Effects of nanoparticle zinc oxide on emotional behavior and trace element homeostasis in rat brain

Article *in* Toxicology and Industrial Health · June 2013

DOI: 10.1177/0748233713491802 · Source: PubMed

8 authors, including:

Salem Amara
Faculty of Sciences of Bizerte
29 PUBLICATIONS **533** CITATIONS
SEE PROFILE

K. Omri
The Faculty of Science of Gabes
52 PUBLICATIONS **592** CITATIONS
SEE PROFILE

J. El Ghoul
Imam Muhammad bin Saud Islamic University
47 PUBLICATIONS **591** CITATIONS
SEE PROFILE

El Mir Mabrouk Lassaad
The Faculty of Science of Gabes
220 PUBLICATIONS **2,179** CITATIONS
SEE PROFILE

Toxicology and Industrial Health
http://tih.sagepub.com/

Effects of nanoparticle zinc oxide on emotional behavior and trace element homeostasis in rat brain

Salem Amara, Imen Ben Slama, Karim Omri, Jaber EL Ghoul, Lassaad EL Mir, Khemais Ben Rhouma, Hafedh Abdelmelek and Mohsen Sakly

Toxicol Ind Health published online 6 June 2013
DOI: 10.1177/0748233713491802

The online version of this article can be found at:
http://tih.sagepub.com/content/early/2013/05/31/0748233713491802

Published by:
SAGE
http://www.sagepublications.com

Toxicology and Industrial Health
1–8

DOI: 10.1177/0748233713491802
tih.sagepub.com

Effects of nanoparticle zinc oxide on emotional behavior and trace elements homeostasis in rat brain

Salem Amara[1], Imen Ben Slama[1], Karim Omri[2], Jaber EL Ghoul[2], Lassaad EL Mir[2,3], Khemais Ben Rhouma[1], Hafedh Abdelmelek[1] and Mohsen Sakly[1]

Abstract

Over recent years, nanotoxicology and the potential effects on human body have grown in significance, the potential influences of nanosized materials on the central nervous system have received more attention. The aim of this study was to determine whether zinc oxide (ZnO) nanoparticles (NPs) exposure cause alterations in emotional behavior and trace elements homeostasis in rat brain. Rats were treated by intraperitoneal injection of ZnO NPs (20–30 nm) at a dose of 25 mg/kg body weight. Sub-acute ZnO NPs treatment induced no significant increase in the zinc content in the homogenate brain. Statistically significant decreases in iron and calcium concentrations were found in rat brain tissue compared to control. However, sodium and potassium contents remained unchanged. Also, there were no significant changes in the body weight and the coefficient of brain. In the present study, the anxiety-related behavior was evaluated using the plus-maze test. ZnO NPs treatment modulates slightly the exploratory behaviors of rats. However, no significant differences were observed in the anxious index between ZnO NP-treated rats and the control group ($p > 0.05$). Interestingly, our results demonstrated minimal effects of ZnO NPs on emotional behavior of animals, but there was a possible alteration in trace elements homeostasis in rat brain.

Keywords

ZnO nanoparticles, emotional behavior, elevated plus-maze, trace elements, rats

Introduction

The rapidly developing field of nanotechnology is becoming a potential source for human exposure to nanoparticles (NPs). In recent years, nanomaterials have found diverse applications in various aspects of human life, from cosmetics and medical products to water purification and solar energy capture (Staggers et al., 2008). With the industrialization and increasing public exposure, nanosized materials have received more concern over their biosafety and underlying effects on the central nervous system (CNS) (Yongling et al., 2012). Zinc oxide (ZnO) NPs are amongst the most commonly utilized nanomaterials in consumer products. Some studies have indicated that ZnO NPs affected functions of different cells or tissues (Osmond and McCall, 2010; Song et al., 2008), biocompatibility (Rasmussen et al., 2010), and neural tissue engineering (Gabriel, 2008), but little was known about the influence on CNS and CNS-related diseases. ZnO NPs were suggested to modulate synaptic transmission *in vitro* (Zhao et al., 2009) and to change the spatial cognition capability. Recent experimental studies of acutely isolated rat hippocampal CA3 pyramidal neurons show that exposure to ZnO NPs results in increases in amplitude of sodium (Na) current and

[1] Laboratoire de Physiologie Intégrée, Faculté des Sciences de Bizerte, Tunisia
[2] Laboratory of Physics of Materials and Nanomaterials Applied at Environment, College of Sciences in Gabes, Tunisia
[3] Department of Physics, College of Sciences, Al Imam Mohammad Ibn Saud Islamic University (IMSIU), Riyadh, Saudi Arabia

Corresponding author:
Salem Amara, Laboratoire de Physiologie Intégrée, Faculté des Sciences de Bizerte, Jarzouna 7021, Tunisia.
Email: amara_salem_fsb@yahoo.fr

excitability of neurons, these mechanisms may contribute to cell apoptosis (Remillard, 2004; Zhao et al., 2009). ZnO NPs increase neuronal excitability by enhancement of Na influx leading to the accumulation of intracellular calcium (Ca). Furthermore, concentration of intracellular Ca is a crucial and essential source of long-term potentiation and long-term depotentiation in both inducing and maintaining phases. Such effects manifest dose dependency but no size-dependent toxicity (Deng et al., 2009).

