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RESEARCH ARTICLE

Effects of Zinc Oxide Nanoparticles on the Expression of Zinc Transporter 1-4 Genes in the Hippocampus of Male Rats Under Acute Stress [1]

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Abstract

Zinc transporters (ZnT) and ZIP proteins maintain Zinc homeostasis in the live organisms. On the other hand, the impacts of zinc oxide nanoparticles (ZnO NPs) on the expression of the *Znt* genes in biological systems were not clear yet. So that in this experimental study we have tried to find the effects of ZnO NPs on *Znt1-4* genes expression in the hippocampus of male rats under acute stress. Adult male rats were divided into groups of control and treated with 5 or 10 mg/kg of ZnO NPs alone and under acute restraint stress for 90 min. The changes in the expression of the selected genes were monitored using real-time qRT-PCR. The ZnT4 protein expression also was measured by Western blotting. Real-time qRT-PCR expression analysis revealed that the *Znt1* gene expression was up-regulated in the stress group, while the expression of the *Znt1* and *Znt4* genes was significantly up-regulated in the group receiving 10 mg/kg of ZnO NPs. Furthermore, in the ZnO NPs 10 mg/kg group under stress, the *Znt2* gene expression was down-regulated, while the *Znt4* gene expression was up-regulated. Moreover, the levels of ZnT4 protein were significantly increased after 10 mg/kg of ZnO NPs injection in the stress and normal groups. According to these results ZnO NPs administration can cause changes in the expression of a number of zinc transporter genes under stress conditions and increases the ZnT4 protein level. Therefore, this is a valuable approach for forecast investigation in biomedicine and pharmacogenetics studies.

Keywords: *Hippocampus, Nanoparticles, Rats, RNA, Zinc*

Akut Stres Altındaki Erkek Ratların Hipokampusundaki Çinko Taşıyıcı Genler 1-4'ün Ekspresyonu Üzerine Çinko Oksit Nanopartiküllerinin Etkileri

Öz

Canlı organizmalarda çinko homeostazını çinko taşıyıcılar (ZnT) ve ZIP proteinleri korur. Öte yandan, çinko oksit nanopartiküllerinin (ZnO NP) biyolojik sistemlerde *Znt* genlerinin ekspresyonu üzerine etkileri henüz tam olarak netlik kazanmamıştır. Bu nedenle, bu deneysel çalışmada, ZnO NP'lerin akut stres altındaki erkek ratların hipokampusundaki *Znt1-4* genlerinin ekspresyonu üzerine etkilerini araştırmaya çalıştık. Yetişkin erkek ratlar, kontrol, yalnızca 5 mg/kg ve 10 mg/kg ZnO NP uygulananlar ve 90 dk'lık kısıtlamaya bağlı oluşan stres süresince 5 mg/kg ve 10 mg/kg ZnO NP uygulananlar olmak üzere gruplara ayrıldı. İlgili genlerin ekspresyonlarındaki değişiklikler gerçek zamanlı qRT-PCR kullanılarak izlendi. ZnT4 protein ekspresyonu ayrıca Western Blot yöntemi ile ölçüldü. Gerçek zamanlı qRT-PCR ekspresyon analizi, stres uygulanan grupta *Znt1* gen ekspresyonunda bir artışın olduğunu, 10 mg/kg ZnO NP uygulanan grupta ise *Znt1* ve *Znt4* genlerinin ekspresyonunda önemli ölçüde bir artış olduğunu ortaya çıkardı. Ayrıca, stres altında 10 mg/kg ZnO NP uygulanan grupta, *Znt2* gen ekspresyonunda azalma saptanırken, *Znt4* gen ekspresyonunda artış belirlendi. Bunun haricinde, 10 mg/kg ZnO NP uygulanan stres ve normal gruplarda uygulamalardan sonra ZnT4 protein seviyeleri önemli ölçüde arttı. Bu sonuçlara göre, ZnO NP uygulaması, stres koşulları altında çinko taşıyıcı bazı genlerin ekspresyonunda değişikliklere neden olabilir ve ZnT4 protein seviyesini artırabilir. Bu nedenle, bu çalışma, biyotıp ve farmakogenetik çalışmaları tasarlamak için değerli bir yaklaşım sunmaktadır.

