

The Effect of Intraventricular Administration of Zinc on Serum LH, FSH, Prolactin, and Testosterone in Male Rats

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Background: Zn is calcium channels blocker and interferes with biological function of calcium ion, and FSH and LH secretion are dependent on calcium ion.

Objectives: The aim of this study was evaluate the effects of supra physiological dose of zinc injection in lateral ventricle (LV) on serum FSH, LH, testosterone, and prolactin (PRL) in male rats.

Materials and Methods: This experiment was performed on 30 Wistar-Albino male rats weighing 220 - 260 g, which were cannulated in LV. Rats were divided into: sham control (SC), control (V), and test (T) groups, randomly. Test group received 10 μ L of artificial cerebrospinal fluid (ACSF) containing 0.06 μ mol zinc sulfate, for 20 days. Group V was given the same volume of ACSF, but group SC did not receive any agent at this time. At the end, animals were anesthetized by diethyl ether, sacrificed and blood samples were collected. Serum gonadotropins and prolactin were measured via rat kits and ELISA methods. Testosterone was measured by ordinary methods. Obtained data were analyzed by SPSS-17 software and Kruskal-Wallis, Mann-Whitney and Bonferroni correction statistical tests. Results were expressed as mean \pm SD. Statistical differences were recognized significant at $P < 0.05$.

Results: Our findings showed that serum LH, food and weight gain in group T significantly decreased but prolactin value in this group increased in comparison to those in other groups.

Conclusions: We conclude that intraventricular (IV) injection of supra physiological dose of zinc sulfate in IV affected on serum LH, prolactin, food intake, and gain of weight in male rats.

Keywords: Zinc; Gonadotropins; Testosterone; Prolactin

1. Background

Zinc is an important ion which participates in different mechanisms such as protein, nucleic acids and hem syntheses, gene expression, and cell division [1, 2]. Zinc is taken into the body along with food, water, and vegetables, and absorbed by Na cotransport in intestinal cells [3]. Zn enters the glomerulus and filtrate reabsorbed along the nephron [4]. Moreover, it is a vital ion for childhood health and its deficiency causes pneumonia, anemia and diminishes the immune system activity and appetite in children [5, 6]. Zinc deficiency reduces the leptin secretion [7] and its serum level correlated to Parkinson disease [8]. Zinc acts as a neuromodulator at excitatory synapses and has a considerable role in the stress response, Parkinson and Alzheimer's patients [9, 10]. Acute zinc toxicity in a dog following ingestion of a metallic object which resulted in marked hemolytic anemia [11]. On the other hands excess infused zinc causes the development of behavioral seizures in a

kindling model of epilepsy in rat [12]. This ion also accumulates in prostate gland and its homeostasis alters in prostitutes [13]. Investigation revealed that administration of zinc diet caused a decrease in the testis and seminiferous tubule size and disruption of spermatogenesis, testosterone serum, LH and FSH in male rats in a period of 96 days [14]. Moreover, administration of zinc sulfate in diet for two weeks caused an increase in serum prolactin level and testosterone in male rats [15], but other studies revealed that Zn administration does not inhibit prolactin) PRL (secretion in hyperprolactinemic patients [16]. On the other hand, investigation showed that the metal ion such as Zn administered in vivo decrease serum testosterone concentration and change serum concentrations of pituitary hormones in animal models [17]. In addition, it has been shown that in vitro Zn administration, at high dose, can be toxic to cells and can cause their death [18], but other studies

showed growth acceleration, testicular development and increase in FSH, LH, and testosterone serum levels after Zn supplementation [19]. Moreover, investigation showed that zinc compounds were more potent for promotion of release of gonadotropins hormone in the ovariectomized animals which pretreated by estradiol and progesterone in a treatment period for 30 days [20]. Zinc is a voltage sensitive calcium channel blocker, accumulates in central nervous system and interferes with biological function of calcium ion and since FSH, LH, and testosterone secretion is dependent on calcium ion [21, 22], hence, alteration in secretion of these hormones will be expectable after administration of zinc. High intake of Zn after first prescription could induce some unknown effects in body systems.

2. Objectives

According to reported studies till now, there was no any agreement for the injection of supra physiological effects of Zn in animal studies, the aim of the present work was to evaluate the in vivo effects of injection of zinc sulfate in unilateral lateral ventricle (LV) on serum FSH, LH, prolactin, testosterone, weight gain, food and water intake in male rats.

3. Materials and Methods

3.1. Animals

This experimental study performed on 30 adult Wistar-Albino male rats, weighing 220 - 260 g and aged 5 - 7 months, which were kept in Animal House of Zahedan University of Medical Sciences. Rats were separately housed in cages (one rat in each cage) and had free access to water and food. Animals were maintained in a room at $24 \pm 2^\circ\text{C}$ with a fixed 12-hour/dark and light artificial light period (timer model: SUL180a, AC220V. China, 6 Am to 6 Pm), and humidity of 45 - 70% and the air was adequately recycled.

