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EFFECT OF ZINC OXIDE NANOPARTICLES ON OXIDATIVE STRESS OF CEREBELLUM IN MALE RATS

RAFIEIRAD M[1]*, VALIPOUR CHEHARDACHERIC S[2]

1, 2: Department of Biology, Faculty of Sciences, Izeh Branch, Islamic Azad University, Izeh, Iran

***Corresponding Author: Rafieirad.m@gmail.com**

ABSTRACT

Due to the wide application of nanoparticles, human body is exposed to oxidative stress damages caused by nanoparticles. The purpose of this study is to investigate effects of zinc oxide nanoparticles on oxidative stress markers of cerebellum among mature male rats. In this experiment, Wistar male rats (200-250g) were divided into saline group (injection control group) and three groups receiving 1.25, 2.5, and 5 (mg/kg) zinc oxide nanoparticles. Immediately after administering the drug, the rats were beheaded, their brains were removed, and the oxidative stress and anti-oxidant marker such as malondialdehyde and thiol groups in the cerebellum area were measured. Findings of this study showed that level of malondialdehyde in the groups receiving the zinc oxide dosage of 5 mg/kg significantly decreased and thiol in the groups receiving zinc oxide nanoparticles increased compared to the control group, although this increases was not significant. The conclusion was that changes in the oxidative stress markers, i.e. increase in thiol and decrease in malondialdehyde, were probably due to anti-oxidative effects which could also provide another evidence for the anti-oxidative effects of zinc oxide nanoparticles.

Keywords: zinc oxide nanoparticles, cerebellum; oxidative stress, rats

INTRODUCTION

Zinc oxide nanoparticles are among the most widely used nanoparticles with many applications in industry, medicine, and nutrition **[1]**. In addition to absorption by

inhalation, absorption can also take place via other paths (such as dermis). Absorption via olfactory nerve and blood-brain barrier are the most important paths which have been studied so far **[2]**. This path has been extensively examined for drug delivery to the brain **[3]**. Considering the movement of nanoparticles, permeability of the blood-brain barrier depends on charge of nanoparticles. This factor allows a great number of cationic nanoparticles, compared to neutral and ionic particles, to pass the barrier by totally breaking it **[3]**. So far, few cases of effects caused by ZnO nanoparticles on CNS have been identified **[4]**. Several studies have indicated the useful effects of this compound such that acute prescription of ZnO nanoparticle shows greater and more significant anxiolytic effects within animal models than its conventional type **[5]**. The presence of zinc in the rat cerebellum and the uptake of 65Zn in this area has been known since the 1960s Later, in the eighties, analytical studies on the determination of this metal showed that the cerebellum presents with the second highest amount of Zinc in the Central Nervous System (CNS) **[6]** . Also, chronic consumption of nano-ZnO has a significant antinociceptive effect with and without aerobic exercises among rats, compared to its normal type **[7]**. This issue can provide a new approach in the related treatment **[8]**. Zinc element is one of the most important elements of ZnO nanoparticles whose anti-oxidant effects have been well examined in some studies **[9]**. Zn also acts as an anti-oxidant in the central nervous system, particularly in the brain **[10]**. Compared to other soft tissues, human brain contains a considerable amount of Zn **[11]**. Deficiency in Zn is related to a higher level of tissue oxidative destruction as well as ROS formation [12]. For this reason, it seems that Zn separated from compounds such as nano-zinc oxide or zinc chloride is responsible for changes in physiologic processes. Therefore, nano-zinc oxide can have two or more effects which, bedsides its receptive effects, make its oxidative and anti-oxidative applications inside the body necessary and worthy of investigation. Generally, one destructive aspect of oxidative stress reactions is the production of ROS which contains free radicals and peroxides and their decrease would increase the life span **[12]**. Disorders caused in the oxidative state are among the consequences of producing peroxides and free radicals, which causes damage to cellular components including proteins, lipids, and DNAs **[13]**. Lipid peroxidation

refers to the oxidative destruction of lipids. ROS decreases the unsaturated lipids and produces malondialdehyde (MDA). Malondialdehyde is an organic compound and a reactive aldehyde with the chemical formula $CH_2(CHO)_2$, which is introduced as the final product of lipid peroxidation **[14]**. Thiol groups (-SH) are also sensitive (responsive) to oxidative damage and their decrease is an important sign of oxidative stress **[15]**. Therefore, the purpose of this study is to investigate oxidative stress markers in the cerebellum area among mature male rats.

