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Oral zinc oxide nanoparticles attenuate chronic stress induced alterations in metabolic profile and antioxidant activity in male rats

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Abstract

The dynamic mechanism of coping with stress at different body, organ, tissue and even cellular levels is a life critical process. Improving such capability means a better life quality. Oral supplementation of various therapeutics and natural agents has been widely implemented. Biomedical applications of nanomaterials have gained a lot of attention due to their extensive and important biological features and biomedical uses. All living beings require the trace metal zinc. Although, in the gut, dietary ZnO is poorly absorbed, zinc oxide nanoparticles (ZnO-NPs) can be considered a suitable substitution as it has an increased surface reactive area, bioavailability, and absorbability. The purpose of this study was to investigate the protective role of ZnO-NPs on metabolic and antioxidant activity after chronic application of multiple stressors. Forty adult male albino rats with initial mean body weight (200 ± 10) g were randomly divided into 4 equal groups (10 for each) as follow: Group one: control (Handled), Group two: Zinc oxide Nanoparticle (oral 3mg/kg Bwt ZnO-NPs), Group Three: Chronic restrain stress (CRS), Group Four: Chronic restrain orally administered with Zinc oxide nanoparticle (CRS + oral 3mg/kg Bwt ZnO-NPs). After 60 days of chronic restrain for 3 hrs/day, rats were sacrificed. Application of chronic restrain stress showed significant elevated in total cholesterol, triacylglycerol, corticosterone, MDA and glucose levels and reduced total antioxidant capacity, free fatty acid in comparison with control group. Oral administration of ZnO-NPs in chronic stressed rats significantly reduced Total Cholesterol, Free fatty acid, corticosterone, MDA, glucose levels and improved HDL-c, total antioxidant capacity and insulin levels compared to stressed group. Overall, ZnO-NPs administered orally may improve the body's antioxidant and metabolic functions.

Keywords: Zinc oxide Nanoparticles; Chronic Restrain Stress; Oxidative Stress; antioxidant activity

1. Introduction

Stress has an impact on many parts of our body and generates a variety of physiological changes, which appear as symptoms including headache, nausea, heartburn and exhaustion1. Stress has serious and enduring negative effects affect the growth rate, feed intake, body weight, libido and productivity of the animal 2. Acute and chronic stress can be classified depending on how long it lasts. Both acute and chronic stress induces a known physiological and biological problems, in particular, because of their substantial influence on the generation of reactive oxygen species (ROS) 3.

Adrenocorticotrophic hormone (ACTH) is released by the anterior pituitary in response to stress, and the hypothalamus then releases corticotropin-releasing hormone (CRH), which ultimately causes an increase in the synthesis and release of corticosteroids, including corticosterone (CORT), in rats. Different stress reactions are triggered in tissues upon CORT

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release, which then feeds back negatively on the production of CRH and ACTH from brain and pituitary. 4. External stimuli including heat, shock, noise, radiation, hyperoxia, toxins, and physical activity increase the formation of free radicals (ROS). Normal cells create modest amounts of ROS, but their accumulation can disrupt macromolecules including lipids, proteins, carbohydrates, and DNA structures 5. Thus improving the antioxidant defenses is effective therapeutic strategy for preventing free radical production and oxidative damage 6.

Zinc is a trace element that is important to all living things, including human and rodents. Substantial research has been done to understand how zinc operates both physiologically and biochemically. More than 300 distinct enzymes, cell-signaling proteins and transcription factors have zinc as a component, which supports the body's regular immunological processes. It also modifies protein metabolism, protects the integrity of cell membrane, and aids in controlling cell differentiation and proliferation 7. Because zinc wasn't very bioavailable, zinc oxide nanoparticles (ZnO-NPs)are considered suitable substitutions as it has an increased surface reactive area, bioavailability and absorbability, as well as cover the needs of the animals accordingly 8. The uses of ZnO-NPs extends also to industrial applications 9. The study's goal is to determine whether or not ZnO-NPs can decrease the oxidative stress generated on by exposure to chronic restraint stress and study the potential ameliorative effects of ZnO-NPs against metabolic imbalance.

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2. Methods

2.1. Animal care

40 Adult male albino rats with initial mean body weight (200 ± 10) g were utilized in the study. Rats were obtained from laboratory animal unit of the Faculty of Veterinary Medicine, Zagazig University. Rats were kept in a 24±1 ºC ambient temperature environment with a 12-hour light/dark cycle. and 35-50% humidity for two weeks before the beginning of the experiment for acclimatization. standardized chow and the experiments included unlimited access to tap water. The care and use of the animals confirmed to the rules of the Institutional Animal Care and Use Committee (IACUC) of Zagazig University, Egypt (Approval no: ZU-IACUC/2/F/94/2022).

