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Alteration of neuropharmacological behavior by elemental zinc in healthy adult mice

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ABSTRACT: The role of elemental zinc has been documented for different types of disorder. The present study's objective was to evaluate zinc supplements (zinc sulphate heptahydrate) effect on neurological behavior in mice models. The study was conducted on mice for 28 days. Effect on neurological behaviors was assessed by conducting various behavioral tests, including Elevated plus maze (EPM) test, Hole-board test, Hole cross test, and Open field test. After the experimental period, the zinc supplement group spent less time in the open arm ($p<0.05$), indicating an anxiety state. This result was supported by a reduced level of head dipping activity ($p<0.05$) in the elevated plus maze test. Again, the mice in the supplemented group showed an increased number of movements between the holes ($p<0.05$) in the hole-board test followed by the reduced number of squares crossed ($p<0.05$) in the open field test compared to the control group. These findings support the notion of anxiety state, lack of neophilia or decreased locomotion activity, or less sedation. Based on the present study's findings, it can be concluded that all the behavioral tests indicate that regular treatment with zinc may show an impaired effect on the brain leading to mood and behavioral disorders by interfering with the zinc regulations homeostasis. So, zinc mineral should be supplemented to users only having specific zinc-deficit disease conditions.

KEYWORDS: Zinc; central nervous system (CNS); locomotion; neophilia; anxiety.

1. INTRODUCTION

Zinc (Zn), being a member of essential trace elements, plays a critical role in different biological processes such as cellular metabolism and immunological response [1,2]. Zinc's altered level has been documented for pathophysiological changes in many diseases like liver functions, diabetes, etc. [3-5]. Besides, about 1.5% of the total zinc in the human body is present in the brain. Almost 10% of total brain zinc exists in the synaptic vesicle. Zinc may be released from synaptic vesicle on excitation and play a role in synaptic signaling modulation [6]. Although several roles of zinc associated with neuronal functions have been studied, but it remains ambiguous. Different studies suggest that dietary zinc deficiency may induce learning and memory impairment that ultimately contributes to developing a state of anxiety and other behavioral disorders [7-10]. Some studies have also reported that zinc supplements may have adverse effects on the central nervous system and cause memory deficit, anxiety, and mood disorders [11-13]. Moreover, there is little information about zinc supplementation on neurological activity in a healthy volunteer.

The central nervous system's activity evaluates the living body's external behavior, including humans and animals. The evaluation of animal models' neurological behavior is based on the assumption that behavioral changes in animals are comparable to behavioral changes in humans. The neuro-pharmacological screening includes various tests to depict the central nervous system's functions and behavior, including sensory and motor function, emotional responses, autonomic reflexes, and cognition [14]. Considering all the things, we aimed to study zinc supplementation in neurological behavior using healthy adult mice to get more information.

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2. RESULTS

2.1. Results of the elevated plus maze test

The effect of zinc supplementation on mice's neurological behavior in the elevated plus maze test is shown in table 1. The elevated plus maze test, duration spent, and many open and closed arm entries were significantly different between groups. Time spent in the open arm for the zinc-supplemented group was 64.2± 12.85 s, whereas mice of the control group spent 123.8 ± 8.24 s, significantly higher ($p<0.05$). Again, the number of entries in the open arm for the zinc-supplemented group (5.6 ± 1.152) was also significantly ($p<0.05$) less compared to the control group (12.8 ± 0.335). Time spent in the closed arm for the zinc-supplemented group (230.2 ± 11.24 s) was more ($p<0.05$) compared to the control group (170 ± 5.70 s).

Table 1. Effect of zinc supplementation in elevated plus maze test.

Test Group	Foods	Time spent in open arm (s)	No of entries in open arm (s)	Time spent in closed arm (s)
Control	Only Normal feed (daily)	123.8 ± 8.24	12.8 ± 0.335	170 ± 5.70
Zinc Supplement	6.5 mg/kg elemental Zinc+ Normal feed (daily)	64.2± 12.85	5.6 ± 1.152	230.2 ± 11.24
p value		0.002*	0.001*	0.001*

[a] Values are presented as mean± SEM, where n=5. *p< 0.05 vs control (student's t test).

2.2. Results of the hole-board test

The effect of zinc supplementation on the head dipping activity in the hole-board test is presented in Figure 1. In the hole-board test, the values of the mean head dipping counts were different between groups. There seems a significant ($p<0.05$) reduction of head dipping activity of the zinc supplemented group (11.8 ± 4.35) compared to that of the control group (48.6 ± 4.58).

