

EFFECT OF ZINC SUPPLEMENTATION OF PREGNANT RATS ON SHORT-TERM & LONG-TERM MEMORY OF THEIR OFFSPRING

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ABSTRACT

Objective: To see the dose dependent effects of zinc chloride on the short-term and long-term memory in a shuttle box (rats).

Methodology: Six pair adult wistar rats were taken for this experiment. One group of pregnant rats received a daily oral dose of 20mg/kg Zn as zinc chloride and the remaining groups received a daily oral dose of (30, 50, 70, 100mg/kg) zinc chloride for two weeks by gavage. One month after birth, a shuttle box was used to test short-term and long-term memory. Two criteria were considered to behavioral test, including latency in entering dark chamber and time spent in the dark chamber.

Result: This experiment showed that oral administration of ZnCl₂ with (20, 30, 50 mg/kg/day) doses after 2 weeks at the stage of pregnancy, can improve the working memory of their offspring (p<0.05). Where as ZnCl₂ with 30mg/kg/day dose has been more effective than other doses (p<0.001). But rat which received ZnCl₂ with 100mg/kg/day at the stage of pregnancy, has shown significant impairment in working (short-term) memory of their offspring (p<0.05) and there was no significant difference in reference (long-term) memory 3 for any of groups.

Conclusion: This study has demonstrated that zinc chloride consumption with 30mg/kg/day dose for two weeks at the stage of pregnancy in rats, has positive effect on short-term memory on their offspring. But consumption of enhanced zinc 100mg/kg/day in pregnant rats can cause short-term memory impairment. On the other hand, zinc supplementation such as zinc chloride has no effect on long-term memory.

KEY WORDS: Zinc, Gestation, Offspring, Memory, Rat.

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INTRODUCTION

Several studies have suggested a functional role for zinc in the mental function. For instance, zinc supplementation improves cognitive behavior in school-age Children.¹⁻² Zinc is one of the most abundant oligoelements in the living cell. It appears tightly bound to metallothioneins, loosely bound to some metalloproteins and nucleic acids, or even as free ion. Small amounts of zinc ions (in the nanomolar range) regulate a plentitude of enzymatic proteins, receptors, and transcription factors; thus, cells need accurate homeostasis of zinc ions. It is also vital for normal brain development, particularly concerning the hippocampal function.^{3,4} In fact, it has been

established, using both animal experiments and human studies, that inadequate parental zinc status can result in a variety of deleterious effects on the offspring. The most obvious connection between clinical or sub-clinical zinc deficiency and fetal teratology seems to⁴ relate directly to its central role in all processes concerning cell differentiation and replication.

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 plant foods contain high phytic acid and fibre content. Also, every day dietary staples 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.⁵ Studies with pregnant rats has found, that zinc deficiency will lead the offspring to be born with gross congenital malformations encompassing every organ system of the body. In addition, inadequate zinc during prenatal period had been particularly linked with low birth weight.⁵ Zinc ions are essential in the brain tubulin phosphorylation and in the induction of tubulin to form transport sheets, as well as in increasing the number of neurofilaments. 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.⁶ At the molecular level, zinc is unevenly distributed in the brain with the highest concentration in the olfactory bulb and hippocampus.^{3,7} In the hippocampal region, zinc participates in neurotransmission. Glutamatergic vesicles in the mossy fiber region contain ionic zinc, which is released with glutamate when the neurons are stimulated.³ In vitro studies indicate that the GABA (α -amino butyric acid) and NMDA (N-methyl-D-aspartate) receptor, which participate in memory formation, are modulated by zinc.⁸⁻¹⁰ Many investigators have evaluated the association 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.⁴

Because zinc is an essential dietary element associated with cognition and deficiency of this vital element at the stage of gestation it is able to impair seriously the fetal growth, brain size, total brain count, learning and memory dysfunction, As such the present study was conducted to test the effects of different doses of zinc supplementation of pregnant rats on learning, short & long-term memory of their offspring and find out the effective and adverse dose of this vital element on cognitive behaviors.

MATERIALS AND METHODS

Animals: Six pair adult wistar rats were taken and each pair was individually housed in stainless cages at the temperature of (23 ± 2) °C, and a 12-h light/dark cycle: 7:00 a.m. light on, 7:00 p.m. light off.³ All animals were provided from Ahwaz University of Medical Science animal house in Iran. Then pregnant rats were separated from male rats and they were divided into six groups. One group was control group with free access to food and water, and five groups drank zinc 6 chloride in different doses (20, 30, 50, 70, 100mg/kg/day) for two weeks by gavage methods at the stage of pregnancy. The amount of time required for this study was 8 months.