Anahtar sözcükler: *Hipokampus, Nanopartiküller, Rat, RNA, Çinko*

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Introduction

Psychological stress (PS) is considered as the risk-increasing factors for the central nervous system (CNS) diseases. The stress-related problems can have detrimental effects on regulation of inflammatory and immune processes. The latter in turn is claimed to have the potentials to exacerbate depression, autoimmune, coronary artery disease with the possibility of developing some kinds of cancers [1]. Stressful environmental conditions can more often than not, affect zinc homeostasis and alter its spread throughout various body organs, particularly the brain [2].

Zinc (Zn) is considered as a vital micronutrient with several crucial catalytic and regulatory roles especially for efficient brain functioning [3]. This is because of the extracellular and intracellular zinc transport in the hippocampal mossy fibers during neutral activities [4]. Zinc deficiency on the other hand, is instrumental in bringing about irregular glucocorticoid secretion. This, in turn, is seen as a major contributory factor to the emergence of various types of neurobehavioral complications. These include certain cases of Alzheimer and related brain malfunctioning like epilepsy [5]. Endowed with its anti-anxiety, anti-depression, anti-inflammatory, anti-edematous and analgesic functionary, Zinc can be widely employed as a novel therapeutic intervention agent in order to deal with certain behavioral complications [6].

Zinc transport is differently regulated based on dietary zinc levels and physiological conditions through several zinc transporters in different tissues [7]. Results of clinical and experimental observations clearly relate any dysregulation in the zinc transporters expression to the development of cancer, asthma, diabetes, brain malfunctioning and mental illnesses, such as depression [8].

Zinc homeostasis maintenance in mammals is principally achieved by two types of proteins such as the ZIP (SLC39) and Zn transporters (ZnT). The ZIP proteins increase intracellular zinc by transporting extracellular zinc into the cells, while ZnT (SLC30) proteins transfer zinc out of the cytoplasm to the organelles or the extracellular matrix [9].

As the first member of ten proteins in the ZnT family, ZnT1 is located on the plasma membrane of various tissues like brain [10]. It exports cytosolic zinc ions to extracellular space. Under the circumstances of zinc deficiency, the amount of ZnT1 in brain decreases markedly. This is the case, bearing in mind that ZnT1 prevents zinc aggregation in cytoplasm under different physiological conditions [11]. ZnT2 is a component of intercellular acid vehicle. It is often expressed in intestine, kidneys and testis in rats, though not so often in brain [12]. ZnT3 plays a very vital role in several neurotransmitter signaling pathways in the hippocampus [13]. ZnT2 and ZnT3 proteins transport cytoplasmic zinc into vesicular components that in turn facilitate decreasing the intercellular zinc [3]. ZnT4 by nature ensures zinc homeostasis maintenance in various intercellular organelles of tissues like mammalian glands and brain. It facilitates zinc transport into trans-Golgi network. It is also instrumental in transferring Zn^{2+} ion into milk during lactation process [14].

The unique physiochemical nature of nanoparticles renders their universal application in biotechnological and medical science arena [15]. Nanoparticles of oxidized elements like zinc oxide (ZnO NPs) have also been employed in a wide spectrum of technological and medical purposes [16]. Research observation shows that MgO and ZnO nanoparticles treatment grows the glutamate level under stressful conditions. The ZnO NPs also increase zinc and Nr2a expression in rat's hippocampus [17]. The acute restraint stress method decreases zinc level in serum with a simultaneous increment in hippocampus [18].

While several studies have investigated the effects of ZnO NPs in mice and rats, the effects of ZnO NPs on the expression of zinc transporter genes in the hippocampus under restraint stress is an important and new goal. Therefore, in the current study, we evaluated the effects of ZnO NPs on the mRNA expression of the *Znt1*, *Znt2*, *Znt3,* and *Znt4* genes in the hippocampus of male rats under acute restraint stress and normal conditions. In addition, the ZnT4 protein was selected to determination its level in hippocampal tissue.

