Applied Nanoscience
https://doi.org/10.1007/s13204-019-01093-3

ORIGINAL ARTICLE

Neurotoxicity of ZnO nanoparticles and associated motor function deficits in mice

Atif Yaqub[1] · Ijaz Faheem[1] · Khalid Mahmood Anjum[2] · Sarwar Allah Ditta[1] · Muhammad Zubair Yousaf[3] · Fouzia Tanvir[1] · Chand Raza[1]

Received: 20 April 2019 / Accepted: 19 June 2019

Abstract
Nano-sized zinc is extensively used by various industries and exposure of humans to these nanoparticles is increasing by the day. Mounting evidence suggests that health hazards are induced by metallic nanoparticles. However, at present, there is insufficient information concerning the impairment of motor functions due to exposure to metallic nanoparticles, particularly zinc oxide (ZnO) nanoparticles. The present study evaluates the toxic effects of ZnO nanoparticles in Swiss albino mice. Motor functions impairments were monitored using beam balance, and pole and footprint tests. Results showed that ZnO nanoparticles cause deficits in normal motor functions. Histopathological investigations revealed significantly ($p < 0.01$) increased motor cortex nuclear size probably resulting from neuroinflammation/neuronal damage. Overall, it is concluded that ZnO nanoparticles induce neurotoxicity resulting in motor function impairments.

Keywords Metallic nanoparticles · ZnO nanoparticles · Motor function impairment · Histopathology · Neurotoxicity

Introduction

In recent years, nanotechnology has emerged as an allied science with a diverse range of applications. This field of science has a significant impact on almost all areas of society and all industries. Nanoparticles (NPs) have at least one dimension in the range of 1–100 nm (Albanese et al. 2012; Chaturvedi and Dave 2018; Salisbury et al. 2018; Sharma et al. 2009b). This technology can potentially revolutionize every field of science and open new frontiers in this century (Dasgupta and Ranjan 2018; Rico et al. 2011). However, people working in industries, such as communications electronics, automobile, aerospace, paint, and chemical are at higher risk of being exposed to an ample amount of nanoparticles (Jachak et al. 2012; Kulvietis et al. 2011; Sharma et al. 2009b; Zhang et al. 2012). Hence, the risk of exposure of people to NPs is high in the environments, where NPs persist.

Nanoparticles have been recognized for their expected biological utility, including nanomedicine and biological science (Akakuru et al. 2018; Groneberg et al. 2006; Lanone and Boczkowski 2006), but they may pose serious health effects (Chaturvedi and Dave 2018). In recent years, hazardous toxic effects of NPs on the human body have been growing to higher levels; enormous use of nanomaterial-based consumer products or building materials in the market has made easy their way to pass through biological membranes and in humans; and NPs have potentially toxic effects and significant pathological consequences (Brooking et al. 2001; Lockman et al. 2004; Nel et al. 2006; Subramaniam et al. 2018; Xie et al. 2012; Yang et al. 2013). Nano-sized materials have received considerable attention regarding their biosafety and underlying effects on the central nervous system (CNS) (Xie et al. 2012), due to their industrialization and increasing public exposure (Wang 2004).

Nanoparticles may enter the brain, especially the cerebellum, cortex, and olfactory bulb via the nervus olfactorius when they are deposited on the mucosal membrane of the nose (Mistry et al. 2015; Oberdörster et al. 1995; Tian et al. 2015), or through some other routes and induce neurotoxicity in neurons within the CNS (Salisbury et al. 2018).

✉ Atif Yaqub
Atif@gcu.edu.pk

[1] Department of Zoology, Government College University, Lahore, Pakistan

[2] Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Lahore, Pakistan

[3] Department of Biological Sciences, F.C. College University, Lahore, Pakistan

Published online: 27 June 2019

Nanoparticles have adverse effects on health particularly on the nervous system in living beings due to induction of the generation of reactive oxygen species leading to oxidative stress (Karmakar et al. 2014; Nel et al. 2006). Furthermore, they cause pathological changes and inflammatory responses (De Jong and Borm 2008). Hence, NPs have certain hazardous effects on the brain tissue (Li et al. 2009; Wang 2004).

Zincite (ZnO) as a mineral is present in the earth crust (Sabir et al. 2014). It is nearly insoluble in water and appears as a white powder. Zinc oxide nanoparticles (ZnO-NPs) are being used enormously in various fields, such as cosmetics, medicine, engineering, and water treatment. The powder, ZnO, is widely used as an additive in many materials including glass, pigments, plastics, sealants, lubricants, adhesives, ointments, ceramics, cement, rubber, foods, ferrites, batteries, and fire (Wang 2004).

Zinc has been reported to be a necessary element for systemic physiology, including cellular signaling pathways and enzymes regulation and also on the brain (Takeda 2000). The novelty suppressed feeding test was performed to determine dietary zinc deficiency in mice, and it caused stress like behavior and increased response time to eat (Whittle et al. 2009). Exposure to cells to ZnO induces ROS generation and an increase in Zn^{2+} concentration stimulates the production of these radicals (Kukic et al. 2014). Exposure of cells to ZnO nanoparticles causes mitochondrial dysfunction (Guan et al. 2012; Jeng and Swanson 2006; Karbowski and Youle 2003). It has been reported that Zn^{2+} is significantly associated with cell damage and cytotoxicity was most likely via Zn^{2+} dissolution (Deng et al. 2009). Exposure to ZnO nanoparticles may result in impairment of long-term memory (Feng et al. 2015). ZnO nanoparticles may also interact with the cell markers on their surfaces and affect the transcription of proinflammatory cytokines. These nanoparticles may get accumulated in important organs, such as lungs, heart, kidney, liver, and brain resulting in toxic outcomes (Saptarshi et al. 2015). This study was designed to investigate the effects of ZnO nanoparticles on the behavior of the animal in relation to motor impairment and neurotoxicity.

Materials and methods

All the chemicals were purchased from Sigma-Aldrich supplied by quality vendors and used without further purification. The deionized and double distilled water was used in all experiments.