2.2. Zinc oxide nanoparticle synthesis and preparation (ZnO-NPs)

ZnO-NPs have been prepared as previously described and reported 10. By means of X-ray diffraction (XRD), the phase characterization and morphology of nanoparticles were examined using the XPERT-PRO Powder Diffractometer device., with 2 theta (20^{O} - 80^{O}), with Minimum step size 2Theta: 0.001, and at wavelength (Kα) = 1.54614^{O}. and JEOL JEM-2100 high resolution transmission electron microscope at an accelerating voltage of 200 kV as shown in (Figure 1). The TEM image of Zinc oxide revealed that the prepared zinc oxide in nanoscale with average size 30± 3nm and spherical like shape.

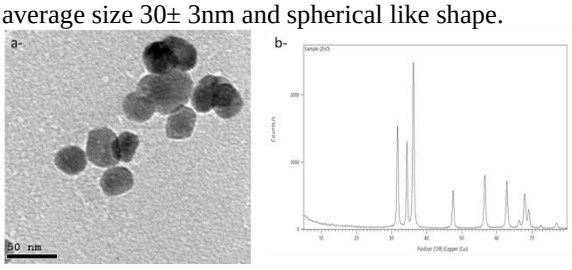

Figure 1. Characterization of ZnO-NPs (a-b): a- Morphology of the formed ZnO-NPs pictured by transmission electron microscopy (TEM) and; b- X-ray diffraction (XRD) pattern of the ZnO-NPs

2.3. Experimental design

40 experimental rats were used, and they were divided into four equal groups (10 for each) at random. ; Group one: control (handle for only 2-3 minutes and orally administered with distilled water at 1 ml/kg Bwt via gastric tube), Group two: Zinc oxide Nanoparticle administered group (handled for 2-3 minutes/day and orally administered ZnO-NPs 3mg/kg Bwt via gastric tube) 11, Group Three : Chronic restrain stress (CRS) as previously reported in 12 with modifications and orally administered with distilled water at 1 ml/kg Bwt via gastric tube. Group Four: Chronic restrain stress (CRS) as previously reported in 12 with modifications and orally administered ZnO-NPs 3mg/kg Bwt via gastric tube. Oral administration of ZnO-NPs or distilled water (1ml/kg Bwt) was performed at 8:00 am. Animals were stressed 3 hrs/day, 7 days a week for 60 days. The operation for immobilization was done out from 9:00 am to 12:00 pm. The stress protocol involved exposure to one of seven different restraint stressors with random sequence to avoid habituation. For induction of acute stress, the remaining 10 rats were restrained as acute stress for 3 hours directly before blood collection.

2.4. Sample collection

At the end of the experiment, 24 hours after the last induction of restraint stress, blood samples were collected via median eye canthus from fasted rats without anticoagulant for separation of serum via centrifugation at 3000 r.p.m for 20 minutes, the collected serum was stored at -20oC until analysis of biochemical parameters. After that, all experimental animals were then euthanized.

2.5. Biochemical measurements

A glucometer was used to measure blood sugar level (URight blood glucose meter TD-4251). Total cholesterol (TC) levels in the blood, triacylglycerol (TAG), high-density lipoprotein-cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), and very low-density lipoprotein-cholesterol (VLDL-c) were determined as previously reported 13, 14 using kits

provided from Spectrum, Egyptian Company for Biotechnology, Cairo, Egypt. Free fatty acid (FFA) was spectrophotometrically measured using Biovision FZscreen TM (BioVision, Inc., USA) as previously reported 14. The serum insulin and corticosterone levels were determined by commercially available specific rat insulin and corticosterone ELISA kits (Mybiosource and RayBiotech, respectively) according to manufacturing instruction using a DNM–9602 ELISA reader (Beijing Perlong Medical Inst. Ltd., China).

2.6. Data analysis and statistics.

Statistical analysis and figures were produced using GraphPad prism 7.0 software (GraphPad Software Inc., United States). Statistical comparisons between groups were performed using ANOVA followed by post hoc Tukey test. A P-value < 0.05 was described as statistically significant.