Material and Methods

Animals and Treatment Groups

This study was performed on thirty male Wistar rats (200±20 g) from the Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz. The rats were kept separate with free access to water and food, except during the experiment. Room temperature (22-24°C) and a 12 h light/dark cycle were maintained throughout the study. Animals were randomly classified into six treatment groups, each containing five rats (N = 5) [19]. Animal groups were: control (Saline 0.9%), ZnO NPs (5 and 10 mg/kg), Saline + stress 90 min and ZnO NPs (5 and 10 mg/kg) + stress 90 min.

Experimental rats were raised in an environment that was in strict accordance with the "Guide for the Care and Use of Laboratory Animals". The use of these experimental animals was approved by the "Shahid Chamran University of Ahvaz (Approval No. EE/96.24.3.88369/SCU.AC.IR)".

Preparation of ZnO NPs Compound

The spherical morphology of ZnO NPs (US Nano Co., Texas, USA) was determined by scanning electron microscopic (SEM) images (Hitachi S4160., Co, Japan) and the particle sizes were ranged from 10 to 30 nm. ZnO NPs solution was prepared in an ultrasonic bath by sonication for 16 and shaking for 1 min before each injection. Two doses of

ZnO NPs suspension (5 and 10 mL/kg) were administered through intraperitoneal injection (IP), while the control group received 0.9% saline (1 mL/kg).

Acute Stress Induction

To induce acute stress, rats were placed in semicircular Plexiglas tubes (19.5 × 4.5 × 6 cm), where they were not able to move for 90 min. After stress induction, the rats received 5 or 10 mg/kg of ZnO NPs suspension or saline. Corticosterone hormone levels in serum were assessed to ensure induction of stress in rats.

Tissue Collection and Expression Analysis

Two hours after the ZnO NPs injection or stress induction, all rats were euthanized with ether, and the hippocampal tissue of their brains was excised. Trizol Reagent (Thermo Fisher Scientific, USA) was used to extract total RNA according to the manufacturer's protocol, and the extracted RNA was stored at -80°C. The RNA concentration of each sample was measured using a NanoDrop™ 2000/c spectrophotometer. In addition, RNA integrity was assessed using electrophoresis on a 1% agarose gel containing SafeStain (CinnaGen), and the 28 s, 18 s, and 5 s bands were observed. Extracted RNAs were treated using DNaseI (Takara Bio, Japan). Furthermore, the Primescript™ RT reagent kit (Takara Bio, Japan) was used for the reverse transcription of RNA to cDNA. A total of 1 µL of random hexamers (100 µM) and 1 µL of the oligo(dT) primer (50 µM) were added to 1 µg of DNase-treated RNA, and RNase-free water was added to a final volume of 5 µL, followed by incubation at 65°C for 5 min. Subsequently, 1 µL of the reverse transcriptase enzyme (1 U/µL) and 4 µL of 5X buffer were added, and RNase-free H_2O was added to a final volume of 20 µL, followed by incubation at 37°C for 25 min for cDNA synthesis, and incubation at 80°C for 5 s to deactivate the enzyme. Then, the cDNA was stored at -20°C. For real-time PCR, the following reagents were mixed as noted: 8 µL of SYBR Green I (Takara Bio, Japan), 1 µL each of forward and reverse primers, 2 µL of cDNA, and 3 µL of DNase-free water. Conventional real-time PCR was performed using the ABI 7900HT system. The thermocycling conditions were: 95°C for 30 s; 40 cycles of 95°C for 5 s and 60°C for 30 s; and a dissociation stage of 95°C for 15 s, 60°C for 60 s, and 95°C for 1 s. In this study, we used beta actin (act-β) as a housekeeping gene for normalizing the expression analysis. Primers for the act-β, *Znt1*, *Znt2*, *Znt3*, and *Znt4* genes in rats were designed by Gen Script online tool according to the cDNA sequences by Gene Bank *(Table 1)*. To validate the real-time qRT-PCR data, melt curves were plotted, and the accuracy of the curves was confirmed for each analyzed gene and primer dimer strands.

