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Exposure to zirconium nanoparticles induce oxidative stress in albino mice

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ABSTRACT

Zirconium (Zr) is identified as one of the metal compounds that have been employed in biosensors, dentistry, cancer treatment and nephrology for a various purpose such as hemodialysis, peritoneal dialysis due to the low toxicity. This study aimed to detect the malondialdehyde (MAD) concentrations in the blood and liver after 14 and 28 days of orally exposure to 400 and 500 mg/kg bw ZrO_2 NPs in mice. The twenty-five albino mice were divided into five groups and each group comprises five animals. The first group considered as a control and the other four groups as a treatment. At the ends of each period of exposure, blood samples were collected from the heart and liver samples were collected to measure the level of MDA. Comparison with the control group, the average animals body weight was statistically significant reduced after 28 days of exposure to both concentrations of ZrO_2 NP, with time effect. The level of MDA revealed a significant elevated in blood and liver tissue with both doses and times of exposure compared to the control with concentration and time effects. In conclusion, the current results revealed that ZrO_2 NPs can produce an increased oxidative damage in mice and damage the tissue structure of organs.

Keywords: ZrO_2 NPs, Oxidative stress, MDA, Mice.

Introduction

Zirconium, known as zircon (Zr), which is classified as a transition metal with an atomic number of 40. The zirconium name has Arabic origin and derived from the word Zargon, meaning golden in color (Abd El-Ghany et al., 2016). According to study by Saridag et al. (2013), Zircon is archetypally a gray-white element that is not commonly found in nature as a pure metal form. Zirconia has become a topic of significant interest and inquiry across various fields and disciplines due to its advantageous properties, including biocompatibility, high resistance to temperature and corrosion, fracture toughness, mechanical strength, excellent optical and insulator characteristics with a high refractive index, low solubility in water, lipophilic nature, and strong chemical stability (Aguilar, 2013).

Several studies have found that the use of zirconia nanoparticles in biomedical applications is considered safe by some, while others have raised concerns about potential risks, indicating the need for further research (Han et al; 2020). Recently Zr NPs has been utilized in the medical field as a nanocarrier for targeted drug delivery. This application has been supported by the established of zirconia bioactivity (Nagy et al., 2016; Hichem et al., 2022). Zirconium compounds have also been employed in nephrology for various purposes such as peritoneal dialysis, hemodialysis, hemofiltration, anti-cancer medication researches, deodorants, and antiperspirants (Hosseinzadeh et al., 2019). Piconi (2014) reported that over 500,000 zirconia orthopedic materials for total hip replacement ball-heads were initially produced. Recent advancements in nanotechnology have expanded the applications of Zr NPs in the biomedical sector, particularly in dental implants and bridges as well as crowns due to its biocompatibility, minimal corrosion, and aesthetic appeal (Nishihara et al., 2019).

Asadpour et al. (2014) reported that using of nano-ZrO_2 might have cytotoxic effects such as suppression of cell growth, DNA damage, and programmed cell death. Whereas, Ye et al. (2018) study illustrated that nano ZrO_2 could produce reactive oxygen species (ROS) and apoptosis in Osteoblast-Like 3T3-E1 Cells, which may have an effect on osteogenesis.

Previous research demonstrated the distribution of nano ZrO_2 in different organs such as the kidney, spleen, lung, heart and brain, and it found that exposure to these materials might cause alteration of gene expression, oxidative stress and cell death in different organs (Sun et al., 2019).

The oxidative stress in living organisms generate from the imbalance between the generation of ROS and the organism's capability to neutralized them (Vona et al., 2021). The variation between over reactive molecules and frail endogenous defense causes in damage to cellular structures and molecules such as proteins, lipids, and DNA, eventually contributing to the pathogenesis of an inclusive range of diseases. The available of ROS in small amounts turn as signal transduction molecules driving the cell activities and also run the cell protection (Janssen-Heininger et al., 2008). Whereas, the over amount of ROS (as in inflammation) may activate the generation of additional extremely reactive species (Vaziri ,2008).