Synthesis and characterization of ZnO nanoparticles

ZnO nanoparticles were prepared according to the previous protocol (Zak et al. 2011) with slight modifications. Following this protocol, triethanolamine (TEA) polymer was used to terminate the growth process of ZnO nanoparticles. Sodium chloride (0.9%) was used to dissolve these particles and was sonicated for 15 min. Characterization of these nanomaterials was conducted using various techniques and facilities. Ultraviolet–visible spectroscopy was performed using Thermo Fisher Scientific spectrophotometer (GENESYS 10S UV–Vis) in the range of 200–800 nm. Morphology of these nanoparticles was examined with the help of Scanning Electron Microscopy (SEM) using JEOL (JSM-6480LV) instrumentation with an acceleration voltage of 15 kV. Size analysis was performed by BT-90 nanolaser particle size analyzer.

Animals and experimental protocols

Adult male Swiss mice (10–12 weeks, body weight ranging from 28 to 40 g) were obtained from Animal House Facility of the Government College University, Lahore, Pakistan. All the experimental procedures were conducted after receiving approval from the ethical committee for animal use at the Government College University, Lahore, Pakistan (GCU-IIB-366). Mice were housed in temperature controlled (22 ± 1 °C) facility, 12:12 h light/dark cycle, and was provided commercially purchased diet and water. Mice were acclimatized to the laboratory conditions. All doses expressed in milligrams per kilogram (mg/kg) were orally administered in a volume of 10 ml/kg animal body weight using oral gavage. The experimental mice were divided into three groups with each group containing six mice. One group of mice was treated with low dose (250 mg/kg) and one with high dose (500 mg/kg) once a day for 21 consecutive days, while the third group was left untreated to serve as a control. For dosing, accurately weighted ZnO nanoparticles were suspended in distilled water, and were vortex mixed and sonicated for 3 min prior to dosing.

Body weight

Body weight of each experimental mouse was taken on a daily basis. For this, each mouse was lifted gently by holding from its tail and was placed on the weight balance.

Behavioral assessment of motor function

Mice in all groups were subjected to behavioral assessment of motor activities through beam walking to compare fine motor coordination, pole climb-down, and stride length measurements.

Beam balance walking

Motor coordination of mice was assessed by measuring the ability of the mice to traverse 1 m long and 50 cm elevated

beam to reach an enclosed safety platform (Tung et al. 2014). The beams consisted of 1 m long strips of 1 cm diameter wood. The beams were placed horizontally, 50 cm above the bench surface, with one end mounted on narrow support and the other end attached to an enclosed box (20 cm square) into which the mouse could escape. The latency to traverse beam was recorded for each trial. Analysis of each measure was based on the mean scores of two trial/animal/day for 21 days.

Footprint test

The footprints were taken to compare the distance between a proceeding and preceding hind limb. For this purpose, a 50-cm-long, 10-cm-wide runway (with 10-cm-high walls) into the arms of T-maze was used. A fresh sheet of white paper was placed on the floor of the runway. The footprint patterns were analyzed for stride length (measured in centimeters). Stride length was measured (with the help of a vernier caliper) as the average distance of forwarding movement between each stride of the left paw. The mean value of each set of three values was used in subsequent analysis.

Pole test

Pole test is a commonly used behavior test used to evaluate motor dysfunction (Brooks and Dunnett 2009). This test was performed after dosing with ZnO nanoparticles. In this test, mice were placed at the top of a 50 cm vertical pole with a diameter of 1 cm. The recording was started when the animal began the turning movement. The total time to descend to the floor (T total) was recorded. The test was repeated for two trials per animal/day in each set for 21 days.

Tissue processing and histological examination

For histological examination, mice from each group ($n = 3$) were deeply anesthetized with ketamine and transcardially perfused. Following perfusion, the brains were carefully dissected by the bone-cracking method and were post-fixed in 10% buffered formalin. Tissue processing was done to remove water from tissues and serial coronal sections of 4 µm thickness were made in the horizontal plane using a microtome (CM1900, Leica, Nussloch, Germany). Sections of each sample were achieved and placed in the water bath at 45–50 °C for melting and washing away the paraffin wax. Egg albumin-coated glass slides were used for mounting the sections of each tissue on the slides. After proper placing the specimen in the center of the slides, slides were heated on the hot plate for 5 min. These slides were given washes in the xylene solution again to remove any extra paraffin from the specimen. Specimens fixed on slides were given 3 min washes through baths in descending grades of ethanol: i.e., 100, 90, 70, 50, and 30% for further dehydration. Finally, slides were washed with tap water for removing the extra alcohol from the specimen. After dehydration, staining of the specimen was performed with the help of eosin and hematoxylin stains. Eosin stained the cytoplasm with pink color, whereas hematoxylin stained (purplish color) nucleus of the cell.

Microscopy and statistical analysis

Prepared slides of the brain tissues from all the samples were observed using an Olympus microscope under 10X objective and bright field micrographs were taken out for all the samples with the help of attached camera to the microscope. The area corresponding to motor cortex was imaged. Histological differences between the brain tissues of the control and treated groups were observed and finally analyzed for neurotoxicity. All images were converted into 8-bit resolution and subjected to the same threshold settings to yield optimum signal-to-noise ratio. A rectangle of fixed area (length: 500 µm × width 250 µm) was adjusted to 150 µm deep the cerebellum's motor cortex outer margin. The nuclear area was measured using 'Analyze Particles' function of Image software and results were exported to excel result sheet. Neurobehavioral data were analyzed for statistical differences using two-way ANOVA with Bonferroni post hoc analysis. For histological data, one-way ANOVA was applied to investigate statistical differences between ZnO nanoparticle-treated vs vehicle control groups. All data were represented as mean ± SEM and the number of mice analyzed per group is shown in the results accordingly.

Results

Synthesis and characterization of ZnO nanoparticles

The synthesized nanoparticles of ZnO were spherical in shape, with a size range of 30–40 nm (Figs. 1 and 2), UV–visible spectroscopy yield peak at 380 nm, generally the characteristic range of the ZnO nanoparticles.