3.2. Experimental Design and Protocol

Thirty adult Wistar-Albino male rats were randomly selected from laboratory animals from Zahedan University of Medical Sciences. After a week of habituation, animals were weighed (first weight), and then anaesthetized with intra peritoneal (i.p.) injection of ketamine 150 mg/kg (Gedeon Richcer chemical works, Hungry) and midazolam 0.1 mg/Kg. All rats were cannulated in the lateral ventricle unilaterally according to Paxinos Atlas using stereotaxic system (Narishige sp-5b Japan). Stainless steel guide cannulae (21-1.2 gauge) were unilaterally implanted by AP = +1.4 mm from bregma, DV = +3.4 mm from surface of the brain and ML = ± 2 mm from midline [23]. After recovery period, animals were divided into sham control (SC), vehicle (V), and test (T) groups, randomly (N=10 per group). Test group received 10 μL (as a dose upper than physiological dose and lower

than toxic dose) of zinc sulfate (Merck, Germany) in lateral ventricle by a Hamilton syringe daily (8 - 10 am) for 20 days [24, 25]. Control group B were given the same volume of ACSF with pH = 7.4, (effect of volume) and the control group A did not receive any agent (effect of cannulation). At the end of the experiment, after an overnight fasting (12 - 14 hours), all animals were weighed GOTTL. KERN and SOHN and ds 0.01 g Japan digital balance (final weight) then sacrificed under deep diethyl ether (Merck Germany) anesthesia and blood samples were collected from cervical vessels.

3.3. Ethical Approval

The present study followed the leaders and procedure of the Iranian Council on animals, and it received institutional ethical approval from the committee for Animal Research of Zahedan University of Medical Sciences (3922).

3.4. Biomarker Analysis

Serum was obtained (BH-1200 type Iran) and stored at -70°C for further analyses. FSH, LH and prolactin serum levels were determined by sensitive rat insulin kit (Cusabio Biotech Co. Ltd), using double antibody enzyme-linked immunosorbent assay (ELISA) and serum testosterone was measured by usual laboratory (Mino bine human kit USA) methods. Food and water intake was measured daily. All measurements were blind.

3.5. Statistical Analysis

The results were analyzed by SPSS-17 software, using non parametric statistical tests, Kruskal-Wallis, Mann-Whitney and Bonnferroni correction. Results were expressed as mean \pm SD. Statistical differences were considered significant at $P < 0.05$.

4. Results

Our results showed that LH concentration value, in test group (T) (2.6 ± 1.3 ng/mL) were significantly decreased compared to those of SC (7.44 ± 5.52 ng/mL) and vehicle group (7.77 ± 6.77 ng/mL) ($P < 0.014$) (Table 1). In Addition food intake in group T (14.21 ± 1.15 g) were significantly decreased compared to those of SC (15.89 ± 2.52 g) and group V (16.23 ± 2.37 g) ($P < 0.021$) (Table 2). The weight gain in group T (235.55 ± 11.33 g) which received zinc sulfate in lateral ventricle, significantly decreased compared to those of other groups (SC = 271.27 ± 13.25 g, V = 269.9 ± 18.73 g) ($P < 0.032$, $P < 0.014$) (Table 2). On the other hand, prolactin value in test group T (13.03 ± 10.1 ng/mL) significantly increased in comparison with other groups (SC = 2.03 ± 3.44 ng/mL, V = 5.85 ± 4.3 ng/mL) ($P < 0.014$) (Table 1). Our results in the present study for FSH and testosterone serum value and the amount of water intake did not show any significant difference between all groups.

Table 1. Intra Ventricular of Zinc on Serum FSH, LH, PRL, and Testosterone Values in Sham Control, Vehicle and Test Groups ^a

	Shame Control (SC) ^b	Vehicle (V) ^b	T-Test ^b	P Value ^c
FSH, ng/mL	35.15 ± 10.31	21.22 ± 12.83	11.55 ± 5.21	0.263
LH, ng/mL	7.44 ± 5.52	7.77 ± 6.77	2.6 ± 1.3 ^d	0.014
Prolactin, ng/mL	2.03 ± 3.44	5.85 ± 4.3	13.03 ± 12.1 ^e	0.014
Testosterone, nmol/L	2.62 ± 2.22	2.28 ± 1.85	1.95 ± 1.52	0.58

