



## RESEARCH ARTICLE

### Zinc Oxide Nanoparticles Effect on Oxidative Status, Brain Activity, Anxiety-Like Behavior and Memory in Adult and Aged Male Rats

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#### ABSTRACT

Zinc is participating in different physiological processes and it is a functional or structural constituent of many proteins. As many pieces of evidence point to that in the elderly the zinc brain homeostasis is affected. The current work studied the zinc oxide nanoparticles (ZnO NPs) effect on the oxidative status and brain activity in adult and aged Sprague-Dawley rat brain. Forty male Sprague-Dawley rats (6 months and 24 months old rats) were used in this study; 10 of each age category served as a control and 10 rats of 6 and 24 months old rats were treated with zinc oxide nanoparticles (ZnO NPs) orally. Morris water maze and elevated plus maze performance tests were used for neurophysiological evaluations of memory and anxiety-like behavior, respectively. Brain malondialdehyde (MDA), reduced Glutathione (GSH) levels, glutathione peroxidase (GPx), superoxide dismutase (SOD) activities, serum and brain zinc and nitrite contents and creatine kinase (CK), acetylcholine esterase (AChE), and lactate dehydrogenase (LDH) activities were estimated. Control aged rats revealed high level of memory impairment, anxiety and oxidative stress damage which were not found in the control adult rats. ZnO NPs treatment significantly ameliorated the aging-induced cognitive impairment, anxiety and oxidative stress damage. The findings of the present study indicate a protective effect of ZnO NPs against aging-induced cognitive impairment, anxiety and associated oxidative damage recommending the use of ZnO NPs for boosting brain function and protecting the brain from further decay.

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#### INTRODUCTION

Researchers in neurobiology are not only trying to study the different aspects of brain aging but also developing beneficial strategies to help to maintain the brain compensatory capacity and to prevent neurological disorders (McCord and Aizenman, 2014). Aging is widely recognized by a gradual and marked loss of function, with progressive aging the nerve cells in the brain and cerebral blood flow decrease (Nuttall and Oteiza, 2014). Reactive oxygen species (ROS) are reported to have a damaging role in the beginning and progress of neuronal injury and nerve cell contains a group of enzymes and antioxidants to buffer the ROS after their production (Lizama-Manibusan and McLaughlin, 2013). Therefore, oxidative stress can occur either from an increased production of ROS, a defect in the antioxidant system, or both. Uncontrolled

ROS production can have a marked deleterious effect on nerve cells during aging (McCord and Aizenman, 2014).

Zinc (Zn) is a well-known essential trace element that is highly distributed and plentiful. It plays an important role in many cellular functions including neurotransmission, gene regulation, structural maintenance and enzymatic activity (Wang *et al.*, 2016). Zn was recorded to protect the blood-brain barrier from oxidative damage and prevent the progress of neurodegenerative diseases (Shim *et al.*, 2014). It was reported that age-related changes is frequently associated with a defect in the physiological functions of the brain and subsequently a decline in the memory performances, this could be related dysfunctions affecting the availability of Zn ion inside the cell (McCord and Aizenman, 2014).

It is an established fact that nanotechnology is now incorporated into various sciences like biology, chemistry, medicine and physics. Recently, ZnO NPS have drawn the

attention of many biotechnologists as it has useful properties with potentially unlimited therapeutic applications (Yan *et al.*, 2011). The Zn supplementation beneficial effect for brain function is undergoing scientific debate in the process of aging and age-related neuronal disorders. Some authors believe that zinc administration enhance myelination (Corona *et al.*, 2010), while others believe that it has harmful effects leading to the accumulation of zinc ions in post-synaptic neurons which leads to nerve cell toxicity (Attia *et al.*, 2018).

The present work was planned to study the effect of ZnO NPs on brain oxidative status, some enzymatic biomarkers, Neurophysiological evaluations of memory and cognitive function by using Morris water maze (MWM) and anxiety-like behavior by using the elevated plus maze (EPM) in adult and aged male rats.