Free radicals initiate the lipid peroxidation process within the body, leading to the production of malondialdehyde (MDA) due to the peroxidation of polyunsaturated fatty acids in cells. Elevated levels of free radicals lead to excessive production of MDA, which is widely recognized as an indicator of oxidative stress and the antioxidant status in individuals (Gawet et al.; 2004). Shi et al. (2014) found that intraperitoneal injection of various doses of zirconyl chloride in mice for 30 days caused increased the level of MDA in the kidney and decreased the activities of SOD and GSH-Px as well as histological changes in the kidney tissue such as inflammatory cells infiltration and renal tubular epithelial cells swelling. Since no comprehensive study has been conducted on the relationships between ZrO_2NPs exposure and malondialdehyde (MDA) concentration, this research was undertaken to detect the concentration of MDA in blood and liver following exposure to different concentrations of ZrO_2 NPs for 14 and 28 days.

Materials and Methods

Characters of ZrO2NPs: The ZrO_2 NPs employed as a white powder with a purity of 99% and a particles size of 40 nm from US Research Nanomaterials. Inc, USA. These particles have spherical shape (Figure 1).

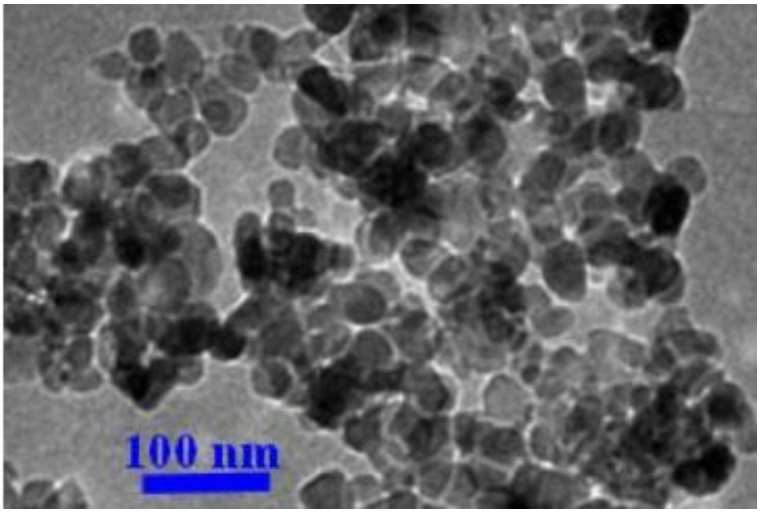

Figure (1): Showed the shape of ZrO_2 NPs according to the company which have spherical shape.
https://www.us-nano.com/inc/sdetail/356

Concentration preparation: Two concentrations of ZrO_2 NPs (400 and 500 mg/kg /body weight) were selected to be administrated orally to the albino mice for 14 and 28 days. Each concentration was prepared by using distilled water to dissolve the ZrO2NPs powder. The suspension of ZrO_2 NPs was agitated for 30 min. in an ultrasonic bath and then mixed before being administrated to mice.

Treated animals: Twenty-five albino mice, weighing between 20-30 g, were purchased from the Iraqi

Center for Cancer Research in Baghdad. All procedures involving animals were approved by the Al-karkh university of Ethics Committee of the Science college, Iraq. Animals were fed with mice pellet and drunk tap water and housed in a standard laboratory condition. Mice were randomly divided into five groups (N = 5 in each). For fourteen and twenty-eight days, 0.1 ml of orally suspension was administered to the following groups: group I (GI) served as the control group and received distilled water; group II (GII) received 400 mg/kg ZrO_2 NPs for 14 days; group III (GIII); received 500 mg/kg ZrO_2 NPs for 14 days; group IV (G IV) received 400 mg/kg ZrO_2 NPs for 28 days; and group V (G V) received 500 mg/kg ZrO_2 NPs for 28 days. After the end of each duration of treatment, mice were weighted and then anesthetized. Blood samples were collected from the heart and then put into an Eppendorf tube and immediately kept in an ice box. After that, mice were sacrificed and the liver tissue were harvested and washed. The blood and liver samples were then stored until use at -20 °C for measuring the level of MDA.