Apparatus: The apparatus used for passive avoidance response training of their offspring was shuttle box that consisted of two adjacent Plexiglas compartment of identical dimensions (27*14.5*14)cm. Two compartments were separated by a guillotine 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 2.5cm hurdle. The floor consisted of 6mm diameter stainless-steel rods spaced 1.7cm between centers. The rods were connected to shock generator which could deliver to either compartment a scrambled foot shock, a flashing light (7.5W) was fixed to the outside wall of the white chamber.

Procedure: Day one (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 second later the guillotine door was raised. Upon entering the dark compartment the door was closed and a 1.5mA constant current shock was applied for 2 second, after 20 second the rat was removed from the dark compartment and placed in to home cage. For testing short-term and longterm memory, 48 hour (two days) after passive avoidance response training, the rat was placed in illuminated chamber and 30 second later the guillotine door was raised and the latency of entering the dark compartment (step-through latency)⁷ and the time spent there during 5 minutes was recorded.^{7,11} We also did this procedure 30 days after passive avoidance response training, for testing long-term memory. *Data Analysis:* Data were analyzed by one way analysis of variance (ANOVA) followed by post hoc test. The level of significance was set at $p < 0/05$.

RESULTS

Our data shows that in step-through latencies 48 hour after training shows significant difference between rats where mothers received Zncl₂ (20mg/kg/day) at the stage of pregnancy and control group ($P < 0.05$). In addition, in this step there was significant difference between rats where mothers received Zncl₂ (30mg/kg/day) at the stage of gestation and control group ($P < 0.001$) and also between rats where mothers received Zncl₂ (50mg/kg/day) and control group ($P < 0.01$). There were no significant differences between control group and rats where mothers received (70,100mg/kg/day) (Figure-1). But 30 days after training there was no significant difference between control group and any of groups where mothers received zinc chloride in different doses at the stage of gestation (Figure-2).

On the other hand, statistical analysis of data in the time spent in the dark chamber, shows significant difference between rats where mothers received Zncl₂ (20mg/kg/day) at the

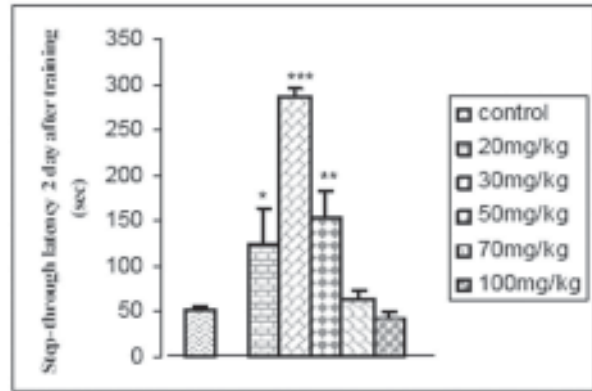


Fig-1: Effect of zinc chloride on step-through latency 2 day (48 hour) after training
* $P < 0.05$ n = 10 ** $P < 0.01$ *** $P < 0.001$

stage of pregnancy and control group ($P < 0.01$) 48 hour after training, and rats where mothers received (30mg/kg/day) Zncl₂, shows significant difference with control group ($P < 0.001$). But in this step, there was no significant difference between control group and rats which their mothers received (50, 70mg/kg/day) zinc at the stage of gestation, where as significant difference between rats which their mothers received (100mg/kg/day) Zncl₂ and control group ($P < 0.05$) (Figure-3). But 30 days after training, there was no significant difference between control group and any of groups where mothers received zinc chloride in different doses at the stage of gestation (Figure-4).

DISCUSSION

The effect of zinc chloride on learning task and memory is controversial. Also, there are

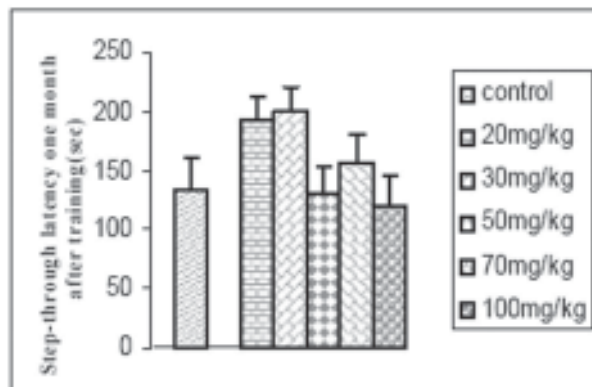


Fig-2: Effect of zinc chloride on step-through latency one month after training n = 10