## MATERIALS AND METHODS

Forty healthy Albino male rats, weighing  $190 \pm 10$  g and aged 6 months and 28 months were divided according to their age into four equal groups of 10 rats each. These age groups represent adult and aged rats, respectively. The animals were from the Research Medical Institute, Alexandria University, Egypt. Rats were acclimatized for 2 weeks and were housed in plastic cages kept in a room with light cycle, natural humidity and room temperature 22-25°C. Animals had free access to a standard pellet diet and water *ad libitum*. The guidelines of animal care of the National Institutes of Health (NIH) were in agreement with the execution of the current experiments.

Rats in the first and third groups (control adult and control aged, respectively) received distilled water by gavage (2 ml/kg BW) for 30 days. Rats in the second and fourth groups (ZnO NPs adult and ZnO NPs aged, respectively) received ZnO NPs (Zinc oxide nanodispersion; particle size <100 nm; Sigma-Aldrich Ltd., New York, USA) 5 mg/kg BW (Pasupuleti *et al.*, 2012) in 2 ml/kg BW by gavage for 30 days. Neurophysiological evaluations of memory function by using Morris water maze (MWM) and anxiety-like behavior by using elevated plus maze (EPM). Retro-orbital bleeding technique was used for collection of blood samples, they were left to coagulate, then the samples were centrifuged at 3000 rpm for 15 min, the serum was obtained and kept at -25 °C for Zn, CK, LDH, AchE and nitrite measurements. At necropsy, brain tissues were collected on ice to determine tissue Zn, CK, LDH, AchE and nitrite, MDA, GSH, GPx, and SOD concentrations.

### Neurophysiological evaluation

**Morris water maze (MWM):** MWM was used to assess the acquisition and retention of a spatial navigation task (Ihalainen *et al.*, 2016). On day 22 post-ZnO NPs administration, in a circular water pool, rats were trained to swim to a central platform. Initial acquisition latency (IAL) was recorded as the time taken by a rat to reach the platform. After training, the visual platform was hidden and following 24 h (day 23) and 8 days (day 30) after IAL, each rat was examined for the retention of the previously learned task. First and second retention latency (1st RL and 2nd RL) was recorded as the time to reach the hidden platform on days 23 and 30, respectively.

**Elevated Plus Maze (EPM):** The EPM was performed according to Torabi *et al.* (2013). Open arm time % OAT: [(time in open arm/ time in open + closed arm) × 100] and the open arm entries % OAE: [(number of open arm entries/ number of open + closed arm entries) × 100] were used for the evaluation of anxiety. Locomotor activity (LA) was measured as the distance proceeded in close and open arms in 5 min.

**Zinc, biochemical, antioxidant and oxidative stress analysis:** Zn concentration was analyzed according to (Simsek *et al.*, 2016). The activities of CK (EC: 2.7.3.2), LDH (EC: 1.1.1.27), and AChE (EC: 3.1.1.7) were determined using commercially available kits (Biodiagnostic, Egypt) following the manufacturer's instructions. Nitrite level was estimated according to Green *et al.* (1982). Part of brain tissues were kept frozen at -70°C for measurement of the levels of MDA, GSH and activities of GPx and SOD. They were determined according to the method of Yoshioka *et al.* (1979), (Sedlak and Lindsay, 1968), (Paglia and Valentine, 1967) and (Marklund and Marklund, 1974), respectively.

**Statistical analysis:** Results are expressed as mean ± SE. Statistical evaluations were done using ANOVA followed by Duncan's multiple range test. Statistical significance was set at P<0.05.