MATERIALS AND METHODS

In this experimental study, Wistar rats within the weight range of 200-250 g were provided from Laboratory Animal Proliferation Center, Jondi Shapour University of Medical Sciences, Ahvaz, Iran, and were stored under standard conditions at 22±2 ^oC and humidity of 55-60%. Four animals were kept in each cage with the photoperiod of 12 h lightness and 12 h darkness and sufficient food and water. Before beginning the experiments and in an attempt to familiarize the rats with the laboratory condition, they were put in separated cages for one week. Then, the animals were randomly divided into the following groups: 1) Saline-recipient group (injection control group) (n=7): they only received 10ml/kg saline and then were examined for oxidative stress markers; 2) three groups receiving different amounts of ZnO nanoparticles (1.25, 2.5, and 5 mg/kg) who were examined for the oxidative stress markers after receiving the drug. All the tests were conducted on the rats according to code of ethics in research approved by Vice Chancellor for Research and Technology, Islamic Azad University. A sufficient amount of nano-ZnO, a product of Lulitech (Germany) for the daily needs, with the size of 50-80 nm was dispersed in 0.9% saline before the tests by ultrasonic bath device for 15 min. Also, before each injection, again the mixture was vibrated by the shaker device for 1 min [4]. The drugs were administered intraperitoneally on a single dose basis **[16]**.

Biochemical evaluation of oxidative stress:

To investigate oxidative stress markers, the animals were euthanized. Then, their brains were removed and the biochemical analysis was conducted on the cerebellum. Level of MDA was measured on the basis of reaction with thiobarbituric acid (TBARS). MDA is a lipid peroxidation marker, formed by free radicals. MDA enters the reaction with TBA to produce a red complex with the maximum amount of absorption at 532 nm wavelength.

3ml phosphoric acid (1%) and 1ml TBA (0.6%) were added to 0.5 ml of a homogenous solution in a centrifugal tube and the mixture was placed in a boiled water bath for 45 min. After the mixture was cooled, 4ml n-butanol was added to it and the mixture was stirred for 1 min and then was centrifuged at 2000g for 20 min. The colorful layer was transferred to a new tube to measure its absorption at 532nm wavelength. TBARS level was determined using 3,3,1,1-tetra-methoxy-propan as a standard reference. MDA standard curve was also prepared. For plotting the standard curve, it is necessary to prepare MDA standard solution and measure its light absorbance using spectrophotometer. 0.5ml of the standard tetra-methoxy-propan solution with the concentrations of 0.5, 1, 2, 4, 6, 8, and 10 micro molar (mM) was taken and 3ml of 1% phosphoric acid was added. The rest of the process was carried out similar to MDA evaluation process **[17]**.

Evaluation method of the amount of thiol groups (-SH): Sum of thiol groups was calculated using DTNB (Ellman's reagent) as the reagent. This reagent reacts with –SH groups to produce a yellow-colored complex with maximum absorption at 412nm wavelength. 1ml of tris-EDTA buffer (pH=8.6) was added to 50µl homogenous solution and absorptivity of the samples was read separately at the wavelength of 412 nm against tris-EDTA buffer (A1). Then, 20µl of DTNB reagent (10 mM in methanol) was added to the mixture and absorptivity was read after 15 min (at room temperature) (A2). Absorptivity of DTNB reagent was also read as a blank (B). Sum of thiol concentration (millimole) was calculated using the following equation **[17]**:

Sum of thiol concentration (millimole)=(A2-A1-B)×1.07/0.05×13.6

RESULTS AND DISCUSSION

Data were presented as mean ± standard error of mean (mean±SEM). Results were analyzed using SPSS v.21 software. In order to examine the results within different test groups, one-way ANOVA tests and LSD post-hoc test were used. In all the cases, differences between the groups were reported to be significant ($P<0.05$).