ZnO nanoparticle-treated mice

Body weight measurements of nanoparticle-treated mice demonstrated very slight change in body weight (Fig. 3). Mice fed upon 250 mg/kg of their body weight showed a slight decrease in body weight; however, this change in weight was negligible when compared with control and higher dosed (500 mg/kg of body weight) groups of mice:

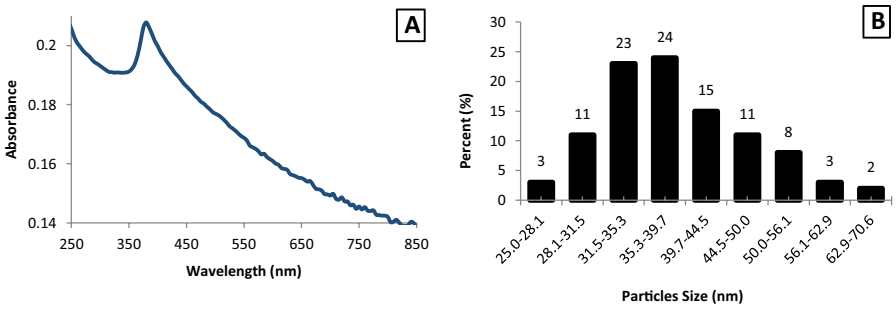

Fig. 1 **a** UV–visible spectrum of the ZnO nanoparticles. **b** Size distribution map

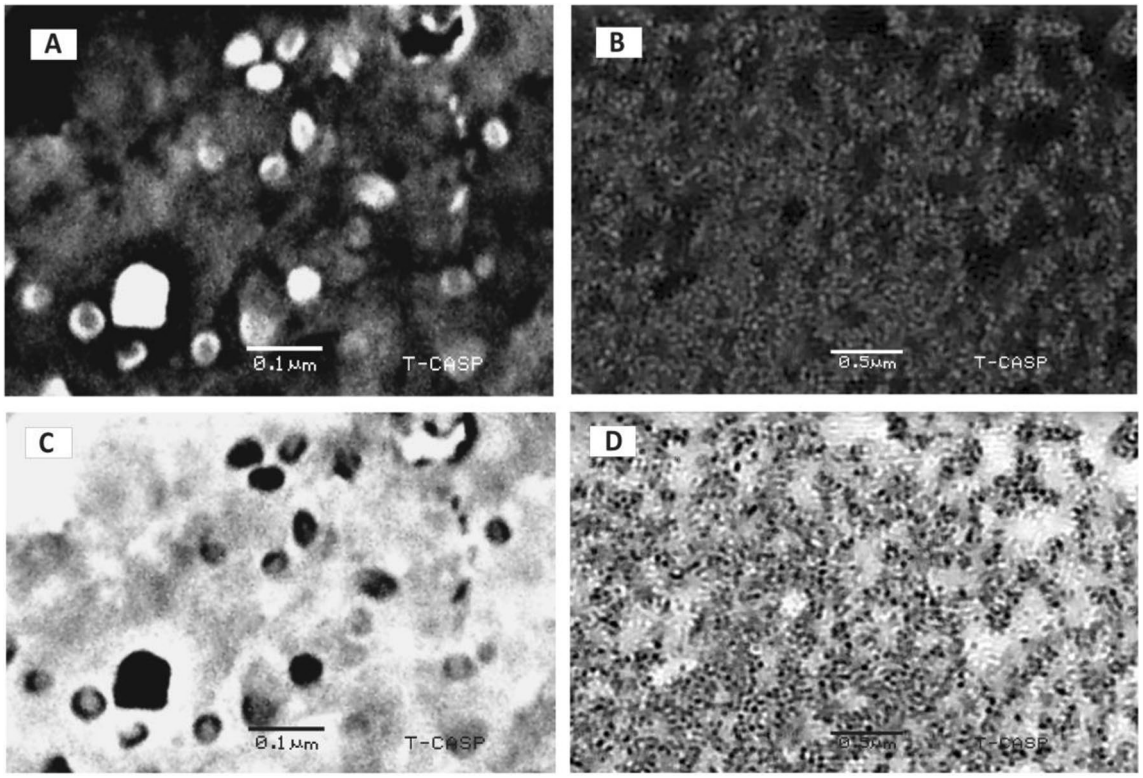

Fig. 2 **a**, **b** Simple SEM micrograph of ZnO nanoparticles; **c**, **d** SEM micrograph of ZnO nanoparticles with inverted color

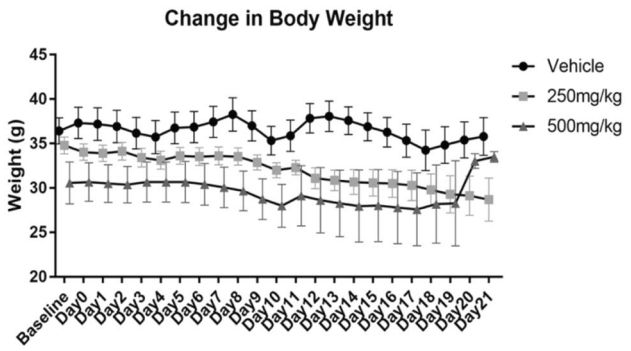

Fig. 3 Mean body weight of mice, ZnO-nanoparticle-treated groups, and the non-treated control group

Motor behavior of ZnO nanoparticle-treated mice

Baseline values revealed that mice in all groups crossed the elevated beam within 7–7.8 s. However, following the first dose of ZnO nanoparticles, mice in treated groups took a longer duration of time to cross the beam and it was significantly different for 250 mg/kg dose group compared to the control group. Similarly, after the first, second, and third weeks of ZnO-nanoparticle treatment, mice demonstrated prolonged duration to cross the beam compared to the control group (Fig. 4).