## RESULTS

### Neurophysiological evaluations

**Alteration in memory performance in MWM:** Control aged rats had a significantly delayed mean acquisition latency (on day 22) and retention latencies (1<sup>st</sup> and 2<sup>nd</sup> RL on day 23 and 30, respectively) compared to the control adult group. These data suggested that senescence caused a significant memory impairment. While ZnO NPs significantly improved the cognitive performance (increased memory retention) for the 1<sup>st</sup> and 2<sup>nd</sup> RL on days 23 and 30, respectively, compared to the control aged rats. However, ZnO NPs treatment in adult rats did not alter acquisition latency nor retention latencies compared to control adult animals that did not show initial signs of cognitive impairments like the aged ones (Fig. 1).

**Alteration in anxiety-like behavior in EPM:** Control aged rats have significantly decreased OAT, OAE and LA (P<0.05) compared to the control adult group, indicating an anxiogenic effect. ZnO NPs treatment significantly improved this anxiogenic effect (P<0.05) in the aged rat's group. However, ZnO NPs treatment in adult rats did not influence OAT, OAE and LA compared to control adult animals (Fig. 2). These results suggested that ZnO NPs has an anxiolytic effect in aged animals with anxiety but did not have the same effect on adult animals that did not show initial signs of anxiety like the control aged group.

### Zinc and enzymatic biomarkers of brain functions:

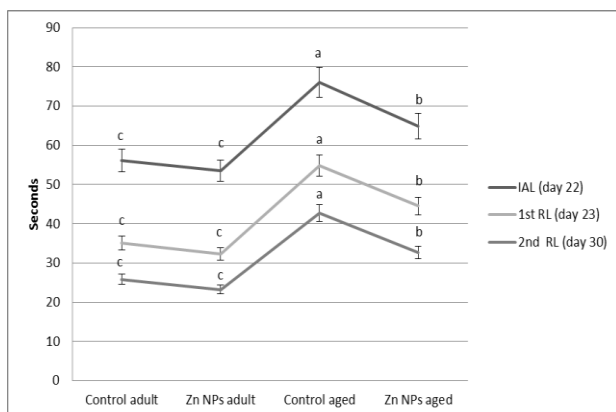
Data presented in Table 1 revealed that serum and brain zinc levels decreased significantly (P<0.05) in the control aged group compared to the control adult group but the administration of ZnO NPs to aged rats improved these parameters. Also, ZnO NPs administration to adult rats

increased serum and brain zinc levels significantly ( $P<0.05$ ) compared to the control adult group. Control aged rats showed a significant decrease in serum and brain CK and AChE activity and an increase in serum and brain nitrite and LDH activity, as compared with control adult rats ( $P<0.05$ ; Table 1). ZnO NPs improved these parameters in aged rats, which were near the observed data in the control adult group. Administration of ZnO NPs to adult rats did not significantly change serum and brain AChE, CK, nitrite activities and brain LDH while reducing serum LDH activity.

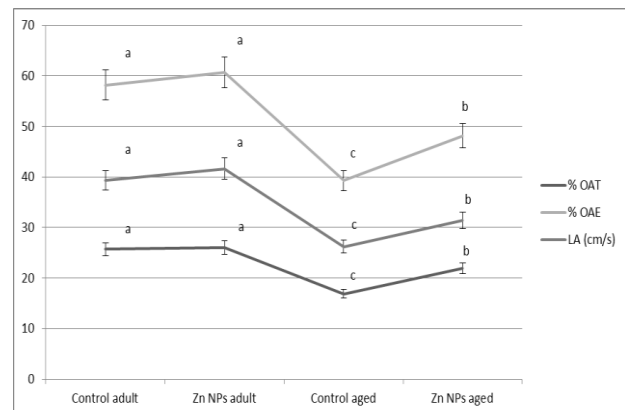
**Oxidative stress and antioxidant finding:** A state of oxidative stress was recorded in the control aged group with a significant increase in the level of brain MDA accompanied by a reduction in the activities of antioxidant enzymes; SOD and GPX, and the level of GSH in brain when compared with control adult group ( $P<0.05$ ; Table 2). Administration of ZnO NPs in aged and adult rats significantly reduced the MDA level with a concurrent increase in the activity of antioxidant enzymes and GSH level.