RESULTS

Biochemical analysis of oxidative stress markers

Standard curve: In order to plot the standard curve, it is required to prepare the standard MDA solution and measure light absorptivity using spectrophotometer. 0.5ml

of the standard tetra-methoxy-propan solution with the concentrations of 0.5, 1, 2, 4, 6, 8, and 10 micro molar (mM) was taken and then 3ml of 1% phosphoric acid was added to it. The rest of the process was carried out similar to the MDA evaluation process.

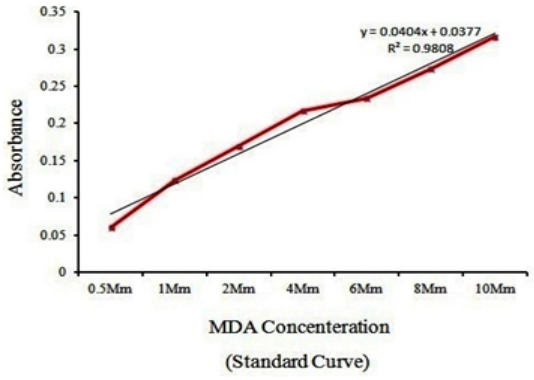

Figure 1: Standard curve for calculating the amount of MDA

Amount of malondialdehyde (MDA), as a marker, showed the lipid peroxidation in the cerebellum area of the rats receiving saline and in the cerebellum area of the rat groups receiving different dosages of nano-ZnO. As shown in the figure, difference in the amount of MDA was not significant between the cerebellum area of the rats receiving 1.25mg/kg nano-ZnO and saline group, while a significant decrease ($P<0.001$) was observed in the MDA amount of cerebellum in the rats receiving 5mg/kg dosage of nano-ZnO compared to the saline group.

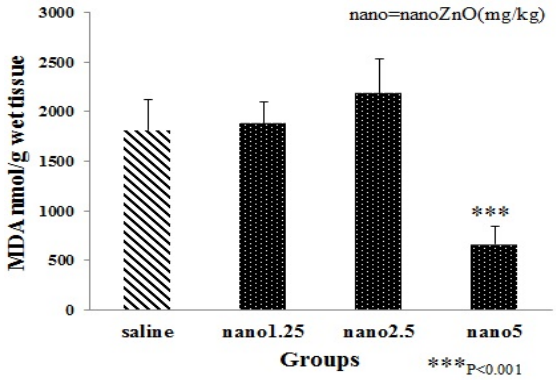

Figure 2: Comparing the amount of malondialdehyde (MDA) in cerebellum area of the rats receiving saline and rat groups receiving different dosages of nano-ZnO

Total content of thiol groups (-SH): This section shows the total content of thiol groups (-SH) in the cerebellum region of male rats receiving saline and male rats receiving different dosages of ZnO nanoparticles. As shown in this figure, total

content of thiol group in cerebellum of the rats receiving different dosages of ZnO nanoparticles increased; however, it did not show any significant increase compared to the saline group.

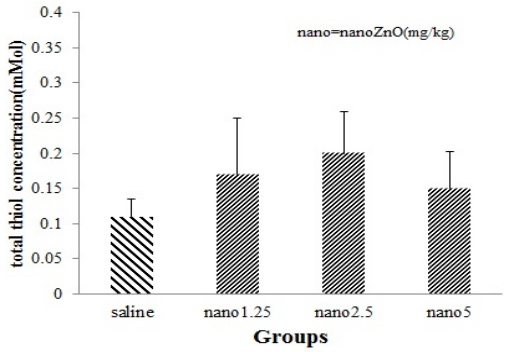

Figure 3: Comparing the total amount of thiol groups in cerebellum of the rats receiving saline and rat groups receiving different dosages of nano-ZnO