^a N = 10.^b Values are presented as mean ± SD.^c P < 0.05 is considered significant.^d Kruskal-Wallis, Mann-Whitney, and Bonferroni correction statistical tests, the LH concentration in test group T was significantly decreased compared to that of others groups.^e Kruskal-Wallis, Mann-Whitney, and Bonferroni correction statistical tests, the prolactin concentration in test group T was significantly increased compared to that of others groups.**Table 2.** Intra Ventricular of Zinc on Weight, Food and Water Intake in Sham Control, Vehicle and Test Groups ^a

	Shame Control (SC)	Vehicle (V)	T-Test	P Value ^b
Final weight, g	271.27 ± 13.25	269.9 ± 18.73	235.55 ± 11.33 ^c	< 0.032, < 0.014
Food intake, g	15.89 ± 2.52	16.23 ± 2.37	14.21 ± 1.15 ^d	< 0.021
Water intake, mL	44.55 ± 3.44	45.89 ± 4.3	42.68 ± 5.1	< 0.58

^a N = 10.^b P < 0.05 is considered significant.^c Based on Kruskal-Wallis, Mann-Whitney, and Bonferroni correction statistical tests, the final weight in group T was significantly decreased compared to that of others groups.^d Based on Kruskal-Wallis, Mann-Whitney, and Bonferroni correction statistical tests, food intake in group T was significantly decreased compared to that of others groups.

5. Discussion

The results obtained from the present study showed that the injection of supra physiological dose of zinc sulfate solution (which was lower than toxic dosage) in lateral ventricle in male rats (test group) caused a significant decrease in the serum level of LH, food intake and weight gain, although in this group PRL value significantly increased compared to other groups. The comparison of FSH and testosterone values did not show a significant difference among all groups. According to our knowledge, there was no any similar study till now to compare our results with other studies. The study of Madureira et al. showed that the chronic oral Zn administration in a human study with prolactinomas did not show any changes in serum PRL levels after oral Zn administration (37.5 mg), during a treatment period of 60 days [16].

Our results showed that serum PRL level in group T increased compared to other groups. It seems that this controversy may be attributable to the factors such dose, duration and species specific differences between animal and human. The studies of Kochman et al. showed that zinc-compound more potently promoted the FSH release in the ovariectomized female rats [20]. The study of Karaca et al. indicated that administration of zinc supplementation increased serum FSH, LH, and testosterone values in patients who suffered from Prasad syndrome

[19]. This controversy may be a result of differences in zinc route administration and difference in sensitivity of these patients in response to zinc components. Since Zn is a voltage sensitive calcium channels blocker and has antiepileptic role in appropriate doses [25, 26], it may be blocked prolactin inhibitor factor secretion in hypothalamus and caused increased prolactin secretion in adeno-hypophysis [27, 28]. Moreover, present results showed that serum LH level was decreased in experimental group but did not show any changes in serum FSH level. Since FSH and LH secretion is promoted by FSHRH and LHRH factors which are produced in separated zones in hypothalamus nucleoli [27, 29], it is probable that Zn inhibited LHRH production in hypothalamus but did not affect on FSHRH production. Daniels et al. reported that zinc is a vital component for normal performance of the brain; however, this ion, at high levels, can be toxic to cells and may induce cell death [18]. Although these findings are similar to those of Dissanayake et al. [15], but our findings revealed that serum testosterone value in group T was decreased compared to other groups but statistical analysis did not show any significant changes. The decrease in testosterone secretion may be a result from decrease in secretion of central LHRH and LH, although the peripheral LHRH which may be secreted by Sertoli cell can be improved it [29]. The other study showed that dopamine

secretion and exocytosis is dependent on dopaminergic axonal N calcium concentration [27], it seems that there is the same pathway for synthesis and secretion of central LHRH. Jeong et al. reported that a large numbers of divalent metals such as zinc are voltage-activated T-type calcium channel blockers [21]. In addition, the results obtained from the present study showed that in group T, serum prolactin was increased compared to other groups. This may be due to a result from zinc injection in lateral ventricle which probably blocked N-calcium channels in dopaminergic neurons and hence dopamine secretion stopped and prolactin secretion was increased. Hypothalamus contains a great amount of peptide such as neuropeptide Y (NPY), galanin and other neurotransmitters which manipulate food intake [30]. In addition, our finding revealed that food intake and weight gain in group T were significantly increased compared to other studied groups. It is probable that zinc after injection to lateral ventricle entered the hypothalamic nuclei and inhibited the neurotransmitters secretion such as NY which in turn affected appetite and weight gain.

Our finding showed that injection of supra physiological dose of zinc sulfate in lateral ventricle can effect on serum LH, prolactin levels, gain of weight, and food intake in male rats. However, further studies are recommended to discover the exact mechanism of Zn on neurons.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis, and manuscript writing.

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