3. Results

3.1. Effects on lipid profile.

Application of chronic restraint in male rats resulted in an elevated blood total cholesterol (TC) level markedly when compared to other groups (Fig. 2a). Furthermore, chronic stress markedly increased serum TAG level in comparison to control group, ZnO-NPs group but no significant change in TAG level was noticed while combining chronic stress with oral administration of 3 mg/kg Bwt ZnO-NPs (Fig. 2b). Although no significant reduction in the level of serum HDL-c was noticed following chronic stress induction in comparison to the control group, the combination with orally administered ZnO-NPs significantly increased HDL-c level compared to chronic restraint rats (Fig. 2c). The levels of serum LDL-c and VLDL-c were greatly reduced in chronic restraint group compared to orally administered ZnO-NPs rats, but not in the control rats (Fig. 2d, e). Interestingly, when compared to the control, the chronic stress group's serum FFA level was significantly lower. or ZnO-NPs groups, that was further significantly reduced in the combination of chronic stress with dose of 3 mg/kg Bwt ZnO-NPs orally (Fig. 2f).

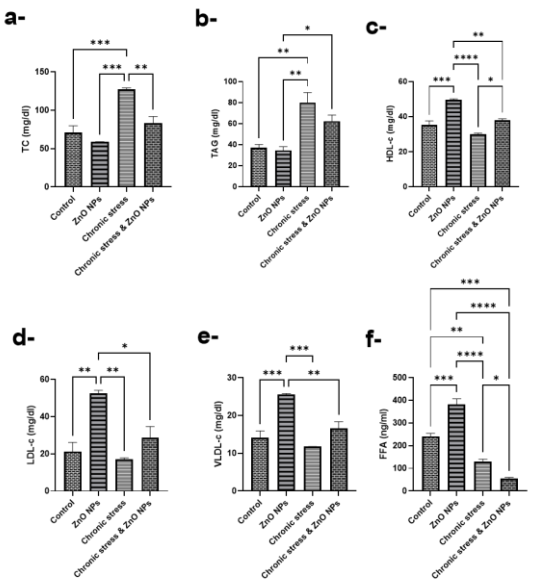

Figure 2. Effect of oral administration of ZnO-NPs (3mg/KgBw.t) on lipid profile of chronic restraint stressed rats (a-f). a-TC, b- TAG, c-HDL-c, d- LDL-c, e- VLDL-c and f- FFA. Values are mean ± SEM of 10 animals per experimental group. Means bearing different superscripts were significantly different at P < 0.05.

3.2. Effects on serum corticosterone level.

A significant elevation in serum corticosterone level in chronic restraint group was noticed when compared to all other groups (Fig. 3a). Such elevation in corticosterone level not occurred due to the acute effect of restraint stress, as acute stress resulted in vastly higher serum corticosterone levels than both control and chronic restraint rats (Fig. 3b). The combination of orally administered ZnO-NPs with chronic restraint significantly reduced serum corticosterone level compared to chronic restraint group (Fig. 3a)

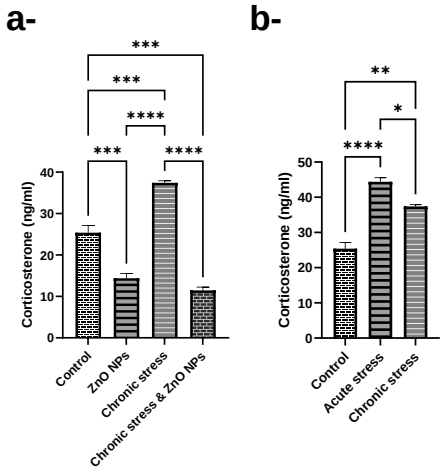

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Figure 3. Effect of oral administration of ZnO-NPs (3mg/KgBw.t) on serum corticosterone level of chronic restraint in male rats (a-b). a- corticosterone level in chronic restraint stress, and b- corticosterone level in chronic stress vs acute stress. Values are mean ± SEM. Means bearing different superscripts were significantly different at P < 0.05.

3.3. Effects on serum insulin and blood glucose levels.

The chronic application of restraint stress caused a marked elevation in both serum insulin and glucose levels compared to control and orally administered ZnO-NPs rats (Fig. 4a, b). The combination of oral administration of ZnO-NPs with chronic restraint significantly augmented serum insulin level compared

to other groups (Fig. 4a). The level of serum glucose significantly dropped in the combination of oral administration of ZnO-NPs compared to chronic restraint group (Fig. 4b).