DISCUSSION

Results obtained in this study showed that acute prescription of ZnO nanoparticles changed the oxidative stress markers such that increasing the amount of drug significantly decreased the MDA, as a lipid peroxidation marker, compared to the group receiving saline, while total amount of thiol groups (-SH) increased. Therefore, by decreasing MDA and increasing thiol groups, this compound showed an anti-oxidative effect in the cerebellum, which was in agreement with the results of previous studies, suggesting that deficiency in zinc is related to a higher level of tissue oxidative damage as well as ROS formation **[12]**. Oxidative stress is caused by the lack of balance between free radicals and oxygen reactive species from one side, and anti-oxidative defense from the other. In other words, in aerobic biological systems, anti-oxidative defense mechanisms are designed to confront the free radicals and oxygen reactive species to neutralize or minimize the destructive effects of these aggressive agents. Some of the elements of this defense system, including superoxide dismutase enzymes, glutathione peroxidase, and catalase, as well as uric acid, bilirubin, and molecules containing thiol group, are formed inside the body, while some others must be supplied by nutrition, such as vitamin C and β-carotene. Under oxidative stress conditions, many macromolecules are damaged and lipid peroxidation, protein and DNA oxidation, enzyme inactivation, and dysfunction of different membranes occur **[18]**. ROS production and oxidative stress induction are among the main mechanisms of the toxicology of environmental nanoparticles.

Large quantities of ROS can be produced, even when only a limited amount of ZnO nanoparticles enter the cells **[19]**. When nanoparticles are subjected to lysosome's acidic environment **[20]**or interfere with oxidative organelles such as mitochondria **[21]**, nanoparticles can directly produce or induce ROS formation.

However, nanoparticles may have interference with proteins containing metal and anti-oxidants (from bodily and intracellular fluids) which might slightly change the surface properties of the nanoparticles and decrease their toxicity **[22]**. While the mechanism via which nanoparticles induce inflammatory effects are not known, it is suggested that they produce reactive oxygen species (ROS) and thus moderate intracellular calcium concentration, activate the transcription factors, and induce production of cytokines **[23]**. Previous studies have shown that subjecting to the low level of nano-ZnO concentration produces genotoxic caused by lipid peroxidation and stress oxidative in epidermal cells **[24]**; on the contrary, findings of this study showed anti-oxidative effects for higher dosage of ZnO nanoparticles in the cerebellum of rats, which was in agreement with the studies on protecting the action of ZnO nanoparticles on cellular membrane and inhibition of lipid peroxidation **[25]**. Experimental evidence has shown that start and progression of neurodegenerative diseases such as Alzheimer, Parkinson's, selection disease, with oxidative stress and accumulation of high concentration of metals (such as copper, aluminum, zinc, and especially iron) in cerebellum areas are related to cellular dysfunction and damage **[26]**. Formation and inhibition of the activity of free radicals in the biological systems are closely related to pathologic disease. In healthy humans, ROS and its related oxidative stress are examined by a combination of anti-oxidative activities **[27]**. Human cells develop a powerful anti-oxidative defense against oxidation reactions. Particularly, they contain enzymatic and non-enzymatic anti-oxidative molecules, including thiol groups [mainly glutathione (GSH)] which are useful in defending. One of the key chemical barriers against damage caused by stress is the oxidation balance and sulfhydryl (SH)/disulfide reduction where thiol groups with low molecular weight are reversibly oxidized to disulfide and/or protein in combination with disulfide in response to an oxidative stress **[28]**. Although currently there are limited

information about environmental toxicology and effect of human toxicology of nanomaterials, considering chemo-physical properties of nanomaterials, it is predicted that these materials have interaction with biological components and have large influences on macromolecular behavior and properties, cells, and body of a living organism **[29]**. Due to great diversity of nanomaterials compared to other conventional chemical materials, they have a large number of unique properties **[30]**. Therefore, it is recommended to closely investigate the exact mechanism of this nanoparticle in the presence of effective factors.

CONCLUSIONS

Thiol groups (-SH) are responsive to oxidative damage and their decrease is an important sign of oxidative stress **[15]** and this group has an important role in cellular anti-oxidative defense system **[31]**. Also, it decreases the level of MDA by consuming Zn nanoparticles, thus, it can be suggested that the changes are probably caused by anti-oxidative effects which could be another evidence for anti-oxidative effects of ZnO nanaoparticles.

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