ZnO nanoparticles caused significant motor impairments in the treated groups during dose administration (Figs. 5

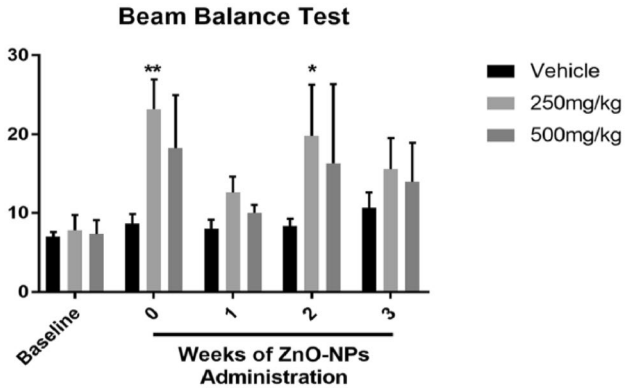

Fig. 4 ZnO nanoparticle-treated mice revealed deficits in motor coordination. Beam balance test demonstrated increased time span in ZnO-nanoparticle-treated groups compared to vehicle-treated mice. Mice at low dose 250 mg/kg demonstrated significant ($p<0.05$) prolonged durations to cross beam length just following first dose and at week 2. $N=4$–6; data represented as mean ± SEM; *$p<0.05$; **$p<0.01$; Two-way ANOVA followed by Bonferroni post hoc analysis

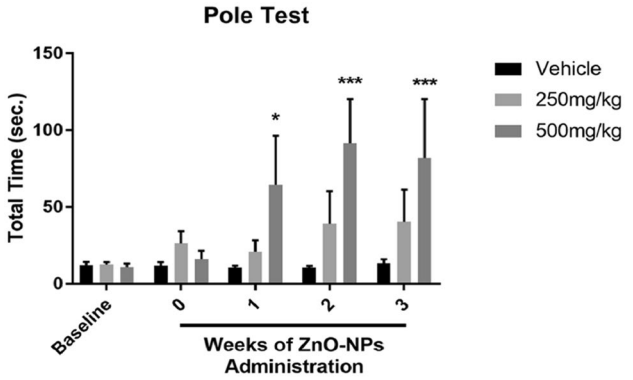

Fig. 5 ZnO-nanoparticle-treated mice showing deficits in motor coordination. Dose administration groups show increased time duration than the vehicle-treated group. A significant increase in time during the first, second, and third weeks was observed. Increase in time is more significant at weeks 2 and 3. Increase in total time is relatively greater in high-dose (500 mg/kg)-treated group than low-dose (250 mg/kg)-treated group. $N=4$–6; data represented as mean ± SEM; *$p<0.05$; ***$p<0.001$; Two-way ANOVA followed by Bonferroni post hoc analysis

and 6). Mice showed that total time and turn time increased during the first week of dose administration and it became significantly different during the second and third weeks of dose administration in the higher dose-receiving group as compared to non-treated vehicle control.

Stride length measurements depicted gait impairments in the form of decrease stride length showing poor motor coordination following the first dose of ZnO-NP (250 mg/kg and 500 mg/kg)-treated groups comparing to vehicle control, as shown in Fig. 7. Similarly, we observed significant

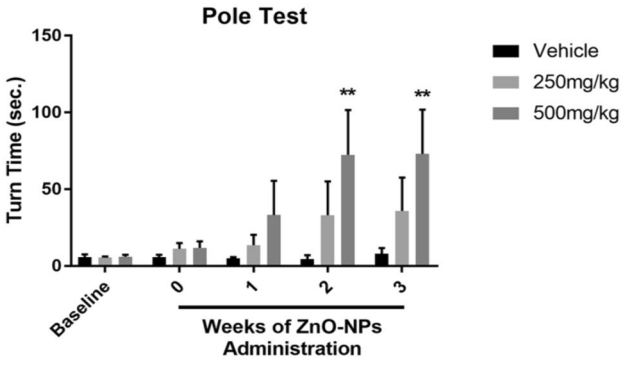

Fig. 6 ZnO-NP-treated mice show a deficit in motor coordination. Turn time is increased to a significant extent at weeks 1 and 2 in both high-dose (500 mg/kg)-treated group and low-dose (250 mg/kg)-treated group and it remained almost constant at week 3 as compared to week 2. Increase in time is relatively more in high-dose-treated group than low-dose-treated group. $N=4$–6; data represented as mean ± SEM; **$p<0.01$; Two-way ANOVA followed by Bonferroni post hoc analysis

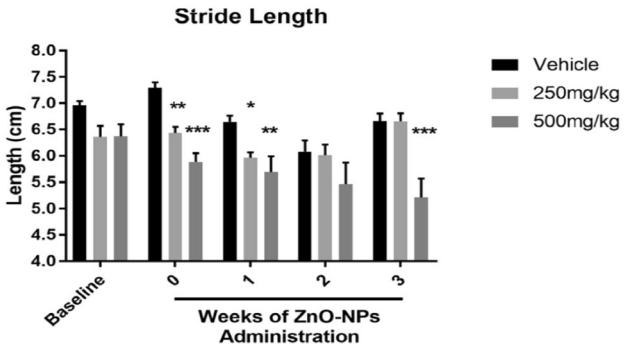

Fig. 7 ZnO-NP-treated mice show gait impairments in the form of decreased stride length showing poor motor coordination. Significant ($p<0.05$) reduction at the first and the third weeks was observed. Decreased stride length as compared to vehicle-treated control. $N=4$–6; data represented as mean ± SEM; *$p<0.05$; **$p<0.01$; Two-way ANOVA followed by Bonferroni post hoc analysis

($p<0.05$) reduction or stride length reduction at weeks 1 and 3. However, a non-significant reduction was observed at a higher dose (500 mg/kg).

ZnO-NPs' administration enhances nuclear size in motor cortex neurons

Motor cortex neurons of mice forebrain control the somatic activities. We reasoned that motor functional impairments depicted in ZnO-NP-treated mice might be the result of some adverse effects of ZnO-NPs on the CNS neurons (primarily involved in voluntary activities). Thus, following the 21 days of dosing and study completion, mice brain was harvested and processed for analyzing motor cortex neurons of the primary motor cortex area (Fig. 8).