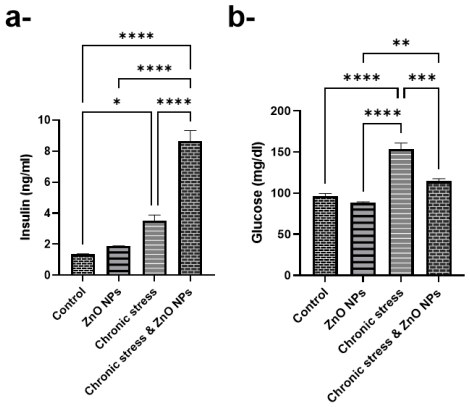

Figure 4. Effect of oral administration of ZnO-NPs (3mg/KgBw.t) on serum insulin and blood glucose levels of chronic restraint in male rats(a-b). a- serum insulin, and b- fasting blood glucose. Values are mean ± SEM. Means bearing different superscripts were significantly different at $P < 0.05$.

3.4. Effects on serum total antioxidant capacity and malonaldehyde (MDA) levels.

Oral administration of ZnO-NPs (3mg/KgBw.t) showed significantly increase in serum TAC when be in comparison with the control group (Fig. 5a). Chronic restrain stress group showed significantly decrease in TAC when be in comparison with the control group (Fig. 5a). Chronic restrain stress & ZnO-NPs group showed significantly increase in TAC when compared to chronic restrain stress (Fig. 5a). Oral administration of ZnO-NPs (3mg/KgBw.t) showed significantly decrease in MDA when compared to control group (Fig. 5b). Chronic restraint stressed group showed significant increase in serum MDA when compared to other groups (Fig. 5b). Chronic restraint stress & ZnO-NPs group showed significant reduction in MDA when compared to chronic restraint stressed group (Fig. 5b).

4. Discussion

Smaller than 100 nm nanoparticles are known as nanoparticles (NPs), which can be considered a key player in the modern era of biomedicine, therapy, and disease prevention. Nanoparticles can quickly enter the bloodstream and circulate through the blood to various organs. ZnO-NPs receive more attention in commercial and biomedical applications due to their antibacterial, anti-inflammation, anticancer, anti-diabetic actions as well as increased surface reactive area, bioavailability, and absorbability 15.

The release of catecholamines from the adrenal medulla, corticosteroids from the adrenal cortex, and adrenocorticotropin from the anterior pituitary are three significant adaptive hormonal responses that are stimulated by stress. 16 . When an organism is exposed to a wide range of stresses, the sympato-adrenal and hypothalamic-pituitary-adrenocortical systems interact extensively to maintain the internal environment. 17 . On the 15th day, there is a considerable increase in fasting plasma corticosterone concentrations as well. On the 30th day, these concentrations recover to levels those before the stress exposure.On the 15th and 30th experimental days, the weights of the stressed animals are considerably lower than the controls18.

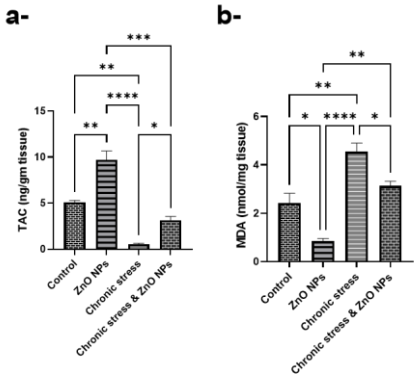

Figure 5. Effect of oral administration of ZnO-NPs (3mg/KgBw.t) on serum total antioxidant capacity and malonaldehyde (MDA) levels of chronic restraint in male rats(a-b). a- total antioxidant capacity, and b-malonaldehyde (MDA) level. Values are mean ± SEM. Means bearing different superscripts were significantly different at $P < 0.05$.

Chronic stress is a condition caused by the prolonged application of any stressor. Stress is known to impact the physiological function of the brain and spinal cord, the reproductive system, digestive system, neuroendocrine system, and cardiovascular system. Corticosterone and blood glucose levels that are increased, according to biochemical research are key stressors 19. The induction of stress induced hyperglycemia in rodent has been reported and verified as a model 20. Yet, the mechanism explaining, it is yet unknown how CRS leads to insulin-resistant. According to studies 21, showed that chronic stress activates the HPA axis.. The HPA axis activation led to an increase in glucocorticoid production. Insulin resistance is brought on by glucocorticoids in the circulation, which limit the effectiveness of insulin signaling in peripheral tissues 22. According to our research, serum corticosterone levels have significantly increased. in chronic restraint group was noticed compared to all other groups. Interestingly, oral administration ZnO-NPs in handled or chronic restraint rats significantly reduced serum corticosterone level compared to control or chronic restraint stressed rats.