Trace elements are involved as essential parts of many physiological functions in the brain's biochemistry such that deficiency or excess of these metals resulted in CNS disorders (Madsen and Gitlin, 2007). Trace elements are essential for normal brain functions. Tiny amounts of these elements help in the formation of neurotransmitters and involved in the antioxidant defense and intracellular redox regulation and modulation of neural cells (Fayed, 2010). Recently, Yongling et al. (2012) reported that ZnO NPs could ameliorate the behavioral and cognitive impairment in mice with depressive-like behaviors, probably by upregulating neuronal synaptic plasticity and functions. Moreover, ZnO NPs may regulate ionic homeostasis and the physiological functions of neurons and have potential influence on CNS, which shed light on the possible application and treatment in neurotransmitter system disorders in CNS (Zhao et al., 2009).

The exact effect of ZnO NPs on biological systems has not yet been adequately clarified. In order to answer the question of safety of ZnO NPs injection into the organism and their use in medicine as substance for biopreparations, the recourse to animal models is clearly needed to evaluate the effect of ZnO NPs on brain function and behavior. Therefore, the concentration of trace elements in the brain reflects the physiological importance of these elements in the brain function. To meet our aim, we investigated in the *in vivo* study the effect of ZnO NPs on the neurobehavioral performance in plus-maze test and the concentration of essential trace elements (zinc (Zn), iron (Fe), Ca, Na, and potassium (K)) in the brain of Wistar rats.

Materials and methods

Animals and treatment

Male Wistar rats (SIPHAT, Tunisia), weighing 150–155 g at the beginning of the experiment were randomly assigned to control or ZnO NP-treated rat groups ($n = 6$ in each group). Animals were housed in groups of 5 or 7 in cages at 25°C, under a 12:12 light–dark cycle (lights on at 7:00 a.m.), with free access to food and water.

We prepared ZnO NPs suspension using physiological saline solution (9% sodium chloride (NaCl)). The powdered ZnO NPs (20–30 nm) were dispersed in the fresh sterilized physiological saline solution, and the suspension was ultrasonicated for 20 min to disperse completely as well as possible (Ultrasonic Liquid Processor: Sonicator 4000, Misonix, Farmingdale, New York, USA). ZnO NPs suspension was vortexed for 1 min before injections. Treated group received a moderate dose of ZnO NPs (25 mg/kg) by intraperitoneal (i.p.) injection every day for 10 days. The control group received a similar i.p. injection of physiological saline solution (0.9% NaCl). Treated and control groups were used to evaluate anxiety behaviors using elevated plus-maze test 24 h after receiving the latter injection. Animals were cared for in compliance with the code of practice for the Care and Use of Animals for scientific purposes. The experimental protocols were approved by the Faculty Ethics Committee.

NPs preparation and characterization techniques

The nanocrystalline ZnO aerogels were prepared by dissolving 2 g of zinc acetate dehydrate ($(ZnCH_3COO)_2{\cdot}2H_2O$) in 14 ml of methanol under magnetic stirring for 2 h. The water for hydrolysis was slowly released by esterification of acetate with methanol. NP aerogel was obtained by supercritical drying in ethyl alcohol (EtOH) (El Mir et al., 2007, 2008). The crystalline data were obtained by x-ray diffractometry (Bruker D8 Advance; 40 kV, 30 mA, Germany). To determine the lattice parameters of the different phases, the diffraction, x-rays were collected by scanning between $2\theta = 5°$ and 70° in 0.02° steps. To examine the size and morphology of oxide nanocrystals, transmission electron microscopy (TEM; JEOL JEM-200 CX, JEOL Co., Japan) operating at 200 kV was used. The sample preparation for TEM observation was as follows: the powder was first put in EtOH, and the ultrasonic dispersed solution was dropped on a Copper (Cu) net. For photoluminescence measurements; the 337.1 nm laser line of a Laser Photonics LN 100 nitrogen laser was used as an excitation source. The emitted light from the sample, collected by an optical fiber on the same side as the excitation, was analyzed with a Jobin-Yvon

Spectrometer HR460 (French company of optical instrumentation and Spectroscopy) and a multichannel CCD detector (France) (2000 pixels). The photoluminescence excitation measurements were performed on Jobin-Yvon Fluorolog 3-2 spectrometer (France) equipped with 450 W xenon lamp as the excitation source. The emission spectra were corrected for the spectral response of the excitation source. The low temperature experiments were carried out in a Janis VPF-600 (France) Dewar with variable temperature controlled between 78 and 350 K (El Mir et al, 2007, 2008).

