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Evaluation the Anxiolytic Effects of Zinc Supplemented Diet in the Elevated Plus-Maze Test

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Abstract: In an experiment, the emotional and exploratory behaviors of Wistar rats to dietary zinc supplementation from zinc sulfate monohydrate (ZnS), Zinc oxide (ZnO) and zinc Methionine (ZnM) were studied in an elevated plus maze test. One hundred-twenty rats were assigned to 10 dietary treatments including: Basal diet (BD) + 50 mg kg^{-1} ZnS, BD + 100 mg kg^{-1} ZnS, BD + 200 mg kg^{-1} ZnS, BD + 50 mg kg^{-1} ZnO, BD + 100mg kg^{-1} ZnO, BD + 200 mg kg^{-1} ZnO, BD + 50 mg kg^{-1} ZnM, BD + 100 mg kg^{-1} ZnM, BD + 200 mg kg^{-1} ZnM and Basal diet (without Zn supplementation). These parameters including number of crossing of arms, time spent in open arms (%), time spent in closed arms (%) and number of rearing were recorded for 5 min. Although, the actual mechanism of zinc effectiveness in the body processes is still unclear, but, it appears to be related to the increase in accumulation of excitatory mediates or the decrease in accumulation of inhibitory mediates in hippocampus and or the decrease in function of GABA ergic mechanisms.

Key words: Emotional behaviors, elevated plus maze, supplemental zinc, rat

INTRODUCTION

Zinc (Zn) is widely distributed throughout the body and plays an essential role in many vital processes. It is the co-factor for several enzymes (e.g., carbonic anhydrase and superoxide dismutase) and influences the metabolism of important hormones such as insulin and gonadotropins (Roth and Kirchgessner, 1994). Approximately 90% of the total brain zinc exists as zinc metalloproteins, especially in the telencephalon. Furthermore, recent reports have indicated that zinc deprivation during periods of rapid development critically impairs animal behavior, mental and brain functions (Halas *et al*., 1983, 1986).

Zinc is commonly added at supplemental levels to many formulated animal diets (Wedekind and Baker, 1990). The supplemental zinc has been traditionally added in form of inorganic salts, such as zinc oxide or zinc sulfate. However, considerable reports for feeding organic trace minerals instead inorganic forms to animals for the benefit of higher bioavailability have been recently published (Henry *et al*., 1992; Rojas *et al*., 1996).

The objective of this study was to evaluate the behavioral responses of young rats to zinc supplementations in both organic and inorganic supplements in an elevated plus maze test.

MATERIALS AND METHODS

Diets: The basal diet for young rats was formulated according to Reeves (1997) recommendation (AIN-93G purified diet, Table1) without zinc supplementation. The control group was fed a zinc-adequate diet (40 mg of Zn kg^{-1} of diet DM). The other groups of rats were fed the basal diet supplemented by different supplemental zinc compounds including zinc sulfate monohydrate (ZnS), Zinc oxide (ZnO) and zinc Methionine (ZnM) in the following dietary treatments:

- Basal diet (BD, Table 1).
- BD + 50 mg kg^{-1} ZnS.
- BD + 100 mg kg^{-1} ZnS.
- BD + 200 mg kg^{-1} ZnS.
- BD + 50 mg kg^{-1} ZnO.
- BD + 100 mg kg^{-1} ZnO.
- BD + 200 mg kg^{-1} ZnO.
- BD + 50 mg kg^{-1} ZnM.
- BD + 100 mg kg^{-1} ZnM.
- BD + 200 mg kg^{-1} ZnM.

Animals: One-hundred twenty weaned male Wister rats (4 weeks old, 112±13 g body weight) were randomly

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Table 1: Composition of the basal diet

Ingredient	Amount (g kg^{-1})
Cornstarch	370
Soybean oil (no additives)	80
Skim milk powder	350
Cellulose	50
Sucrose	100
AIN-93G Mineral mix	35
AIN-93G Vitamin mix	10
L-Cystine	3
Choline bitartrate (41.1%)	2
Tert-Butylhydroquinone (TBHQ), mg	14

assigned to one of the above-mentioned treatments. The animals were housed in the stainless still cages located in a light (the lighting from 07:00-19:00), temperature (22±2°C) and moisture controlled room. Rats were given free access to pelleted diets and distilled water throughout the experiment (60 days). The experiment took place under quiet conditions and low light (50 lux). The experimental conditions were adjusted according to the rules of local committee for animal ethics.

Apparatus: The plus-maze was built as described by Pellow *et al.* (1985). It consisted of 4 arms, each 50 cm long and 10 cm wide. Two of the arms were open (i.e., without side and end walls), whereas the 2 closed arms had 20 cm high side and end walls with an open roof. The arms were arranged such that the 2 arms of each type were opposite each other. All 4 arms were connected to a 10×10 cm center square. The plus-maze was elevated to a height of 50 cm above the floor.

Procedures: On the day 60, each rat was individually placed at the center of the plus maze facing one of the open arms and the following parameters were registered for 5 min: Number of crossing of the arms, transitions between arms, time in open arms (%), time spent in the open arms relative to total time spent in the plus-maze, expressed as a percentage, time in closed arms (%), time spent in the closed arms relative to total time spent in the plus maze, expressed as a percentage, rearing, rise onto the hind legs. All the floors of this apparatus were cleaned with ethyl alcohol and dried with study towels continuously.