Table 1. *List of primers used in this study*

Gene ID	Gene Bank Accession Number	Sequence for Forward Primer (5'–3') and Reverse Primer (5'–3')
Act-β	[NM_031144.3]	F- TATCGGCAATGAGCGGTTCC
		R- AGCACTGTGTTGGCATAGAGG
Znt1	[NM_022853.2]	F- ACCAGGAGGAGACCAACAC
		R- CTCAACTTCTCTGGCTCTGC
Znt2	[NM_001083122.1]	F- GCACCTTCCTCTTCTCCATC
		R- GTAAGGCTTCCACACCATCC
Znt3	[XM_008764526.2]	F- TCAGCACCTTCCTCTTCTC
		R- GTGGTAAGTAAGCGTCAGC
Znt4	[NM_172066.1]	F-AGTCGTTGATGAAGATAGAAGATG
		R- CGAATGTGTTCAGCAAGAGG

ZnT4 Western Blotting

For western blot analysis, hippocampal tissue isolated from rats, after washing with cold PBS buffer PBS, in a micro-tubes with RIPA lysis buffer containing complete protease inhibitor cocktail (Santa Cruz, USA) homogenized. Then, complete lysis was performed for 1 h on ice bucket. Protein concentrations were determined using a Bradford assay. 20 µg of each tissue lysate was boiled for 10 min in a 2x sodium dodecyl sulfate sample buffer and run via 10% polyacrylamide gel electrophoresis (SDS-PAGE). After electrophoresis proteins were transferred to Polyvinylidene Fluoride (PVDF) paper. The paper was incubated with first anti-ZnT4 monoclonal antibodies (orb227160) diluted 1:200 in PBS. Secondary antibody, goat anti-mouse IgG-HRP (Bio-Rad, USA) was used and diluted 1:1000 in PBS. Approximately 5% skim milk in Tris Buffered Saline plus 0.1% Tween-20 (TBST) was used as an antibody blocking and dilution buffer. Act-β (Bio-Rad, USA) was used as an endogenous reference to determine the relative density of ZnT4 protein in the sample. Finally, with the addition of chemiluminescence (Najm biotec, Iran) to the PVDF paper ZnT4 protein was detected. Digital image analysis was accomplished by importing the images into the image analysis software Image j.

Data Analysis

The data are expressed as mean ± standard error of the mean (S.E.M.), and the graphs are plotted using MS Excel. Student's t-test was used for the comparison of the unpaired data means using the Instat3 software application in real-time PCR and western blot data analysis. The results were considered as statistically significant when P-values were <0.05. Moreover, Relative Expression Software Tool (REST, version 2009) was used to detect alterations in the expression of the *Znt1*, *Znt2*, *Znt3,* and *Znt4* genes versus the act-β gene in different groups, and relative gene expressions were calculated as $2^{-\Delta\Delta ct}$. Gene expression analysis for the target and act-β genes was repeated three times for each sample. We prepared several dilutions of every cDNA before analysis, and the efficiency of the expression analysis was determined through standard curves.

Results

The Znt1, Znt2, Znt3, and Znt4 Expression in the Hippocampus Tissue

Our data showed a significant up-regulation in the *Znt1* gene expression in the group receiving restraint stress for 90 min and saline (P=0.0021), and the group receiving 10 mg/kg of ZnO NPs compared to the control group (P=0.0008). However, the results of this study did not show any significant difference in terms of the *Znt1* gene expression between the group receiving 5 mg/kg of ZnO NPs and the control group (P=0.6668), and between the group receiving 5 and 10 mg/kg of ZnO NPs with restraint stress compared to the group receiving restraint stress for 90 min and saline (P=0.1355 and P=0.4027, respectively) *(Fig. 1-A)*.

In the current study, there was a significant down-regulation in the *Znt2* gene expression in the group receiving 10 mg/kg of ZnO NPs with restraint stress compared to the group receiving restraint stress for 90 min and saline (P=0.0001). However, the *Znt2* gene expression did not differ significantly between the group receiving restraint stress for 90 min and saline (P=0.1803), the group receiving 5 and 10 mg/kg of ZnO NPs compared to the control group (P=0.2534 and P=0.1643, respectively), and the group receiving 5 mg/kg of ZnO NPs with restraint stress compared to the group receiving restraint stress for 90 min and saline (P=0.1296) *(Fig. 1-B)*.