Determination of trace elements content

The day following behavioral testing, the animals were killed and the brain samples were removed with no previous perfusions. Brains were rapidly washed with cold phosphate-buffered saline, weighed, and then immediately frozen on dry ice and finally digested in 2 ml of concentrated nitric acid in pressurized Teflon containers at 160°C for 3 h. The concentrations of trace elements (Zn, Fe, Ca, Na, and K) in the brain were determined with an atomic absorption spectrometer (Avanta, GBC, Scientific Equipment, Victoria, Australia). After cooling at room temperature, samples were diluted with 10 ml of deionized water (Congui et al., 2000). Trace element analyses were performed using acetylene gas as fuel and air as an oxidizer. Operational conditions were adjusted to yield optimal determination. The calibration curves were prepared separately for all the trace elements by running suitable concentrations of the standard solutions. Digested samples were aspirated into the fuel rich air–acetylene flame and the concentrations of the trace element were determined from the calibration curves. Average values of three replicates were taken for each determination. Suitable blanks were also prepared and analyzed in the same manner. The detection limits for Fe, Zn, Ca Na, and K were 0.05, 0.008, 0.025, 0.04, and 0.05 ppm, respectively. Trace elements concentration was calculated in micrograms per gram of the dry mass of tissues.

Emotional behavior testing

The elevated plus-maze test was used according to the previously published methodologies (Frye et al., 2000; Maaroufi et al., 2009; Pellow et al., 1985; Roy and Chapillon, 2004). The maze was made of clear painted wood. The arms were 50 cm long and 10 cm width, and the apparatus was elevated at a height of 60 cm. The closed arms were surrounded by a 50 cm wall, while open arms had 0.5 cm edges in order to maximize open arm entries (Treit et al., 1993). The test was 5 min long and began with the placement of a rat in the center of the maze, with its head facing toward open arm. The time spent in the different parts of the maze (i.e. open arms, closed arms, and central part) was recorded along with the number of entries into closed and open arms (Pellow and File, 1986; Rodgers and Dalvi, 1997). In addition, total activity into the maze was evaluated *via* the total number of arm and central part entries. Since total activity was found to be different among groups, a ratio for open arm entries and open arm time was also calculated. The maze was cleaned with a 10% alcohol solution between each animal.

Data presentation and statistical analysis

Data were analyzed using Stat View 512+ software (Abacus Concept Inc., Piscataway, NJ, USA). Means were given with SEM and were subjected to the unpaired Student's t test. The level of significance was set at $p < 0.05$.

Results

Animal observation and effect on body weight

No toxic signs or mortality was observed related to ZnO NPs zinc injection. Also, there were no significant changes in the body weight and the coefficient of brain (as the ratio of tissues wet weight (mg) to body weight (g)) between the exposed rats and the control group (Figures 1 and 2).

Brain trace elements concentration

Zn concentrations in control and ZnO NPs-exposed group are shown in Figure 3. Subacute ZnO NPs treatment caused no significant increase in Zn content in the homogenate brain; values were assessed as 439.0 ± 174.5 versus 258.85 ± 44.0; $p > 0.05$. In contrast, the same treatment significantly decreased the Fe level (15.84 ± 4.25 vs. 28.84 ± 4.17; $p < 0.05$; Figure 4) and the Ca^{2+} content (210.7 ± 41.63 vs. 403.64 ± 57.76; $p < 0.05$; Figure 5). However, the concentration of Na^{+} (449.33 ± 25.51 vs. 464.47 ± 21.07; $p > 0.05$) and K^{+} (874.84 ± 48.75 vs. 869.21 ± 53.28; $p > 0.05$) remained unchanged (Figures 6 and 7).

