, 2001). But some researches indicate that removing zinc from synaptic vesicles doesn't impair spatial learning, memory, or sensorimotor functions in the mouse. The neuromodulatory effects of zinc are not relevant for the tasks tested, or the mice are able to compensate easily for the absence of synaptic vesicle zinc (Toby *et al*., 2001). On the other hand, some scientists had shown that enhanced zinc consumption causes memory deficits and increased brain levels of zinc, because the influx of toxic amounts of zinc from pre-synaptic vesicles into post-synaptic degeneration neurons seems to be mainly responsible for the neurodegenerative process (Tonder *et al*., 1990). Many scientists in their investigation have shown that zinc doesn't affect in long-term memory. That confirms our results in this research with 100 mg kg^{-1} day^{-1} zinc chloride consumption (Edwars *et al*., 2000; Flinn *et al*., 2005). In other research Tommie *et al*. had shown that, high doses of zinc chloride lead to decline in spatial memory in rats and this decline may be partly explained by an alteration in brain dopamine D1 binding kinetics.

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