Statistic: The data were statistically analyzed by one-way analysis of variance, with the significance level of $p<0.05$. When a main factor was significant, the post hoc comparisons were performed using Duncan test.

RESULTS AND DISCUSSION

Feeding the ZnM increased the number of crossing arms, compared the control significantly (7.33 vs. 5.08,

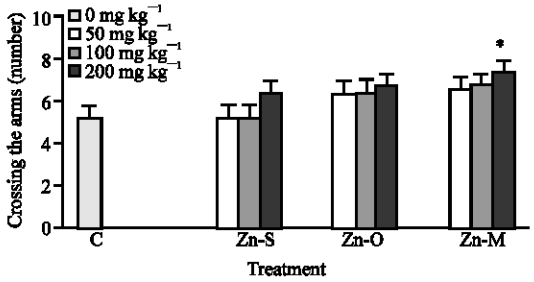

Fig. 1: The number of crossing arms (mean±SEM) by rats fed zinc sulfate (ZnS), zinc oxide (ZnO) and zinc methionine (ZnM) compared to the control group (C). Data are expressed as mean values (±SEM) from each group of minimum 12 rats *p<0.05

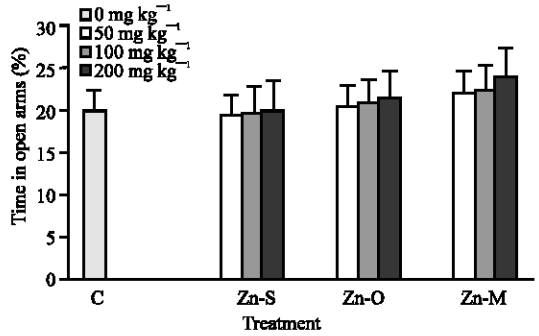

Fig. 2: The percentage of time in open arms (mean±SEM) by rats fed zinc sulfate (ZnS), zinc oxide (ZnO) and zinc methionine (ZnM) compared to the control group. Data are expressed as mean values (±SEM) from each group of minimum 12 rats *p<0.05

Fig. 1). The anxiolytic effect of the ZnM reached a statistical significance level only at its application level of 200 mg kg^{-1} for crossing the arms parameter. There was not a significant difference between the control and zinc supplemented rats in case of rearing numbers (Fig. 2), although nonsignificant increases were observed (19.96, 21.19 and 19.77) at the high level of ZnS, ZnO and ZnM, respectively. There were also no significant differences between the organic and inorganic zinc supplements for the percentage time spent on open arms or on closed arms (Fig. 3 and 4). Figure 2-4 show that the effect of zinc supplements on rearing, percentage time on open arms and percentage time on closed arms were dose-dependent in some degrees.

The possible reasons for this effectiveness can be summarized as follows:

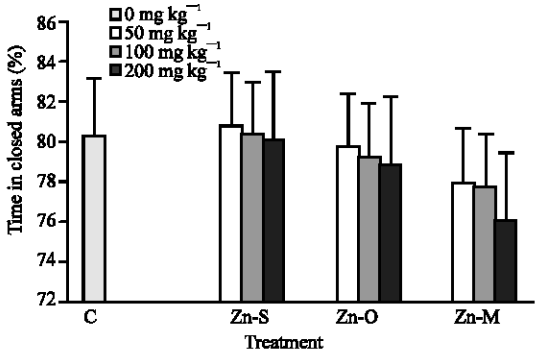

Fig. 3: The percentage of time in closed arms (mean±SEM) by rats fed zinc sulfate (ZnS), zinc oxide (ZnO) and zinc methionine (ZnM) compared to the control group. Data are expressed as mean values (±SEM) from each group of minimum 12 rats *p<0.05

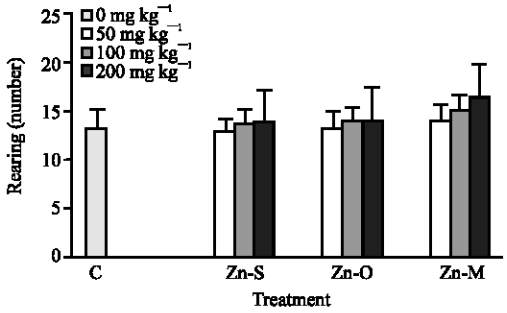

Fig. 4: The number of rearing (mean±SEM) by rats fed zinc sulfate (ZnS), zinc oxide (ZnO) and zinc methionine (ZnM) compared to the control group. Data are expressed as mean values (±SEM) from each group of minimum 12 rats *p<0.05

- Zinc sulfate is a highly available and absorbable supplement. Zinc oxide is less soluble and available for absorption (NRC, 2005). In addition, several investigators have reported that bioavailability of organic forms of zinc in the diet of various animals are higher in comparison with the inorganic zinc forms (Aoyagi and Baker, 1993), probably due to their different metabolism (Ammerman *et al*., 1998; Spears, 1989; Nockels *et al*., 1993).
- The increase in releasing of effective intermediators in the ventral hippocampus when the animals confront a novel environment such as elevated plus-maze can influence the hypothalamic-pituitary-adrenal (HPA) axis activity (Bianchi *et al*., 2003) and led to performing different behavioral responses.

CONCLUSION

The results of the present study indicated that ZnM at a level of 200 mg kg^{-1} was more potent than inorganic supplements in case of crossing the arms parameter. Although, anxiolytic-like effects were observed at this level of application. Therefore, further studies are needed to identify the anxiolytic mechanism and the optimum level of Zn supplementation.

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