In this study, there was not any significant change in the *Znt3* gene expression in different groups, including the group receiving restraint stress for 90 min and saline (P=0.1487), the group receiving 5 and 10 mg/kg of ZnO NPs compared to the control group (P=0.1892 and P=0.1106, respectively), and the group receiving 5 and 10 mg/kg of ZnO NPs with restraint stress compared to the group receiving restraint stress for 90 min and saline (P=0.0617 and P=0.0697, respectively) *(Fig. 1-C)*.

As seen in *Fig. 1-D*, there was a significant change in the *Znt4* gene expression in the group receiving 10 mg/kg of ZnO NPs with restraint stress compared to the group receiving restraint stress for 90 min and saline (P=0.0011). Moreover, the *Znt4* gene expression was significantly up-regulated in the group receiving 10 mg/kg of ZnO NPs compared to the control group (P=0.0005), while the *Znt4* gene expression did not differ significantly between the group receiving restraint stress for 90 min and saline (P=0.0773), the group receiving 5 mg/kg of ZnO NPs compared to the control group (P=0.0761), and the group receiving 5 mg/kg of ZnO NPs with restraint stress compared to the group receiving restraint stress for 90 min and saline (P=0.0605).

Western Blot Analysis

Since the *Znt4* gene expression up-regulated in hippocampus due to receiving 10 mg/kg of ZnO NPs with or

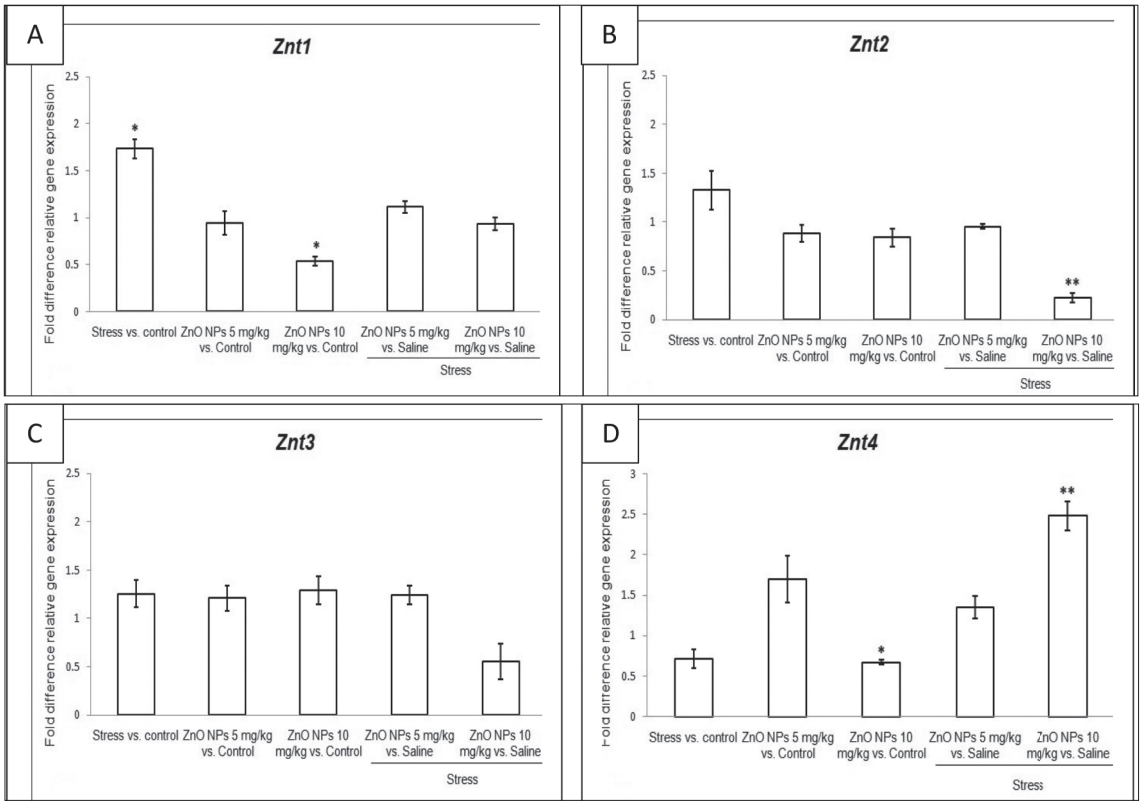

Fig 1. Fold-difference in relative expression of **A-** the *Znt1*, **B-** the *Znt2*, **C-** the *Znt3*, and **D-** the *Znt4* assessed by quantitative Real-Time reverse transcription-polymerase chain reaction, in the hippocampus tissue (* $P<0.05$ vs. the control group, ** $P<0.05$ vs. group receiving acute restraint stress for 90 min and saline). Bars represent mean ± S.E.M. of the genes relative expressions ($2^{-\Delta\Delta ct}$)