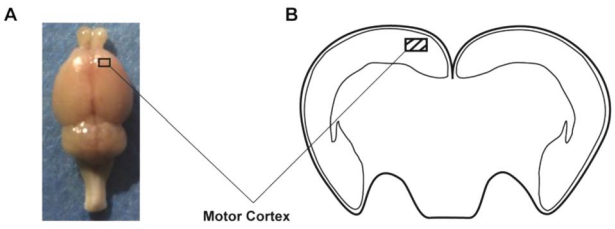

Fig. 8 Brain harvest and location of primary motor cortex area in the cerebral hemisphere (**a**) and diagrammatic representation of the said area in coronal section (**b**)

To our surprise, ZnO-NP-treated mice brain images did not reveal any gross neuronal loss (Fig. 9d) or signs of motor neuronal toxicity comparing to vehicle control group. However, evaluation of the nuclear area of motor cortex area neurons revealed significant enlargements in high-dose-treated mice (Fig. 9e). However, no significant differences were observed in low-dose-treated mice.

Discussion

Characterization of the ZnO nanoparticles

The UV–visible absorption spectra of the ZnO nanoparticles exhibited a prominent absorption peak at 380 nm, which confirmed the formation of ZnO nanoparticles. Talam et al. (2012) had reported the peak at 355 nm for ZnO nanoparticles. SEM images confirmed the formation of spherical-shaped ZnO nanoparticles in the size ranging from 35 to 40 nm). Similar results were also reported by Talam et al. (2012), but the size range reported by them was between 50 and 70 nm; Sharma et al. (2009a) had also reported similar results.

Neurotoxicity of ZnO nanoparticles

Zinc is an essential micronutrient required by animals in trace amounts as a cofactor for various key enzymes (Hänsch and Mendel 2009; Maughan 1999; Soetan et al. 2010); however, its excessive use/exposure may lead to various health-associated problems (Fosmire 1990), one of which may be

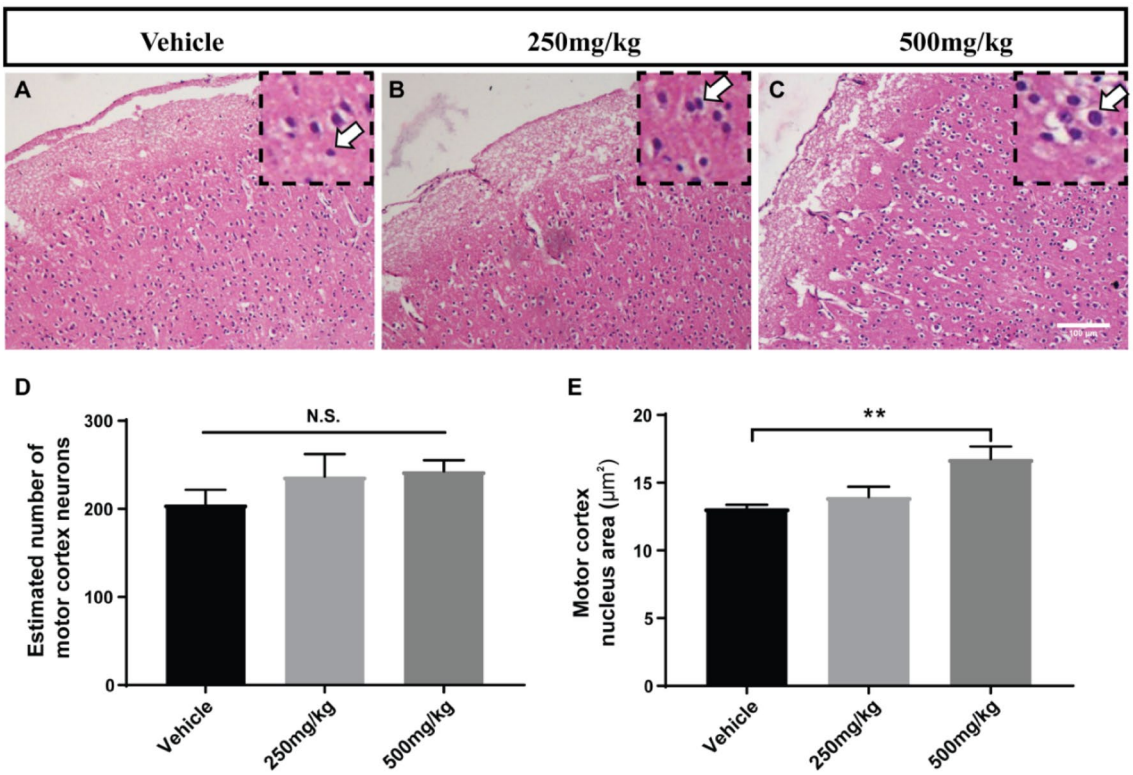

Fig. 9 Histopathology of ZnO-NP-treated mice revealed no change in neuronal density; however, increased nuclear size was observed in the motor cortex area (at least 300 neurons/mouse were analyzed). **a**–**c** Hematoxylin and eosin stained 4 μm-thick brain sections of motor cortex area (**a** vehicle, **b** 250 mg/kg, and **c** 500 mg/kg). **d** No significant differences were observed in ZnO-NP-treated mice with motor cortex neurons comparing to vehicle control. **e** Significant increase in nucleus size of 500 mg/kg treated group was observed. N = 3 animals per group; data represented as mean ± SEM; $^{**}p < 0.01$; one-way ANOVA followed by Bonferroni post hoc analysis

motor functional deficits or neural tissue damages (Migliore et al. 2015).

Oral doses were chosen after careful literature survey. The previous studies used oral doses of the ZnO-NPs for mice at 500, 1000, or 2000 mg/kg per day for 14 days (Patra et al. 2012), 333.3 mg/kg for 5 days (Esmaeillou et al. 2013), 67.1, 134.2, 264.8, or 535.8 mg/kg per day for 13 weeks (Seok et al. 2013), 600 mg/kg or 1000 mg/kg per day for 5 days (Baky et al. 2013), and 5, 50, 300, 1000, or 2000 mg/kg per day for 14 days (Pasupuleti et al. 2012). The LD50 of zinc is 630 mg/kg when rats are orally treated; however, ZnO-NPs are relatively less toxic. The previous studies reported that ZnO-NPs generally induce toxicity at a relatively high-dose level (Choi et al. 2015). Therefore, we choose higher level of two doses, i.e., 250 mg/kg and 500 mg/kg of the BW and one control in our study.