2.3. Results of the hole cross test

The neurological behavior of mice in the hole cross test is given in Figure 2. There is a significant variation in the number of movements between holes among the groups. The zinc supplemented group shows a significantly ($p< 0.05$) increased number of moves between holes (23.6 ± 4.39) compared to the control group (12.6 ± 4.12).

2.4. Results of the open field test

Figure 3 summarizes the effect of zinc supplementation on neurological behavior in the open field test. In this test, the number of movements or squares crossed was different among the groups. There were a significantly ($p <0.05$) reduced number of moves or square crossed by mice on zinc supplementation (44.8 ± 6.39) compared to that of the control group (67.4 ± 5.44).

3. DISCUSSION

Zinc is an essential multi-functional micro mineral that plays many pivotal functions in the human body and present in most of the body's cells. Recently we described the effect of zinc sulphate supplementation on serum lipid profile, oxidative stress, glucose homeostasis and hepatic function in adult healthy mice [15]. Zinc is mainly bound to proteins where it can facilitate the catalytic activity of enzymes. A significantly increased amount of zinc in the central nervous system (CNS) plays a crucial role in synaptic transmission, processing memory, and cognition. The critical step in examining drug action on the central nervous system is to observe the behavioral changes or locomotor, exploratory activity of animals. In the present study, neurological behavior tests are conducted to study zinc supplementation on locomotion or exploratory movement of healthy adult animals after the experimental period of 4 weeks. An equivalent dose of adult human tolerable upper limit of zinc (40 mg/day) was used in this study. Human dose was converted to mice equivalent dose according to the USFDA guideline, 2005 [16].

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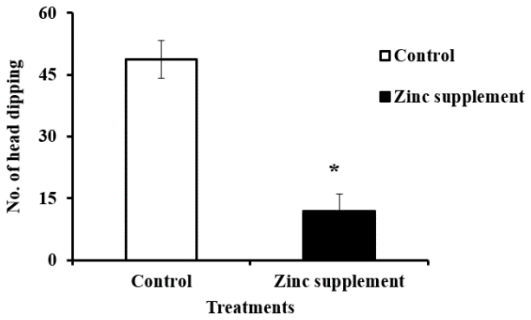

Figure 1. Head dipping activities in hole-board test. Values are presented as mean± SEM, where n=5. *p<0.05, versus Control (Student's t test).

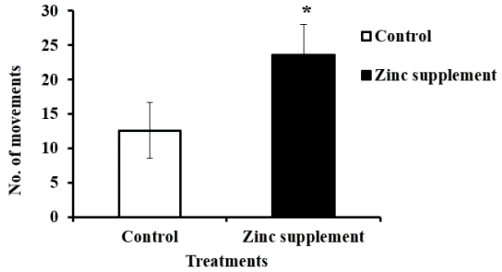

Figure 2. The number of movements between holes in hole cross test. Values are presented as mean± SEM, where n=5. *p< 0.05 versus control (Student's t test).

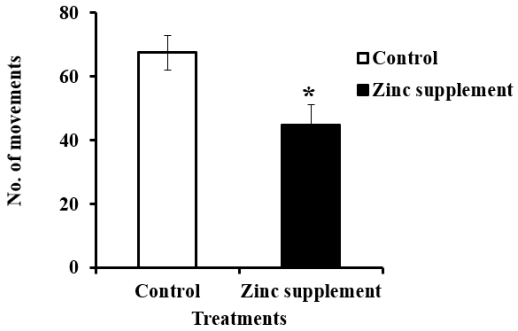

Figure 3. The number of movements in the open field test. Values are presented as mean± SEM, where n=5. * p< 0.05 versus control (student's t test).

The elevated plus maze test is based on the observation that rodents tend to stay in the known or safe place and avoid elevated areas or open places. The avoidance of this open arm behavior is interpreted as anxiety [17]. In this test, the zinc supplemented group's mice tend to avoid the open arm more, and the number of entries in the open arm was also less compared to the control group. The above data represent the high anxiety behavior of mice of the zinc supplemented group compared to that of the control group.

Again, in the hole-board test, high levels of head dipping behavior are assumed to indicate neophilia. In contrast, low levels are supposed to result from a lack of neophilia [18]. Neophilia is the condition of having a strong affinity for novelty or new places. Having a lack of neophilia is assumed to reflect a high anxiety-like state of the animal. In this test, after four weeks of the experiment, the zinc supplemented group mice showed low levels of head dipping activity, reflecting the lack of neophilia or high anxiety state. In contrast, the control group of mice showed neophilia or low levels of anxiety state.