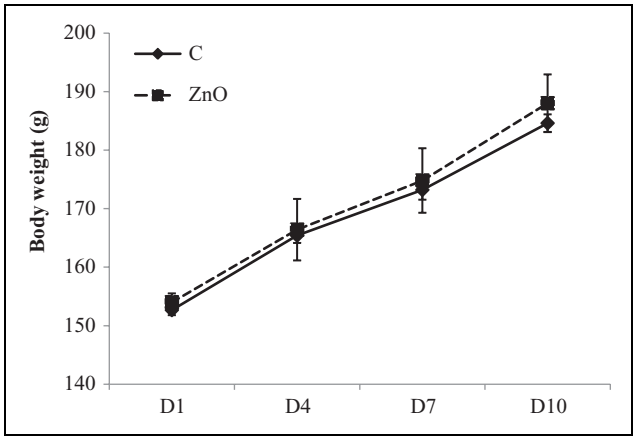

Figure 1. Body weight of control and ZnO nanoparticle-treated rats. Data represent the means ± SEM of six animals per group. ZnO: zinc oxide.

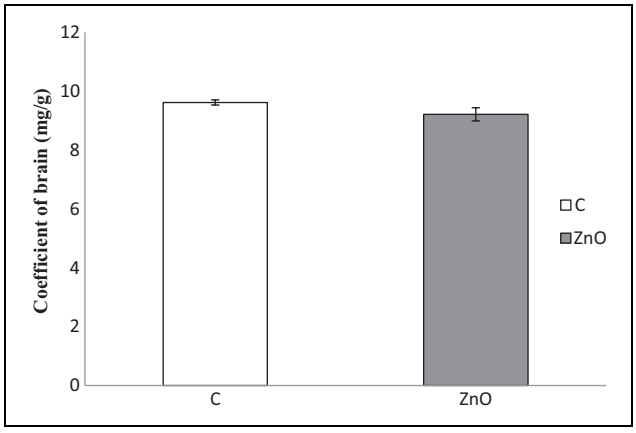

Figure 2. Effect of ZnO nanoparticles treatment on the coefficient of rat brain after 24 h. Data represent the means ± SEM of six animals per group. ZnO: zinc oxide.

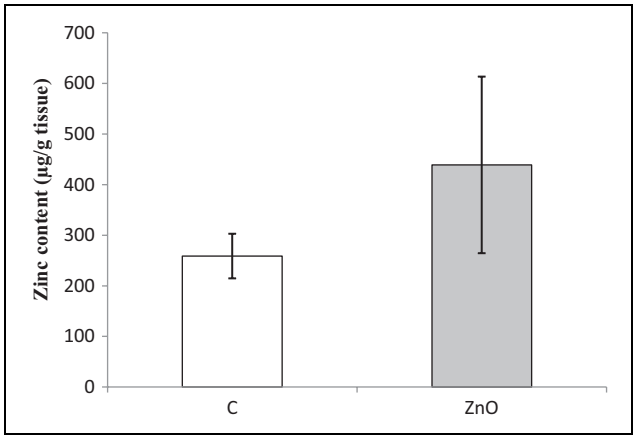

Figure 3. Effect of ZnO nanoparticles treatment on zinc content in the rat brain after 24 h. Data represent the means ± SEM of six animals per group. ZnO: zinc oxide.

Emotional behavior

In the anxiety-related behavior study, there was no evidence of ZnO NPs toxicity at 25 mg/kg in comparison with the control group. There was no statistically significant difference in the emotional behavior parameters analyzed in the plus-maze test such as time in the open arms, time in the close arms, open arm entries, and closed arm entries. However, ZnO NPs exposure decreased the time in the center (28.42 ± 1.74 vs. 127.88 ± 35.19; $p < 0.05$), the second (s) is a time measurement unit (Table 1). Interestingly, subacute ZnO NPs treatment did not change the anxious index (AI), which is expressed as AI = closed arm entries × 100/closed arm entries + open arm entries compared to control group (53.57 ± 8.5 vs. 52.93 ± 8.86; p >005; Figure 8).

Discussion

This is the first description of a multiparametric assay that utilizes the plus-maze test to evaluate the effect of ZnO NPs on the neurobehavioral performance of Wistar rats. This was achieved by the analysis of trace elements content in the homogenate brain. Our results showed no significant differences in body weight and coefficient of brain between treated and the control group. This study suggested that ZnO NPs at this dose (25 mg/kg) could be considered to be relatively nontoxic. By contrast, previous data reported that the toxic effect of ZnO NPs may be attributed to the cytotoxicity of Zn^{2+} ions and the penetration of ZnO NPs into the cell due to the small particle effect (Jin-Hua et al., 2010). However, in the present study, repeated injection of ZnO NPs resulted in a nonsignificant increase in Zn content in the brain. Our model did not indicate clear relationships between ZnO NPs administration and Zn accumulation in brain tissue. In the literature, there was a lack of published data regarding the interactions between ZnO NPs and the blood-brain barrier (BBB). Zn was involved in the protection of BBB against oxidative stress of free radicals and essential for synthesis of coenzymes that mediate biogenic amines synthesis and metabolism (Sandstead et al., 2000; Takeda, 2001). So, high Zn level is essential to maintain homeostasis within the brain and prevent the development of neurological disorders. Recent research has focused on the effects of NPs on the BBB. According to a study by Sharma et al. (2010), administration of silver (Ag), Cu, or aluminum (Al)/aluminum oxide NPs disrupted BBB function and induced brain edema formation. Moreover, Ag NPs induced BBB destruction and astrocyte swelling and caused