## DISCUSSION

The present study aims to clarify the effect of ZnO NPs on some neurophysiological evaluations of memory and anxiety-like behavior, brain enzymatic biomarkers and oxidative status of adult and aged male rats. Enhanced cellular uptake of zinc oxide nanoparticles might be the reason for the increase in zinc levels in serum and brain tissues of ZnO NPs treated groups (Shim *et al.*, 2014). These findings agree with the fact that the nanoparticles have a small size which makes them more absorptive in the tissue due to a large surface area per unit mass. NPs can affect all the body because they can pass through the blood-brain barrier, blood-testes barrier and can pass easily through cell membrane (Wang *et al.*, 2016). Zinc functions as a neurotransmitter as it can play a role in intercellular signaling in the nervous system (Torabi *et al.*, 2013). Kumar *et al.* (2016) provided evidence relating the relationship between zinc homeostasis and the changes occurring in the brain during aging especially zinc deficiency which was reported among the elderly as a common cause of morbidity.



**Fig. 1:** Effect of ZnO NPs on the memory performance in MWM (in seconds) of adult and aged rats. Values are expressed as mean $\pm$ SE of ten animals per group. The values with different superscript letters on the same line significantly differ at  $P<0.05$ .



**Fig. 2:** Effect of Zn NPs on the % OAT, % OAE and LA (cm/s) in EPM of adult and aged rats. Values are expressed as mean $\pm$ SE of ten animals per group. The values with different superscript letters on the same line significantly differ at  $P<0.05$ .

**Table 1:** Effect of ZnO NPs on zinc and enzymatic biomarkers of brain functions in serum and brain tissues of adult and aged rats

	Control adult	ZnO NPs adult	Control aged	ZnO NPs aged
<b>Serum</b>				
Zinc ( $\mu\text{g}/\text{dl}$ )	93.15 $\pm$ 3.43 <sup>b</sup>	115.91 $\pm$ 4.33 <sup>a</sup>	74.24 $\pm$ 2.78 <sup>c</sup>	88.74 $\pm$ 3.94 <sup>b</sup>
CK (U/l)	297.21 $\pm$ 18.12 <sup>a</sup>	307.42 $\pm$ 16.11 <sup>a</sup>	242.31 $\pm$ 12.69 <sup>b</sup>	288.71 $\pm$ 17.97 <sup>a</sup>
LDH (U/l)	540.89 $\pm$ 14.64 <sup>b</sup>	492.81 $\pm$ 13.13 <sup>c</sup>	591.03 $\pm$ 19.08 <sup>b</sup>	534.41 $\pm$ 11.61 <sup>b</sup>
AChE (U/l)	148.68 $\pm$ 4.32 <sup>a</sup>	152.95 $\pm$ 5.41 <sup>a</sup>	118.82 $\pm$ 3.72 <sup>c</sup>	135.42 $\pm$ 4.31 <sup>b</sup>
Nitrite ( $\mu\text{mol}/\text{ml}$ )	74.27 $\pm$ 3.05 <sup>c</sup>	66.23 $\pm$ 6.18 <sup>c</sup>	136.62 $\pm$ 5.78 <sup>a</sup>	101.85 $\pm$ 4.81 <sup>b</sup>
<b>Brain</b>				
Zinc ( $\mu\text{g}/\text{g}$ tissue)	31.85 $\pm$ 2.19 <sup>b</sup>	38.24 $\pm$ 2.78 <sup>a</sup>	21.74 $\pm$ 1.58 <sup>c</sup>	29.23 $\pm$ 1.78 <sup>b</sup>
CK (U/g tissue)	14.34 $\pm$ 1.55 <sup>a</sup>	15.74 $\pm$ 1.58 <sup>a</sup>	6.39 $\pm$ 0.16 <sup>c</sup>	9.51 $\pm$ 0.73 <sup>b</sup>
LDH (U/g tissue)	165.95 $\pm$ 8.13 <sup>b</sup>	158.31 $\pm$ 9.16 <sup>b</sup>	205.91 $\pm$ 13.52 <sup>a</sup>	167.57 $\pm$ 9.51 <sup>b</sup>
AChE (U/g tissue)	216.61 $\pm$ 4.78 <sup>a</sup>	222.54 $\pm$ 4.52 <sup>a</sup>	156.04 $\pm$ 6.71 <sup>c</sup>	187.09 $\pm$ 9.29 <sup>b</sup>
Nitrite ( $\mu\text{mol}/\text{g}$ tissue)	25.99 $\pm$ 2.17 <sup>c</sup>	23.24 $\pm$ 2.34 <sup>c</sup>	43.57 $\pm$ 3.17 <sup>a</sup>	35.14 $\pm$ 2.38 <sup>b</sup>