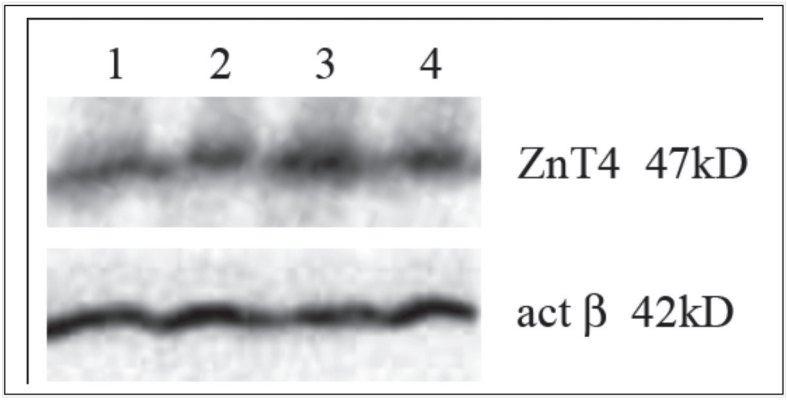

Fig 2. Representing western blot analysis of ZnT4 and act-β (as a control) in the hippocampal tissue of rats (1- The group receiving 10 mg/kg of ZnO NPs, 2- Control group, 3- The group receiving 10 mg/kg of ZnONPs under stress, 4- Stress group). Bands were semi-quantified by image j software and results showed the relative density of ZnT4 protein

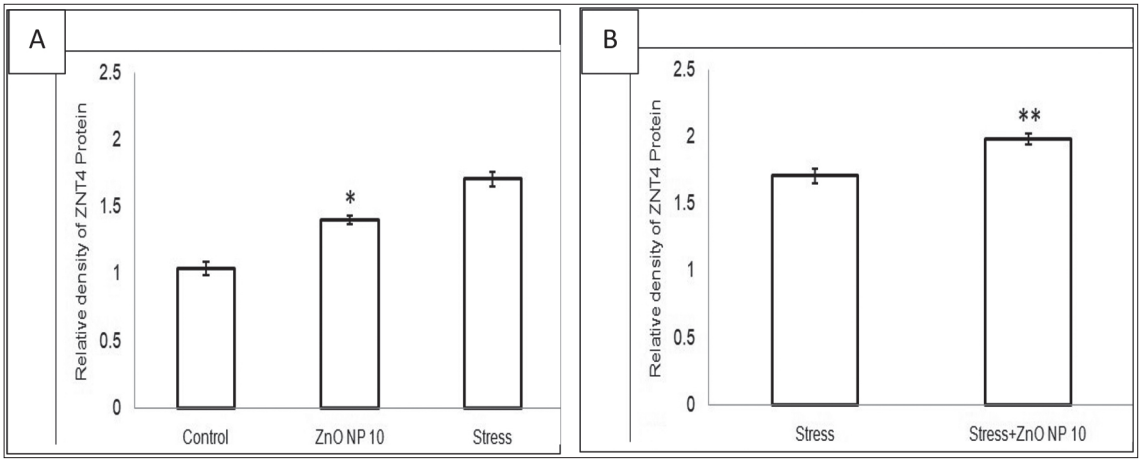

Fig 3. Western blot showing the relative density of ZnT4 protein in hippocampus of **A-** the control group, the group receiving 10 mg/kg of ZnO NPs, and the stress group **B-** the stress group and the group receiving 10 mg/kg of ZnO NPs under stress (* P<0.05 vs. control group, ** P<0.05 vs. group receiving acute restraint stress for 90 min and saline

without stress, the ZnT4 protein level was evaluated using Western blot analysis. The protein level was determined in hippocampal tissue via normalizing with act-β as a control *(Fig. 2)*. Quantitative analysis showed that, in the group receiving 10 mg/kg of ZnO NPs, the level of ZnT4 protein was significantly higher than in the control group (P=0.0004). The ZnT4 protein was significantly differenced in the group receiving 10 mg/kg of ZnO NPs under stress in comparison with the stress group (P=0.0003). Also, no significant change in the level of ZnT4 protein was observed in the stress group compared to the control group (P=0.2207) *(Fig. 3)*.