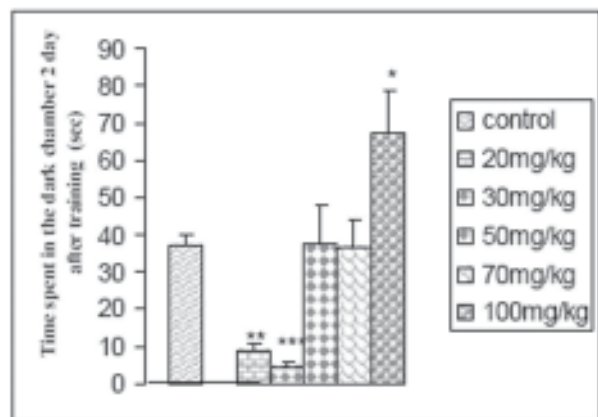


Fig-3: Effect of zinc chloride on time spent in the dark chamber 2 day (48 hour) after training

* P<0.05 n = 10 ** P<0.01 ***P<0.001

different reports about the effect of this essential nutrient, on cognitive behavior and memory, that refer to some of them. In the present study, 6 groups of pregnant rats consumed zinc chloride drinking water in five different doses (20, 30, 50, 70, 100mg/kg/day) for two weeks by gavage at the stage of pregnancy. One month after birth, a shuttle box was used to test short-term & long-term memory of their offspring. The result of this research shows, zinc consumption (30mg/kg/day) for two weeks at the stage of pregnancy, could be more effective on working memory in their offspring than other doses. (figure-1,3) (P<0.001). This result is similar to the result of many other investigators. For instance, Takeda and et al.⁷ have shown that, 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 study, this scientist has reported that zinc deficiency in both humans and animals lead to impairment on passive avoidance learning,⁷ this result confirmed our results in this experiment. Also, Kelleher et al.¹⁰ have 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

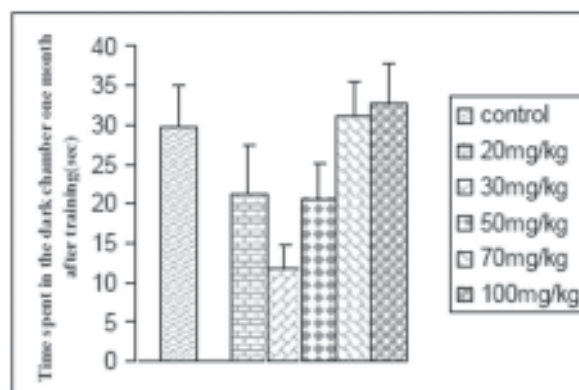


Fig-4: Effect of zinc chloride on time spent in the dark chamber one month after training n = 10

reported from Eugenio and other scientist, zinc is transported from extra cellular compartments in to 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 oxidative stress response and DNA repair.¹² On the other hand, an intriguing aspect related to intracellular zinc ion availability in the long-term potentiation (LTP) a form of synaptic plasticity, implicated as a cellular mechanism subserving learning and memory. The LTP induction, at the mossy fiber-CA3 synapses, is regulated by the release of zinc and by the subsequent entry of zinc in to postsynaptic neurons.^{10,13} In glutamatergic neurons, zinc can also be accumulated in to synaptic vesicles and released, in order to modulate directly NMDA and GABA receptors,^{13,16} both of these receptors are essential for memory functions. Li et al. have reported that, the number of CCK and NOS positive neurons in hippocampal CA1 and CA3 area of zinc deficiency rats were significantly decreased. So, 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.¹⁷ Oxidative stress is associated with the development and progression of several different neuropathologies, including Alzheimer's disease and Parkinson's disease.¹⁸ Zinc is maintaining the integrity of the blood brain barrier (BBB) by 13 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.⁷ 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.¹⁹ On the other hand, some scientists have 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 in to post-synaptic degeneration neurons seem to be mainly responsible for the neurodegenerative process.²⁰⁻²²

Many scientists in their investigations have shown that zinc doesn't effect in long-term memory. That confirms our result in this research with 100mg/kg/day zinc chloride consumption.^{23,24} We hope to get accurate result by conducting extensive research into the function mechanism of this element on central nervous system.¹⁴