Somatic activities are controlled by the primary motor cortex along with associated CNS circuitry. Motor behavioral tests of the ZnO-nanoparticle-administered mice, including beam balance, pole climb-down, and walking track analysis, revealed impaired motor functions compared to untreated mice. Moreover, the functional deficits were more pronounced in mice treated with a higher dose of the nanoparticles, suggesting that ZnO nanoparticles significantly affect the normal motor physiology of mice. Baseline values of the present study are in agreement with the already reported baseline values for beam balance, pole climb-down, and stride length (Zhu et al. 2016) indicating the reproducibility and widely accepted behavioral tests used in the present study.

Observations on processed brain tissue of ZnO-nanoparticle-treated/non-treated mice for observing morphological changes in motor cortex area through hematoxylin and eosin (H and E) staining revealed no prominent changes in gross morphology and number of neurons; however, enlarged nuclei in the sections of ZnO-nanoparticle-treated (at 500 mg/kg) mice were observed. A previous study had described that the motor dysfunctions are associated with neuroanatomical changes in the motor cortex, striatum, and hippocampus neurons, as H and E-stained brain sections of mice with abnormal motor functions revealed altered nuclear morphology (Yoon et al. 2018). Thus, it is very likely that in the current study, ZnO-nanoparticle-induced motor deficits resulting from the altered neuronal morphology of motor cortex cells.

Enlarged nucleus size of motor cortex neurons in ZnO-nanoparticle-treated mice may point to the adjacent tissue damage induced by swelling at higher treatment rate of 500 mg/kg of the nanoparticles; however, at the lower treatment rate of 250 mg/kg, the extent of tissue damage was not pronounced enough to alter the neuronal morphology in the observed area. Results of the present study indicate acute neurotoxicity of ZnO nanoparticles. The findings of neurotoxic effects of ZnO nanoparticles studied by Pole climb test, Beam balance test, Stride length measurement, and histology in the present study.

ZnO-NPs' administration in high dose leads to the DNA damage, due to the release of cytokines, which take part in inflammatory response at various levels. ZnO-NPs disturb the integrity of the DNA molecules and result in DNA fragmentation as cell start apoptosis (Kvietys and Granger 2012). Up-regulation in Fas (death receptor) was also reported in response to ZnO-NPs in the rat (Attia et al. 2018).

DNA damages and cell inflammation is associated with the induction of oxidative stress, which is further associated with the production of free radicals due to nanoparticles exposure. Brains' cells are very sensitive to oxidative stress due to its high metabolism and low level of cellular regeneration (Xiaoli et al. 2017).

The blood–brain barrier (BBB) is very important in connection with the nanoparticles' toxicity in the brain tissue. There are tight junctions present on this extended membrane between the cerebral capillaries and adjacent endothelial cells (Lanone and Boczkowski 2006; Sharma 2009). Nanoparticles on reaching the circulation may alter the permeability of the membrane or induce several cascading molecules leading to the impairment of the tight junctions and hence result in the direct or indirect toxicity in the brain tissues. NPs may also stimulate the vesicular transport, to get entry inside the microenvironment of the central nervous system (CNS) (Karmakar et al. 2014), where they further impair several molecular pathways.

Toxicity of ZnO-NPs is attributed due to the Zn^{2+} ions, which induce ROS and further inflammatory response. In a previous study, ZnO-NPs reported to show dose-dependent toxicity in the neural stem cells (NSCs), and it further credited the Zn^{2+} ions in dissolved form as a toxicant in the cells or culture medium, which induce toxicity by various mechanisms(Deng et al. 2009). Another study on rat hippocampal CA3 pyramidal neurons reported the disturbance in the voltage-gated sodium and potassium pumps induced by ZnO-NPs. Neuronal injuries and apoptosis were induced by higher Na^{+} and Ca^{2+} influx inside the cell, as a result of depolarization by the activation of voltage-gated sodium pumps (Zhao et al. 2009). Altered memory and spatial learning behavior were recorded, as 20–80 nm sized ZnO-NPs (4 mg/kg of the body weight 2 days/week for 8 weeks) injected through intraperitoneal route changed synaptic plasticity (Han et al. 2011).Other studies have shown that Zn^{2+} ions released from ZnO-NPs (Brunner et al. 2006; Heinlaan et al. 2008) and ROS interaction with cell membrane may damage cellular membrane (Brayner et al. 2006; Zhang et al. 2007). Pathological lesions are seen to be developed in spleen, pancreas, kidney, liver, and stomach of the mammalian models when exposed to ZnO-NPs (Wang et al. 2008).

Overall, the present study provides behavioral and histological results, indicating that ZnO nanoparticles do induce functional deficits and underlying associated neurotoxicity of motor cortex neurons. Thus, accumulation of nano-sized zinc particles in the environment is a potential threat to normal motor functions and CNS neurons of associated resident populations.

Conclusion

ZnO nanoparticles are generally toxic nanomaterials that are harmful to the vital organs of the body especially to the nervous tissues, where these nanoparticles may damage some important areas of the brain related to some specific functions, finally impairing that function; hence, proper care and precautions should be taken to avoid their potential intake while handling them.

Acknowledgements The authors are highly thankful to the Department of Physics and CASP (Centre of Advanced Physics) Government College University, Lahore, Pakistan, for providing the facilities for characterization of nanoparticles. Provision of laboratory facilities, model animals, and personnel support by the Department of Zoology, Government College University, Lahore, Pakistan, is dually acknowledged.

Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest.