Discussion

The hippocampus has the highest amount of zinc in the brain and the central nervous system [20]. A great part of the nervous system in hippocampus is affected by stress, bearing in mind that acute stress is instrumental in prompting the zinc level in hippocampus [21,22]. Neurological complaints, depressions and addictions have shown to be stress-related problems [23,24]. The methodology employed in this paper involved deliberations on potential effects of acute restraint stress and ZnO NPs injection on expression of the *Znt1*, *Znt2*, *Znt3*, and *Znt4* genes in hippocampus of various male rats. Each of the latter was subjected to the injection of 5 or 10 mg/kg of ZnO NPs with or without restraint stresses. The effects of 10 mg/kg of ZnO NPs dosages with or without restraint stress on ZnT4 protein expression levels were further assessed.

It was found that the expression of *Znt1* was significantly up-regulated in the observation group subjected to restraint stress. According to the previous studies the acute restraint stress reduces the level of zinc in the serum, while increasing it in the hippocampus [18]. In addition, corticosterone can increase intracellular zinc levels in the hippocampus, causing the production of a type of inactive oxygen in the hippocampal cells [25]. The results are in congruence with the proven homeostasis role of the ZnT1 protein in zinc efflux from the intercellular space. This reportedly acts as an effective protection agent against potential zinc cytotoxicity of nervous system [26].

In this study, there were no significant statistical differences in the expression of studied genes in the different groups subjected to 5 mg/kg of ZnO NPs. However, the expression of *Znt1* and *Znt4* genes was significantly up-regulated in groups subjected to 10 mg/kg of ZnO NPs. Some previous

studies have indicated that ZnO NPs significantly enhances the expression of multiple zinc transporter genes like *Znt1*, *Znt2*, and *Znt4* in mice [17]. Also, it has been reported that zinc deficiency decreases ZnT1 expression in the hippocampus of rats [27]. Considering the role of the *ZnT1* protein in the extraction of cytosolic zinc into the extracellular space and the ZnT4 protein function in the transfer of zinc from the cytoplasm to the Golgi network [11], the increased genes expression in response to ZnO NPs is justified.

In this study, we could indicate that injection of ZnO NPs in the presence of acute restraint stress changed the expression of *Znt2* and *Znt4 in* the rat hippocampus. The ZnT2 protein is responsible for transporting cytosolic zinc to secretory granules and exocytosis [3]. Therefore, by reducing the expression of *Znt2*, the transfer of zinc from the cell is slowed down and zinc is accumulated in the intracellular organelles by the ZnT4 protein [14]. Our results showed that the mutual changes in expression of these two genes could be the cause of zinc homeostasis during stress induction and receiving of ZnO NPs. The close relationship between stress and zinc homeostasis disorder has been previously reported [2]. In conjunction with our results some studies indicated that administration of ZnO NPs under acute stress conditions produce positive effects on behavioral responses [28]. This may indicate the proper accumulation of zinc in the hippocampus by regulation of *Znt2* and *Znt4* genes expression.

In this study, for the first time, we could indicate that acute injection of ZnO NPs in the presence and absence of acute restraint stress could increase the expression of *Znt4* in the rat hippocampus.

Zinc homeostasis in the hippocampus differs from increased zinc levels due to stress or receiving ZnO NPs. During stress, up-regulation in *Znt1* gene expression causes zinc to be exported to the extracellular space. While receiving ZnO NPs, the expression of the *Znt4* gene increases to store the imported zinc in the organelles.