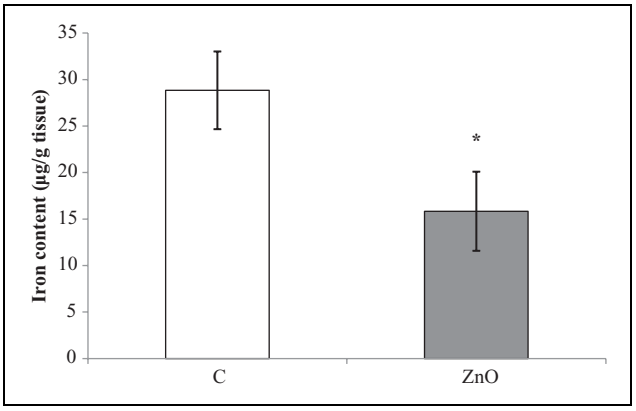

Figure 4. Effect of ZnO nanoparticles treatment on iron content in the rat brain after 24 h. Data represent the means ± SEM of six animals per group. *$p < 0.05$ compared to control. ZnO: zinc oxide.

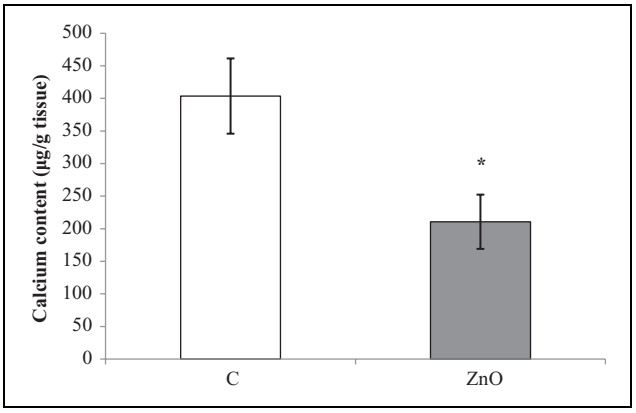

Figure 5. Effect of ZnO nanoparticles treatment on calcium content in the rat brain after 24 h. Data represent the means ± SEM of six animals per group. *$p < 0.05$ compared to control. ZnO: zinc oxide.

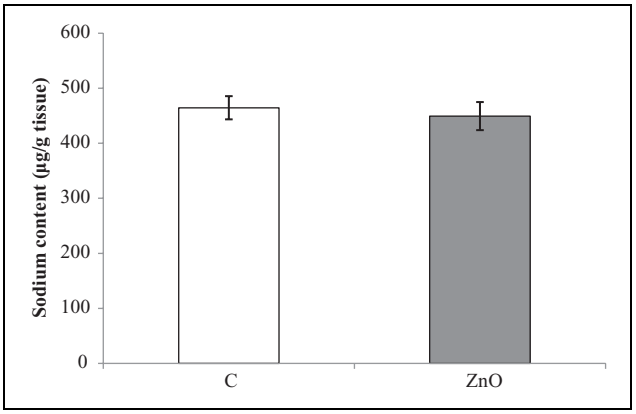

Figure 6. Effect of ZnO nanoparticles treatment on sodium content in the rat brain after 24 h. Data represent the means ± SEM of six animals per group. ZnO: zinc oxide.

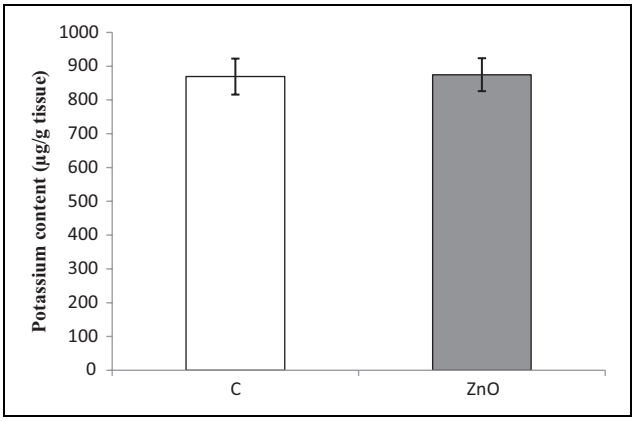

Figure 7. Effect of ZnO nanoparticles treatment on potassium content in the rat brain after 24 h. Data represent the means ± SEM of six animals per group. ZnO: zinc oxide.