References

Akakuru O, Louis H, Oyebanji O, Ita B, Amos P (2018) Utility of nanomedicine for cancer treatment. J Nanomed Nanotechnol 9:481. https://doi.org/10.4172/2157-7439.1000481 **(Page 2 of 6 J Nanomed Nanotechnol, an open access journal ISSN: 2157-7439 Volume 9● Issue 1● 1000481 cytotoxicity which destroys healthy cells in addition to tumor cells. Chemotherapeutics uses NPs as drug carriers which deliver medication directly to tumors and spare the healthy tissues. These nanocarriers)**

Albanese A, Tang PS, Chan WC (2012) The effect of nanoparticle size, shape, and surface chemistry on biological systems. Annu Rev Biomed Eng 14:1–16

Attia H, Nounou H, Shalaby M (2018) Zinc oxide nanoparticles induced oxidative DNA damage, inflammation and apoptosis in rat's brain after oral exposure. Toxics 6:29

Baky N, Faddah L, Al-Rasheed N, Fatani A (2013) Induction of inflammation, DNA damage and apoptosis in rat heart after oral exposure to zinc oxide nanoparticles and the cardioprotective role of α-lipoic acid and vitamin E. Drug research 63:228–236

Brayner R, Ferrari-Iliou R, Brivois N, Djediat S, Benedetti MF, Fiévet F (2006) Toxicological impact studies based on Escherichia coli bacteria in ultrafine ZnO nanoparticles colloidal medium. Nano Lett 6:866–870

Brooking J, Davis S, Illum L (2001) Transport of nanoparticles across the rat nasal mucosa. J Drug Targeting 9:267–279

Brooks SP, Dunnett SB (2009) Tests to assess motor phenotype in mice: a user's guide. Nat Rev Neurosci 10:519

Brunner TJ et al (2006) In vitro cytotoxicity of oxide nanoparticles: comparison to asbestos, silica, and the effect of particle solubility. Environ Sci Technol 40:4374–4381

Chaturvedi S, Dave PN (2018) Nanomaterials: environmental, human health risk. Handbook of nanomaterials for industrial applications. Elsevier, Amsterdam, pp 1055–1062

Choi J et al (2015) Toxicity of zinc oxide nanoparticles in rats treated by two different routes: single intravenous injection and single oral administration. J Toxicol Environ Health Part A 78:226–243

Dasgupta N, Ranjan S (2018) Nanotechnology in food sector. An introduction to food grade nanoemulsions. Springer, New York, pp 1–18

De Jong WH, Borm PJ (2008) Drug delivery and nanoparticles: applications and hazards. Int J Nanomed 3:133

Deng X, Luan Q, Chen W, Wang Y, Wu M, Zhang H, Jiao Z (2009) Nanosized zinc oxide particles induce neural stem cell apoptosis. Nanotechnology 20:115101

Esmaeillou M, Moharamnejad M, Hsankhani R, Tehrani AA, Maadi H (2013) Toxicity of ZnO nanoparticles in healthy adult mice. Environ Toxicol Pharmacol 35:67–71

Feng X, Chen A, Zhang Y, Wang J, Shao L, Wei L (2015) Central nervous system toxicity of metallic nanoparticles. Int J Nanomed 10:4321

Fosmire GJ (1990) Zinc toxicity. Am J Clin Nutr 51:225–227

Groneberg DA, Giersig M, Welte T, Pison U (2006) Nanoparticle-based diagnosis and therapy. Curr Drug Targets 7:643–648

Guan R, Kang T, Lu F, Zhang Z, Shen H, Liu M (2012) Cytotoxicity, oxidative stress, and genotoxicity in human hepatocyte and embryonic kidney cells exposed to ZnO nanoparticles. Nanoscale Res Lett 7:602

Han D, Tian Y, Zhang T, Ren G, Yang Z (2011) Nano-zinc oxide damages spatial cognition capability via over-enhanced long-term potentiation in hippocampus of Wistar rats. Int J Nanomed 6:1453

Hänsch R, Mendel RR (2009) Physiological functions of mineral micronutrients (cu, Zn, Mn, Fe, Ni, Mo, B, cl). Curr Opin Plant Biol 12:259–266

Heinlaan M, Ivask A, Blinova I, Dubourguier H-C, Kahru A (2008) Toxicity of nanosized and bulk ZnO, CuO and TiO_2 to bacteria Vibrio fischeri and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. Chemosphere 71:1308–1316

Jachak AC, Creighton M, Qiu Y, Kane AB, Hurt RH (2012) Biological interactions and safety of graphene materials. MRS Bull 37:1307–1313

Jeng HA, Swanson J (2006) Toxicity of metal oxide nanoparticles in mammalian cells. J Environ Sci Health Part A 41:2699–2711

Karbowski M, Youle R (2003) Dynamics of mitochondrial morphology in healthy cells and during apoptosis. Cell Death Differ 10:870

Karmakar A, Zhang Q, Zhang Y (2014) Neurotoxicity of nanoscale materials. J Food Drug Anal 22:147–160

Kukic I, Kelleher SL, Kiselyov K (2014) Zinc efflux through lysosomal exocytosis prevents zinc-induced toxicity. J Cell Sci 127:3094–4103

Kulvietis V, Zalgeviciene V, Didziapetriene J, Rotomskis R (2011) Transport of nanoparticles through the placental barrier. Tohoku J Exp Med 225:225–234

Kvietys PR, Granger DN (2012) Role of reactive oxygen and nitrogen species in the vascular responses to inflammation. Free Radical Biol Med 52:556–592

Lanone S, Boczkowski J (2006) Biomedical applications and potential health risks of nanomaterials: molecular mechanisms. Curr Mol Med 6:651–663

Li X-b et al (2009) Glia activation induced by peripheral administration of aluminum oxide nanoparticles in rat brains. Nanomed Nanotechnol Biol Med 5:473–479