The *Znt3* gene expression showed no significant change among our different groups. Similarly, *ZnT3* mRNA expressions were not affected in the whole brain of rats during zinc deficiency [27]. It has been shown that the expression of the *Znt1*, *Znt2*, and *Znt4* genes was up-regulated, while the levels of *Znt3* mRNAs were unchanged in the cerebral cortex after transient ischemia [29]. More studies are needed to investigate the effects of different doses and treatment times of ZnO NPs supplementation on the *Znt3* gene expression.

In addition, the ZnT4 protein level was analyzed using Western blot because of up-regulation of the *Znt4* gene expression in hippocampus due to receiving 10 mg/kg of ZnO NPs with or without stress. Results have indicated that ZnO NPs significantly increased ZnT4 protein levels in rats with and without stress. Likewise, Zinc-containing imipramine has reportedly been instrumental in increasing ZnT4 protein level in prefrontal context of mice subjected to stress conditions [30]. It seems that the up-regulation of *Znt4* expression followed by an increase in ZnT4 protein levels is an important mechanism of zinc homeostasis in the hippocampus due to receiving zinc-containing drugs.

The advantage of using the form of ZnO NPs is determined by the fact that other types of zinc, such as the usual ZnO or zinc methionine, have not previously altered the expression of *Znt1*, *Znt2* and *Znt5* genes [31]. ZnT3 protein has been shown to play a protective role against oxidative stress and ER stress (Endoplasmic reticulumstr) in the body [32]. Studies show that mice in which the Znt3 gene has been knocked out are less able to adapt to chronic stress and exhibit anxious and depressive behaviors [33,34]. Sudden changes in zinc signaling, or in other words, sudden changes in zinc levels, induce a certain stress to the cell, which is called zinc stress (zinc stress) and can lead to dysfunction of cells, especially in the nervous system [35].

One of the causes of this problem (zinc stress) is psychological and behavioral stimuli such as psychological stress [36]. Regulating the expression of zinc transporter genes by receiving the appropriate dose of a drug containing zinc nanooxide after induction of stress can prevent dysfunction of the central nervous system. Because changes in the expression of zinc-transferring family genes vary during different psychological stimuli such as psychological stress, physical stress, anxiety, and depression; The study of these genes in various diseases and changes in their expression due to receiving different forms of the element zinc has been considered. On the other hand, the expression of the studied genes is different in different parts of the body due to the difference in the amount of zinc absorption in tissues, cells and intracellular organs [36]. Stress-induced centralization, as well as zinc-containing drugs, does not necessarily lead to a single result of comparing the expression changes of the studied genes with other tissues and cells. Our results in this study showed that, as in depression in rats, stress increased the expression of the *Znt1* gene and did not alter the expression of the *Znt2*, *Znt3* and *Znt4* genes, resulting in a loss of intracellular zinc storage. This can be the cause of stress-induced nervous system disorders due to lack of zinc [36] As a result of receiving zinc oxide nanoparticles under stress conditions, zinc homeostasis is in order to maintain intracellular zinc storage, which is done by altering the expression of *Znt2* and *Znt4* genes. *Znt4* gene expression is mediated by the uptake of zinc oxide nanoparticles under conditions with and without stress to increase cellular storage, which is confirmed by the presence of this protein [37].

Finally, we investigated for the first time the effects of zinc oxide nanoparticles on changes in the expression of four zinc transporter genes in the hippocampus of rats under stress and non-stress conditions, which revealed part of

the mechanism of zinc regulation. These findings can be valuable in the field of pharmacy and medicine. Evaluation results showed the application of ZnO NPs to be valuable in forecasting the necessary investigation in the fields of biomedicine and pharmacogenetics as substantiated by comparable recent researches. The present research showed ZnO NPs to be an effective catalyst to transform the expression of zinc transporter genes in hippocampus of rats subjected to stress and non-stress experimental contributions. Moreover, the zinc homeostasis in rat's hippocampus was shown to vary on its own under restraint stress experimental conditions.

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Author Contributions

MN, AD, planned and designed the research. HG and MK provided help in the experiment. All authors discussed the results and contributed to the final manuscript.

Ethical Principles and Publication Policy

All authors state that the paper presented contains the main results of the research and that the study data have been properly analyzed and prepared for publication using sufficient and appropriate sources.

Declaration of Conflict of Interest

None.

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