neuronal degeneration (Tang et al., 2009). Experimental evidence suggested that the initiation and promotion of neurodegenerative diseases are associated with accumulation of high concentrations of metals like Cu, Al, and Zn, but especially Fe in brain regions associated with function loss and cell damage (Campbell et al., 2005; Liu et al., 2006). Metal homeostasis imbalance and neuronal loss are both present in neurodegenerative diseases. It is not known whether the presence of metals in brain of subjects with neurodegenerative diseases is due to NPs themselves translocating to the brain or their soluble compounds (Cristina et al., 2007). Our investigation showed that subacute exposure to ZnO NPs significantly decreased the Ca concentration in the homogenate brain. However, the Na and K contents remained unchanged. Thilsing-Hansen and Jørgensen (2001) reported the antagonism between Ca and Zn in dairy cows following oral administration of ZnO. There is a little knowledge about whether ZnO NPs have effect on neuronal ion channels, even though Zn functions as a signaling molecule in the nervous system and regulates several voltage-gated ionic conductances, including K^+, Na^+, and Ca^{2+} conductances (Beyersmann and Haase, 2001; Mathie et al., 2006). Wang et al. (2010) showed that ZnO particles selectively inhibit store-operated Ca entry without compromising the homeostatic mechanisms that maintain cytosolic Ca concentrations at low levels. Our investigation reveals that i.p. injected ZnO NPs suspension (25 nm) decreased the Fe concentration in rat brain. The basis for the Fe modulation in rat brain under ZnO NPs is not clear, but may involve impairment of the active transport processes or ion exchange mechanisms that determine Fe concentration in the brain. Previously, Katarzyna and

Table 1. Behavior of control and ZnO NP-treated rats in the plus-maze test.[a]

Variables	Time in the center (s)	Time in the open arms (s)	Time in the close arms (s)	Open arm entries	Closed arm entries
C	127.88 ± 35.19	54 ± 17.73	226.11 ± 17.67	2.57 ± 0.61	2.42 ± 0.66
ZnO NPs	28.42 ± 1.74[b]	15.71 ± 9.91	255.85 ± 10.42	1.42 ± 0.39	1.28 ± 0.19

ZnO NPs: zinc oxide nanoparticles.
[a]Data represent means ± SEM of six animals per group.
[b]$p < 0.05$ compared to control.

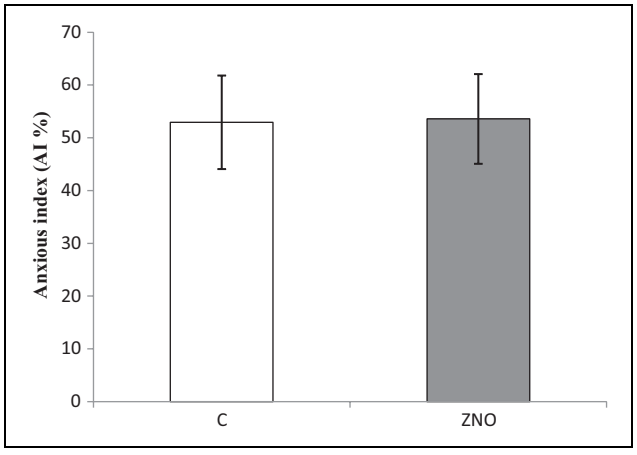

Figure 8. Graphical representation of AI from pulse mice assay of control and ZnO nanoparticle-treated rats. Data represent the means ± SEM of six animals per group. ZnO: zinc oxide; AI: anxious index.

Rebecca (2004) demonstrated an antagonism between Fe and Zn, citing absorptive competition between the two metals at the divalent metal transporter1 as its basis. Recently, Renping et al. (2010) showed that intragastric administration of titanium dioxide (TiO_2) NPs induced a significant decrease in the Fe content in the brain of treated mice. Fe is necessary in many cellular functions, especially in the brain, where it participates in many neuronal processes. In excess, however, Fe is toxic to cells. The brain continuously accumulates iron, resulting in increased iron storage with the age. This effect may lead to oxidative stress. In contrast, Fe deficiency could lead to behavioral deficits particularly during early development and at adult age (Beard et al., 2003; Unger et al., 2007; Youdim et al., 1989). The findings of Renping et al. (2010) indicated that exposure to TiO_2 NPs could possibly impair the spatial recognition memory ability, and this deficit may be possibly attributed to the disturbance of the homeostasis of trace elements, enzymes, and neurotransmitter systems in the mouse brain. Nonetheless, our investigation reported that ZnO NPs did not contribute to declining of the neurobehavioral performance of rats. It is interesting to note that there is no consistent difference in the emotional behavior parameters analyzed in the plus-maze test between ZnO NP-treated rats and the control group. This is in agreement with previous report indicating that ZnO NPs could ameliorate the behavioral and cognitive impairment in mice with depressive-like behaviors, probably by upregulating neuronal synaptic plasticity (Yongling et al., 2012). In contrast, Dadong et al. (2011) showed that long-term exposure to ZnO NPs attenuated the spatial learning and memory ability by the alteration of synaptic plasticity in treated rats. ZnO NPs disrupted the homeostasis of synaptic Zn, which would then lead to hyperactive long-term potentiation and insufficient depotentiation (Dadong et al., 2011). The discrepancy between our study and others is most likely attributed to differences in the sizes or the types of NPs or the treatment methods.