Moreover, an increased number of movements between the holes in the hole cross test represent reduced locomotion activity of the central nervous system and less sedative state, indicating some animal's anxiety behavior [19]. In our study, the zinc supplemented group's mice showed a significantly increased number of movements between holes, reflecting lowered locomotion activity, less sedation, or anxiety-like behavior. In contrast, the mice of the control group reflected enhanced locomotion activity or less anxiety behavior.

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Furthermore, an increased number of movements or increased number of squares crossed in open field tests represent increased exploration activity and lower anxiety-like behavior. In contrast, less square crossed and center activity represent low exploratory behavior and higher anxiety levels [20,21]. Our result revealed that the mice of the zinc supplemented group showed a reduced number of movements or squares crossed in the open field apparatus, reflecting diminished locomotion/exploratory activity or anxiety-like behavior. In contrast, the mice of the control group reflect high exploratory behavior and low levels of anxiety.

Hippocampus is the central processor in the brain that regulates memory and mood. Yang et al. [13] stated that high dose zinc supplementation might cause hippocampus-dependent learning and memory impairment representing anxiety behavior. They also added that high dose zinc administration might lead to unexpectedly specific zinc deficiency in the hippocampus, impairs learning and memory due to the decreased availability of synaptic zinc and Brain-derived neurotrophic factor (BDNF) deficit. Moreover, peroxisome proliferator-activated receptor α (PPARα), a significant regulator for hepatic fatty acid metabolism, is highly expressed in the brain hippocampus to control the memory functions by stimulating the cyclic AMP response element-binding (CREB) mediated memory-related protein. Since PPARα directly regulates fat metabolism, people with abdominal fat levels have depleted PPARα in the liver and abnormal lipid metabolism. At first, these individuals lose PPARα from the liver and eventually from the whole body, including the brain. Therefore, abdominal fat is an early indication of some dementia later in life [22].

Although excess zinc has been documented for the development of obesity and related disorders in adolescents [23], Blazewicz et al. [24] found a negative association of zinc with obesity in adolescents. Our study also observed excess accumulation of abdominal fat in the zinc supplemented group than the control group (Figure 4). Thus, we speculate that the mice in the zinc supplemented group are stealing brain PPAR (to metabolize the excess abdominal fat and eventually develop a state of memory impairment and anxiety by depleting the PPAR (in the brain). Moreover, zinc is involved in Ca^{+2} homeostasis on endoplasmic reticulum (ER) stress-induced neurotoxicity [25]. ER stress has also been reported to be involved in obesity and inflammation [26]. Moreover, Watt et al, [27] reviewed the role of zinc in the development of Alzheimer's disease, a most prevalent form of dementia. This review documented the capability of zinc to bind with amyloid-β (Aβ) for promoting its accumulation and causing neurotoxicity for the pathophysiology of Alzheimer's disease.

The present study indicated that all the behavioral tests stated above support the enhancement of anxiety condition and altered locomotion/exploratory activity of the central nervous system, causing memory, and learning impairment if zinc supplementation is continued for a long time in a healthy individual. That is why if zinc's dietary intake derived from food is sufficient as required of minimum recommendation for the maintenance of health, supplementation of zinc may result in excessive intake of zinc for healthy individuals and may result in zinc toxicity.

4. CONCLUSION

Based on the present research study, it can be concluded that regular treatment with zinc may show increased locomotion activity, the impaired effect on the brain leading to mood and behavioral disorders in healthy individuals. So, zinc should be supplemented to only those users having specific zinc-deficit disease conditions.

5. MATERIALS AND METHODS

5.1. Chemicals and reagents

Zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) from Merck India was used as test material. An equivalent amount of 6.5mg/kg elemental Zn was administered to the mice daily.

5.2. Experimental animals

Male Swiss Albino healthy adult mice (aged between 5 and 6 weeks) were obtained from the animal house of the Department of Pharmacy, Jahangirnagar University, Savar, Bangladesh. Adult healthy ten mice were divided into two groups, each having 5 mice and indicated as control group and zinc supplement group. Both groups of mice having an average weight of 26 g were selected for the experiment. Animals were housed in plastic cages separately with woodcutting as bedding. Standard conditions maintained in animals' cages: relative humidity 55-65%, temperature 24.0 ± 2.0°C, 12 h light-dark cycle. The cages were cleared daily.