Lockman PR, Koziara JM, Mumper RJ, Allen DD (2004) Nanoparticle surface charges alter blood–brain barrier integrity and permeability. J Drug Target 12:635–641
Maughan RJ (1999) Role of micronutrients in sport and physical activity. Br Med Bull 55:683–690
Migliore L, Uboldi C, Di Bucchianico S, Coppedè F (2015) Nanomaterials and neurodegeneration. Environ Mol Mutagen 56:149–170
Mistry A, Stolnik S, Illum L (2015) Nose-to-brain delivery: investigation of the transport of nanoparticles with different surface characteristics and sizes in excised porcine olfactory epithelium. Mol Pharm 12:2755–2766
Nel A, Xia T, Mädler L, Li N (2006) Toxic potential of materials at the nanolevel science 311:622–627
Oberdörster G, Celein RM, Ferin J, Weiss B (1995) Association of particulate air pollution and acute mortality: involvement of ultrafine particles? Inhalation Toxicol 7:111–124
Pasupuleti S, Alapati S, Ganapathy S, Anumolu G, Pully NR, Prakhya BM (2012) Toxicity of zinc oxide nanoparticles through oral route. Toxicol Ind Health 28:675–686
Patra P, Mitra S, Debnath N, Goswami A (2012) Biochemical-, biophysical-, and microarray-based antifungal evaluation of the buffer-mediated synthesized nano zinc oxide: an in vivo and in vitro toxicity study. Langmuir 28:16966–16978
Rico CM, Majumdar S, Duarte-Gardea M, Peralta-Videa JR, Gardea-Torresdey JL (2011) Interaction of nanoparticles with edible plants and their possible implications in the food chain. J Agric Food Chem 59:3485–3498
Sabir S, Arshad M, Chaudhari SK (2014) Zinc oxide nanoparticles for revolutionizing agriculture: synthesis and applications. Sci World J 2014:1–8
Salisbury RL, Agans R, Huddleston ME, Snyder A, Mendlein A, Hussain S (2018) Toxicological mechanisms of engineered nanomaterials: role of material properties in inducing different biological responses. Handbook of developmental neurotoxicology, 2nd edn. Elsevier, Amsterdam, pp 237–249
Saptarshi SR, Duschl A, Lopata AL (2015) Biological reactivity of zinc oxide nanoparticles with mammalian test systems: an overview. Nanomedicine 10:2075–2092
Seok SH et al (2013) Rat pancreatitis produced by 13-week administration of zinc oxide nanoparticles: biopersistence of nanoparticles and possible solutions. J Appl Toxicol 33:1089–1096
Sharma HS (2009) A special section on nanoneuroscience: nanoneurotoxicity and nanoneuroprotection. J Nanosci Nanotechnol 9:4992
Sharma V, Shukla RK, Saxena N, Parmar D, Das M, Dhawan A (2009a) DNA damaging potential of zinc oxide nanoparticles in human epidermal cells. Toxicol Lett 185:211–218
Sharma VK, Yngard RA, Lin Y (2009b) Silver nanoparticles: green synthesis and their antimicrobial activities. Adv Coll Interface Sci 145:83–96
Soetan K, Olaiya C, Oyewole O (2010) The importance of mineral elements for humans, domestic animals and plants—a review. African J Food Sci 4:200–222
Subramaniam VD et al (2018) Health hazards of nanoparticles: understanding the toxicity mechanism of nanosized ZnO in cosmetic products. Drug Chem Toxicol 42:1–10
Takeda A (2000) Movement of zinc and its functional significance in the brain. Brain Res Rev 34:137–148
Talam S, Karumuri SR, Gunnam N (2012) Synthesis, characterization, and spectroscopic properties of ZnO nanoparticles. ISRN Nanotechnol 2012:1–6
Tian L et al (2015) Neurotoxicity induced by zinc oxide nanoparticles: age-related differences and interaction. Sci Rep 5:16117
Tung JY, Gage WH, Poupart P, McIlroy WE (2014) Upper limb contributions to frontal plane balance control in rollator-assisted walking. Assist Technol 26:15–21
Wang ZL (2004) Zinc oxide nanostructures: growth, properties and applications. J Phys Condens Matter 16:R829
Wang B et al (2008) Acute toxicological impact of nano-and submicro-scaled zinc oxide powder on healthy adult mice. J Nanopart Res 10:263–276
Whittle N, Lubec G, Singewald N (2009) Zinc deficiency induces enhanced depression-like behaviour and altered limbic activation reversed by antidepressant treatment in mice. Amino acids 36:147–158
Xiaoli F, Junrong W, Xuan L, Yanli Z, Limin W, Jia L, Longquan S (2017) Prenatal exposure to nanosized zinc oxide in rats: neurotoxicity and postnatal impaired learning and memory ability. Nanomedicine 12:777–795
Xie Y, Wang Y, Zhang T, Ren G, Yang Z (2012) Effects of nanoparticle zinc oxide on spatial cognition and synaptic plasticity in mice with depressive-like behaviors. J Biomed Sci 19:14
Yang Z, Liu Z, Allaker R, Reip P, Oxford J, Ahmad Z, Reng G (2013) A review of nanoparticle functionality and toxicity on the central nervous system. Nanotechnology, the brain, and the future. Springer, New York, pp 313–332
Yoon Y et al (2018) Genetic ablation of EWS RNA binding protein 1 (EWSR1) leads to neuroanatomical changes and motor dysfunction in mice. Exp Neurobiol 27:103–111
Zak AK, Razali R, Majid WA, Darroudi M (2011) Synthesis and characterization of a narrow size distribution of zinc oxide nanoparticles. Int J Nanomed 6:1399
Zhang L, Jiang Y, Ding Y, Povey M, York D (2007) Investigation into the antibacterial behaviour of suspensions of ZnO nanoparticles (ZnO nanofluids). J Nanoparticle Res 9:479–489
Zhang X-Q, Xu X, Bertrand N, Pridgen E, Swami A, Farokhzad OC (2012) Interactions of nanomaterials and biological systems: implications to personalized nanomedicine. Adv Drug Deliv Rev 64:1363–1384
Zhao J, Xu L, Zhang T, Ren G, Yang Z (2009) Influences of nanoparticle zinc oxide on acutely isolated rat hippocampal CA3 pyramidal neurons. Neurotoxicology 30:220–230
Zhu J-W, Li Y-F, Wang Z-T, Jia W-Q, Xu R-X (2016) Toll-like receptor 4 deficiency impairs motor coordination. Front Neurosci 10:33