This research concludes that accumulation of Zn in the brain does not occur after a subacute injection of ZnO NPs. This attests to the absence of abnormalities in the homeostatic regulation system of this metal in the brain through BBB. However, the i.p. injection (25 mg/kg) of ZnO NPs (20–30 nm) could disrupt trace elements homeostasis in rat brain, but this effect is insufficient to promote emotional behavior impairments. Different concentrations and also different durations, that is, longer than 24 h, required further research can be emphasized.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

Beard J, Erikson KM, and Jones BC (2003) Neonatal iron deficiency results in irreversible changes in dopamine function in rats. *Journal of Nutrition* 133: 1174–1179.

Beyersmann D, Haase H (2001) Functions of zinc in signaling, proliferation and differentiation of mammalian cells. *Biometals* 14: 331–341.

Campbell A, Oldham M, Becaria A, Bondy SC, Meacher D, Sioutas C, et al. (2005) Particulate matter in polluted air may increase biomarkers of inflammation in mouse brain. *Neurotoxicology* 26: 133–140.

Congui L, Chicca M, Pilastro A, Turchetto M, and Tallandini L (2000) Effects of chronic dietary cadmium on hepatic glutathione levels and glutathione peroxidase activity in starlings. *Archives of Environmental Contamination and Toxicology* 38: 357–361.

Cristina B, Ivan I, Pacheco B, and Kevin R (2007) Nanomaterials and nanoparticles: sources and toxicity. *Biointerphases* 2(4): 17–172.

Dadong H, Yutao T, Tao Z, Guogang E, and Zhuo Y (2011) Nano-zinc oxide damages spatial cognition capability via over-enhanced long-term potentiation in hippocampus of Wistar rats. *International Journal of Nanomedicine* 6: 1453–1461.

Deng X, Luan Q, and Chen W (2009) Nanosized zinc oxide particles induce neural stem cell apoptosis. *Nanotechnology* 20: 115101.

El Mir L, Amlouk A, Barthou C, and Alaya S (2007) Synthesis and luminescence properties of ZnO/Zn2SiO4/SiO2 composite based on nanosized zinc oxide confined silica aerogels. *Journal of Physica B* 388: 412–417.

El Mir L, Amlouk A, Barthou C, and Alaya S (2008) Luminescence of composites based on oxide aerogels incorporated in silica glass host matrix. *Journal Materials Science and Engineering*: C 28: 771–776.

Fayed A-H (2010) Brain trace element concentration of rats treated with the plant alkaloid, vincamine. *Biological Trace Element* Research 136(3): 314–319.

Frye CA, Petralia SM, and Rhodes ME (2000) Estrous cycle and sex differences in performance on anxiety tasks coincide with increases in hippocampal progesterone and 3alpha, 5alpha-THP. *Pharmacology Biochemistry and Behavior* 67: 587–596.

Gabriel AS (2008) Nanotechnology approaches to crossing the blood–brain barrier and drug delivery to the CNS. *BMC Neuroscience* 9(Suppl 3): S4.

Jin-Hua Y, Yu C, He-Xia Z, Li-Jun S, Chun-Ye L, Jie-Quan L, et al. (2010) Determination, characterization and cytotoxicity on HELF cells of ZnO nanoparticles. *Colloids and Surfaces B: Biointerfaces* 76: 145–150.

Katarzyna K, Rebecca JS (2004) New evidence of iron and zinc interplay at the enterocyte and neural tissues. *American Society for Nutritional Sciences* 134(6): 1295–1298.

Liu G, Mena P, Harris PRL, Rolston RK, Perry G, and Smith MA (2006) Nanoparticle iron chelators: a new therapeutic approach in Alzheimer disease and other neurologic disorders associated with trace metal imbalance. *Neuroscience Letters* 406: 189–193.

Maaroufi K, Ammari M, Jeljeli M, Roy V, Sakly M, and Abdelmelek H (2009) Impairment of emotional behavior and spatial learning in adult Wistar rats by ferrous sulfate. *Physiology and Behavior* 96: 343–349.