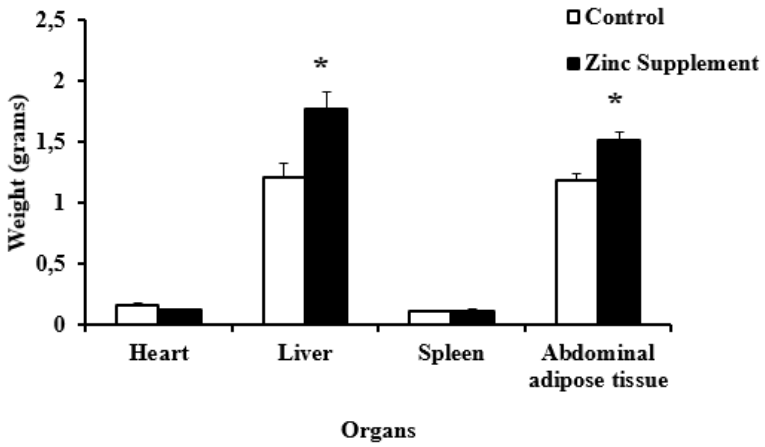

Figure 4. Effects of zinc supplementation on the weight of the organs. Values are presented as mean± SEM, where n=5.* $p < 0.05$ versus control (Student's t test).

5.3. Study Design

The zinc supplement group mice (n=5) were supplemented with 6.5mg/kg of body weight elemental zinc oral daily along with the regular feed. The control group mice were given only regular meals. Both groups of mice were given an equal amount of normal feed, constant access to drinking water, and continuously observed behavior changes. After 4 weeks of treatment, both groups of mice were tested according to the reported neurological and behavioral tests to assess behavioral changes. The study protocol was approved by the ethics committee of Noakhali Science and Technology University (#96/2020).

5.4. Elevated plus maze test

The elevated plus maze (EPM) test [17] apparatus consists of two open arms (35 x 5 cm) crossed with two closed arms (35 x 5 x 15 cm). It is a plus-shaped apparatus. The arms were connected with a central square of 5 x 5 cm. Each mouse of both groups was placed at the junction of the maze's four arms, facing an open arm, and entries and duration were recorded simultaneously for 5 min. After experimental observation of each mouse, the apparatus was washed with 10 % ethanol.

5.5. Hole-board test

The hole-board [18] is a rodent model test for measuring head-dipping activity in experimental animals. The hole-board apparatus consists of an enclosed space. The floor of the device has sixteen holes in a grid pattern where animals dip their heads. Mice were put in the apparatus and allowed to dip their heads through the holes in the floor. For 5 minutes, the frequency and length of 'head-dipping' were reported for each mouse. The apparatus was washed with 10% ethanol after each experimental observation. The 'head dipping of animals into floors is assumed to be a valid measure of the subject's attraction towards novelty or neophilia.

5.6. Hole-cross test

In the Hole-cross test [19], a wood partition is fixed in the middle of a cage having a size of 30 × 20 × 14 cm. A hole of 3 cm diameter is made at the height of 7.5 cm in the center of the cage. Each mouse is placed on one side of the specified instrument. The number of passages through the hole from one chamber to another was counted for 5 min. After each experimental observation, the apparatus was washed with 10 % ethanol.

5.7. Open field test

The open-field test [20] is an enclosed place having a wall of 40 cm to prevent escape. The field is marked with a grid and square crossings. Each mouse of both groups was placed in the field. The number of squares visited by the mice was counted for 3 min. The apparatus was washed with 10 % ethanol after each trial.

5.8. Statistical test

All data are presented as mean ± SEM. The student's t-test was used to determine statistical differences between the control and experimental zinc supplement group. Statistical differences were considered to be significant at $p < 0.05$. All statistical analysis was performed using Microsoft Excel and SPSS Statistics 20.

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Author contributions: Concept – MSH; Design – MAR, MTA, MSB; Supervision – MSH; Resources – MSH, MTA; Materials – – MSH, MTA, MAR; Data Collection and/or Processing – MAR, SA; Analysis and/or Interpretation – MAR, MTA, SA, AMU, MSB; Literature Search – MAR, SA, MSB; Writing – MAR, MTA; Critical Reviews – MSH, MTA, AMU, MSB.

Conflict of interest statement: The authors declared no conflict of interest.

Ethics committee approval: The study protocol was approved by the ethics committee of Noakhali Science and Technology University on the 3rd of August 2020 with the approval number of #96/2020.

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