REFERENCES

1. Penland JG, Sandstead HH, Alcock NW. A preliminary report: effect of zinc and micronutrient repletion on growth & neuropsychological function of urban Chinese children. *J Am Coll Nutr* 1997;16:268-72.
2. Tamura T, Goldenberg RL, Ramey S. Effect of zinc supplementation of pregnant women on the mental and psychomotor development of their children at 5y of age. *J Am Coll Nutr* 2003;6:1512-16.
3. Chu Y, Mouat MF, Grider A. Water maze performance and changes in serum corticosterone levels in zinc-deprived and pair-fed rats. *J Physiol Behav* 2003;78:569-78.
4. Maureen M, Sazawal S, Robert E. Cognitive and Motor Development among Small-For-Gestational-Age Infants: Impact of zinc supplementation Birth Weight, & caregiving practices. *J Pediatric* 2006;113:1297-1305.
5. Tuula E. The adverse effects of zinc deficiency. *J Ortho Mol Med* 1995;10:149-64.
6. Pfeiffer C, Braveman E. Zinc, brain and behavior. *J Biol Psychiatry* 2000;17:513-32.
7. Takeda A, Tamano H, Tochigi M. Zinc homeostasis in the hippocampus of zinc-deficient young adult rats. *J Neuro Chem Int* 2005;46:221-25.
8. Cohen E, Lee W, Nelson N. Zinc inhibition of gamma-aminobutyric acid transporter 4 (GABA) reveals a link between excitatory and inhibitory neurotransmission. *J Proc Natl Acad Sci USA* 2005;24:6154-59.
9. Li Y, Hough CJ, Frederickson CJ. Induction of mossy fiber'!ca3 longterm potentiation requires translocation of synaptically released zinc. *J Neuro Sci* 2001;21:8015-25.
10. Kelleher SL, Chowanadisai W, Lonnerdal B. Maternal zinc deficiency reduces NMDA receptor expression in neonatal rat brain, which persist in to early adulthood. *J Neuro Chem* 2005;94:509-10.
11. Arlene C, Rodriguez WA, Wright TP. Time-dependent effects of fructose on the modulation of reactivated memory. *Prog Neuro Psymemory* 1997;21:649-65.
12. Guojun L, Long-Lian Z, Ling L. Occupational exposure to welding fume among welders: Alteration of manganese, iron, zinc, copper, and lead in body fluids and the oxidative stress status. *J Occup Environ Med* 2004;46:241-48.
13. Eugenio M, Carlo BF, Fiorella M. Brain, aging and neurodegeneration: Role of zinc ion availability. *J Prog Neurobiol* 2005;75:367-90.
14. Baskys A, Reynolds JN, Carlen PL. NMDA depolarizations and longterm potentiation are reduced in the aged rat neocortex. *Brain Res* 2000;530:142-46.
15. Takeda A, Minami A, Seki Y. Differential effects of zinc on glutamatergic and GABAergic neurotransmitter systems in the hippocampus under zinc deficiency. *J NeuroSci Res* 2004;75:225-29.
16. Brown CE, Dyck RH. Modulation of synaptic zinc in barrel cortex by whisker stimulation. *J NeuroSci* 2005;134:355-9.
17. Li J, Yan B, Zhao X. Changes of cholecystokinin and nitric oxide synthase positive neurons of hippocampus in zinc-deficiency rat. *J Hygiene Res* 2004;33:559-61.
18. Braak H, Rub U, Schultz C. Vulnerability of cortical neurons to Alzheimer's and Parkinson's disease. *J of Alzheimer's disease* 2006;9:35-44
19. Toby B, Cole A, Richrd D. Removing zinc from synaptic vesicles does not impair spatial learning, memory, or sensorimotor functions in the mouse. *J Brain Res* 2001;891:253-65.
20. Christopher DS, Viles JH. Solution HNMR investigation of zinc and cadmium binding to amyloid-beta peptide (A β) OF Alzheimer's disease. *J Biochem Biophys* 2005;22:11-40.
21. Chrosniak LD, Smith LN, McDonald CG. Effects of enhanced zinc and copper in drinking water on spatial memory and fear conditioning. *J Geochemical Exploration* 2006;88:91-94.
22. Flinn JM, Hunter D, Linkous DH. Enhanced zinc consumption causes memory deficits and increased brain levels of zinc. *J Physiol Behav* 2005;83:793-803.
23. Edward S, Marilou J, Curtiss D. Learning and memory disabilities in young adult rats from mildly zinc deficient dams. *J Physiol Behav* 2000;37:451-58.
24. Halas ES, Dier MA, Sandstead HH. Learning and memory impairment in adult 381. rats due to severe zinc deficiency during actation. *J Physiol Behav* 2003;30:371-381.