Madsen E, Gitlin JD (2007) Copper and iron disorders of the brain. *Annual Review of Neuroscience* 30: 317–337.

Mathie A, Sutton GL, Clarke CE, and Veale EL (2006) Zinc and copper: pharmacological probes and endogenous modulators of neuronal excitability. *Pharmacology and Therapeutics* 11: 567–583.

Osmond MJ, McCall MJ (2010) Zinc oxide nanoparticles in modern sunscreens: an analysis of potential exposure and hazard. *Nanotoxicology* 4(1): 15–41.

Pellow S, Chopin P, File SE, and Briley M (1985) Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods* 14: 149–167.

Pellow S, File SE (1986) Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. *Pharmacology Biochemistry and Behavior* 24: 525–529.

Rasmussen JW, Martinez E, Louka P, and Wingett DG (2010) Zinc oxide nanoparticles for selective destruction of tumor cells and potential for drug delivery applications. *Expert Opinion on Drug Delivery* 7(9): 1063–1077.

Remillard CV (2004) Channels an essential pathway in programmed cell??Yuan JX-J Activation of K death. *American Journal of Physiology-Lung Cellular and Molecular Physiology* 286: 49–67.

Renping H, Xiaolan G, Yanmei D, Na L, Yi C, Yaling C, et al. (2010) Neurotoxicological effects and the impairment of spatial recognition memory in mice caused by exposure to TiO_2 nanoparticles. *Biomaterials* 31: 8043–8050.

Rodgers RJ, Dalvi A (1997) Anxiety, defence and the elevated plus-maze. *Neuroscience & Biobehavioral Reviews* 21: 801–810.

Roy V, Chapillon P (2004) Further evidences that risk assessment and object exploration behaviours are useful to evaluate emotional reactivity in rodents. *Behavioural Brain Research* 154: 439–448.

Sandstead HH, Frederickson CJ, and Penland JG (2000) History of zinc as related to brain function. *Journal of Nutrition* 130: 4965–5025.

Sharma HS, Hussain S, Schlager J, Ali S F, and Sharma A (2010) Influence of nanoparticles on blood–brain barrier permeability and brain edema formation in rats. *Acta Neurochirurgica* 106: 359–364.

Song W, Wu C, Yin H, Liu X, Sa P, and Hu J (2008) Preparation of PbS nanoparticles by phase-transfer method and application to pb-selective electrode based on PVC membrane. *Analytical Letters* 41(15): 2844–2859.

Staggers N, McCasky T, Brazelton N, and Kennedy R (2008) Nanotechnology: the coming revolution and its implications for consumers, clinicians, and informatics. *Nursing Outlook* 56(5): 268–274.

Takeda A (2001) Zinc homeostasis and functions of zinc in the brain. *Biometals* 14(3–4): 343–351.

Tang J, Xiong L, Wang S, Wang J, Liu L, Li J, et al. (2009) Distribution, translocation and accumulation of silver nanoparticles in rats. *Journal for Nanoscience and Nanotechnology* 9: 4924–4932.

Treit D, Menard J, and Royan C (1993) Anxiogenic stimuli in the elevated plus-maze. *Pharmacology Biochemistry and Behavior* 44: 463–469.

Thilsing-Hansen T, Jørgensen RJ (2001) Serum calcium response following oral zinc oxide administrations in dairy cows. *Acta Veterinaria Scandinavica* 42: 271–278.

Unger EL, Paul T, Murray-Kolb LE, Felt B, Jones BC, and Beard JL (2007) Early iron deficiency alters sensorimotor development and brain monoamines in rats. *Journal of Nutrition* 137: 118–124.

Wang HJ, Growcock AC, Tang TH, O'Hara J, Huang YW, and Aronstam RS (2010) Zinc oxide nanoparticle disruption of store-operated calcium entry in a muscarinic receptor signaling pathway. *Toxicology In Vitro* 24(7): 1953–1961.

Yongling X, Wang Y, Zhang T, Ren G, and Yang Z (2012) Effects of nanoparticle zinc oxide on spatial cognition and synaptic plasticity in mice with depressive-like behaviors. *Journal of Biomedical Science* 19: 14.

Youdim MB, Ben-Shachar D, and Yehuda S (1989) Putative biological mechanisms of the effect of iron deficiency on brain biochemistry and behavior. *The American Journal of Clinical Nutrition* 50: 607–615.

Zhao J, Xu L, Zhang T, Ren G, and Yang Z (2009) Influences of nanoparticle zinc oxide on acutely isolated rat hippocampal CA3 pyramidal neurons. *Neurotoxicology* 30(2): 220–230.