Values are expressed as mean $\pm$ SE. The values with different superscript letters within the same row significantly differ at  $P<0.05$ .

**Table 2:** Effect of ZnO NPs on oxidative stress and antioxidant status of adult and aged rats

	Control adult	ZnO NPs adult	Control aged	ZnO NPs aged
MDA (nmol/g tissue)	80.27 $\pm$ 3.05 <sup>c</sup>	68.93 $\pm$ 3.64 <sup>d</sup>	129.62 $\pm$ 8.37 <sup>a</sup>	105.91 $\pm$ 6.04 <sup>b</sup>
GSH (mmol/g tissue)	37.74 $\pm$ 3.06 <sup>b</sup>	46.35 $\pm$ 2.21 <sup>a</sup>	15.52 $\pm$ 1.56 <sup>d</sup>	23.91 $\pm$ 1.43 <sup>c</sup>
SOD (U/g tissue)	48.14 $\pm$ 4.06 <sup>b</sup>	59.27 $\pm$ 3.38 <sup>a</sup>	26.19 $\pm$ 2.23 <sup>d</sup>	35.73 $\pm$ 2.74 <sup>c</sup>
GPx (U/g tissue)	125.29 $\pm$ 5.11 <sup>b</sup>	147.88 $\pm$ 2.53 <sup>a</sup>	82.17 $\pm$ 3.34 <sup>d</sup>	101.41 $\pm$ 6.56 <sup>c</sup>

Values are expressed as mean $\pm$ SE. The values with different superscript letters within the same row significantly differ at  $P<0.05$ .

McCord and Aizenman (2014) reported that aging is a process associated with oxidative stress, nucleic acid damage, or cell metabolism alteration. The brain of the elderly is mostly associated with neuronal loss, memory impairment and higher susceptibility to neurological disorders. The brain is highly sensitive to damage from free radicals because its low content of antioxidant enzymes, in addition to its high content of polyunsaturated fatty acids which are especially susceptible to peroxidation from free radicals (Chen *et al.*, 2017). In our study, the improvement in the oxidative status of treated aged rats with ZnO NPs approached control adult levels; zinc seems to have an effect on oxidative stress. It was reported that both extremely low and high levels of zinc are associated with oxidative stress, however, intermediate levels were found to be neuro-protective (Aimo *et al.*, 2010) which could give a good explanation for the positive effect of zinc in the present study where a moderate dose of ZnO NPs was used.

SOD metabolizes superoxide anion to hydrogen peroxide ( $H_2O_2$ ), whereas GPx breakdown  $H_2O_2$  ending the cycle of superoxide neutralization. Glutathione is the most abundant non-protein thiol that neutralizes ROS in the brain tissue; it eliminates  $H_2O_2$  (Li *et al.*, 2018). Calcium influx is enhanced by zinc deficiency which leads to activation of nitric oxide synthase combined with dysfunction of mitochondria leads to oxidative stress; overproduction of nitrite in aged mice is associated with neuro-degeneration because of its free radical properties which may cause irreversible cell damage, as they can cause lipid peroxidation in addition to the damage of neurons because of the alterations resulting from cholinergic deficits. These defects are associated also with memory and learning impairments (Zhang *et al.*, 2018).