Although ZnO-NPs have been shown to have antihyperglycemic action, research in this area is limited. As a result, additional in-depth studies are necessary to assess their significance as therapeutic agents following chronic therapies administration. A recent study found that an oral dosage of 1-10 mg/kg for 4 weeks decreased hyperglycemia in type 1 and type 2 diabetes but had no impacton insulin levels in type 1 diabetes.Only at a dose of 10 mg/kg did insulin levels in people with type 2 diabetes rise. , explaining the enhanced glucose tolerance in this model 23. After seven weeks of such oral administration, a comparable impact was seen 24. Other trials in type 1 diabetes, utilizing the same implementing strategy over 28 or 56 days, however, found an elevation in insulin levels along with antihyperglycemic effects 25. In our study, chronic application of restraint stress elevated in both serum insulin and glucose levels. The combination of oral administration of ZnO-NPs with chronic restraint significantly augmented serum insulin level along with antihyperglycemic effects.

Chronic restraint stress has been linked to alterations in blood lipid profiles. Following immobilization in rabbits, a marked increase in the levels of TC, LDL-c, VLDL-c, and TAG in the blood 26 were reported. Such changes were also reported in genetically modified mice 27. In rats, there has been a decrease in HDL-c concentration after immobilization, chronic unpredictable stressors, and restraint 28. Application of chronic restraint in our study increased serum TC, TAG level, did not change LDL-c or VLDL-c and lowered HDL-c and FFA levels. Such results indicate the induction of dyslipidemia. This can be supported by 29 who said that under stress, LDL-c levels rise while HDL-c levels fall.. Rats under continuous restraint stress reported had much lower total cholesterol levels. 30.

The effect of administration of ZnO-NPs on lipid profile is yet unclear. One study revealed that ZnO-NPs demonstrated a significant improvement in TC and TAG levels 31. These anti-hyperlipidemic activities of ZnO-NPs may be related to the nanoparticles' improvingthe affected pancreatic -cells' insulin-like nature . In our study, oral administration of ZnO-NPs increased HDL-c, LDL-c VLDL-c and FFA but not, TC or TAG in handled rats. Oral administration of ZnO-NPs decreased TC and FFA, but not, TAG, LDL-c VLDL-c in chronic restraint stressed rats while increasing HDL-c level. The effect of administration of of ZnO-NPs on lipid profile could be dose / route dependent. Administration of higher levels of ZnO-NPs (25 mg/kg and 50 mg/kg) were found to increase serum levels of triglyceride 32. Administration of comparably lesser levels of ZnO-NPs significantly decreased TAG levels 33.

Any incompatibility between the antioxidant system and ROS generation, as well as any increase in free radical species , would result in oxidative damage to various components in cells, including lipids, proteins, and nucleic acids. Chronic restraint stress may disrupt the oxidant/antioxidant equilibrium, causing a high generation of free radicals while suppressing antioxidant capacity. The current findings, like previous studies, showed that under prolonged restraint stress, levels of the oxidative lipid peroxidation marker (MDA) increased whereas levels of TAC decreased. 34. The oxidative impacts of ZnO-NPs depend on the dose of administration. At a lower dose similar to the one used in our study, 11 reported that ZnO-NPs significantly decreased the MDA level. According to 35, ZnO-NPs can reduce MDA levels, increase antioxidant enzyme activity, and protect cell membrane integrity from oxidative stress damage. Furthermore, ZnO-NPs have a hepatoprotective impact at low dosages either removing free radicals from the environment or stimulating antioxidant processes that detoxify free radicals 36. Although administration of higher levels of ZnO-NPs were suggested to induce oxidative stress owing to a significant decrease in the activity of antioxidant enzymes, and reduced blood TAC level in rats. MDA and total oxidant status (TOS) levels have been shown to increase significantly after exposure to a high concentration of ZnO-NPs 37.

5. Conclusions

In conclusion, this study provides preliminary evidence that exposure of rats to chronic restraint stress induces changes in metabolic performance and lipid profile, hormone leve accelerates lipid peroxidation and oxidative stress. Oral administration of ZnO-NPs at lower doses could be beneficial to releive such alterations. The study can provide insights to researchers for further utilization of NP compounds for therapeutic, preventive and medicinal applications.

6. Conflicts of interest

There are no conflicts to declare.

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