During senescence the age-related disruption in cholinergic function may be partially responsible for short term memory deficits. AChE is a hydrolytic enzyme that breaks the neurotransmitter acetylcholine (ACh) into acetate and choline by which it temporally controls the synaptic activation, and this mechanism is considered to be the main marker of cholinergic metabolism (Leal *et al.*, 2017). With aging the activity of AChE is reduced as observed in the present study, and this is in agreement with Haider *et al.* (2014) who reported that the AChE activity decrease in the elderly and is reflected with brain ACh content; also, functional reduction in the neuron population due to the decrease in the enzyme activity. The low activity of the AChE might be due to the free radical formation during aging which could upset the prooxidant/antioxidant balance inside the brain (Petrov *et al.*, 2018). This could give a good explanation for the positive effect of ZnO NPs (as an antioxidant) on the AChE activity in aged rats; which were near the control adult group range.

Datta and Chakrabarti (2018) suggested that increased brain lactate is a sign of aging and that this lactate accumulation is explained by a shift in the LDH isoenzyme pattern which is found to be related to aging. CK is a crucial enzyme for the brain as it is a high energy consuming tissue; it plays an important role during energy metabolism as it acts as a buffering system of the ATP inside the cell. CK is markedly affected by oxidative stress especially by the thiol group oxidation; it was also

reported that a reduction in the activity of CK is related with neuronal disorders (Ferreira *et al.*, 2017). The significant decrease in the activity of CK in brain of aged rat is consistent with the compromised oxidative status of the brain especially the glutathione depletion recorded in the findings of our study.

The anxiety and the impairment in memory and cognitive function found in the current work could be a normal aging process consequence which is associated with a significant reduction in brain zinc level that could have caused the oxidative stress state and changes in brain enzymatic biomarkers especially the decrease in brain cholinergic activity which is previously recorded in our study. The findings of the present study agree with Torabi *et al.* (2013) who recorded that the ZnO NPs anxiolytic effect is much higher than the conventional form of ZnO. Our findings agree with previous studies which suggest that zinc deficiency has a role in the induction of anxiety, depressive-like symptoms and decreased physical activity (Tassabehji *et al.*, 2008). It was hypothesized that cognitive dysfunction might be caused by the state of oxidative stress (Lizama-Manibusan and McLaughlin, 2013).

It was suggested that zinc deficiency could enhance the incidence for the development of neurodegenerative disease, rather than an excess, as neurogenesis and increased neuronal apoptosis was shown to be affected by zinc deficiency, which can lead to memory and learning impairments (McCord and Aizenman, 2014). Moreover, zinc homeostasis alteration is considered to be an important determinant of the fate of neurons in the aged brain, and is also suggested as predisposing factor for depression, Alzheimer's disease and senescence (Szewczyk, 2013). The most sensitive part of the brain to the overdose as well as deficiency of zinc seems to be the hippocampus. Because it is the part of the brain which plays an important role in memory, learning and neurogenesis, indeed, the effect of administration of zinc or deficiency of zinc on these processes will be critical (Yang *et al.*, 2013).

**Conclusions:** The present study provides evidences for the defensive effect of ZnO NPs administration against aging-induced anxiety, cognitive impairment and associated oxidative stress. Thus, we propose the use of ZnO NPs as a promising treatment for brain damage by restoring the neurological activity induced by aging and other neurodegenerative disorders and for prevention of further brain decay.

**Authors contribution:** Both authors conceived the idea, designed the review, executed the experiment, analyzed the parameters, interpreted the data, wrote up and revised the manuscript.

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