Analysis of malondialdehyde (MDA): The MDA level in blood and liver samples of mice were prepared using the ELISA kit. The method involved the following steps:

1- Adding 300 microliters of homogenize solution to liver tissue and waiting for 10 minutes.

2- Took 200 microliters of whole blood and solution of liver from the bottom of the tube and add it to the plate according to the drawing map to start the experiment and wait for 30 minutes

3. Then washed the plate by a special washing buffer solution.

4- This solution is prepared by adding the entire washing can with 750% distilled water.

5-Added 100 microliters of congated (HRP) and then wait for 30 minutes.

6-Washed the plate by wash buffer

7-After that, adding 50 microliters of TMP inside a dark room, and when the reaction reaches the highest concentration, we stop the reaction by adding 35 microliters of stop solution (acid at a certain concentration)

8- Read the result by Huma reader HS in 450 nm of wavelength.

Statistical Analysis: All data were written as average means ± S.E. The SPSS program (version 20; USA) was used to analyzed the data. the One-way analysis of variance (ANOVA) was performed and followed by Duncan's post hoc tests to reconnoiter the differences among experimental groups. Statistical significance was demonstrated as $p \leq 0.05$.

Results and Discussion

Behavior changes: The current study observed several behavior symptoms such as loss of appetite, diarrhea, lethargy, lack of activity, anorexia, isolation, and frequent eating and drinking water after giving the both doses (400 and 500 mg/kg) of ZrO_2 NPs (Figure 2).

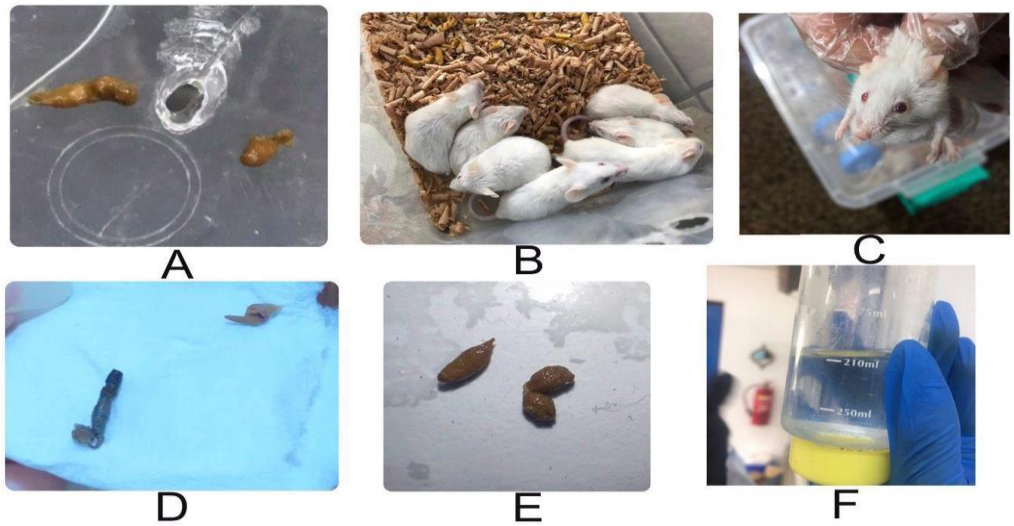

Figure (2): (A) On the fifth day of dosing, it caused diarrhea. (B) After a period of dosing, the animals became withdrawn. (C) After a period of dosing, the eyes became pale. (D) After 28 days of dosing, the color of the stool changed to greenish black. (E) After 14 days of dosing, the color of the stool changes to greenish brown. (F) The level of water consumption of animals over the course of 24 hours.

These symptoms were more evident after five days of exposure, and their severity elevated as the exposure time and concentration increased. The stool colors were altered to a greenish- brown in animals treated for 14 day and to a greenish -black color in animals treated for 28 days. These symptoms might be attributed to accumulate of this substance in the stomach and intestines, resulting dysfunction in the digestive system.

The current findings are agreed with Al-Musawi (2022) study that observed loss of appetite, lethargy, and increased water consumption of mice after 14 days of orally exposure to nanoparticles (CuO NPs), particularly following administration of the dose. Similarly, Al-Al Zerjawe, (2019) study showed that the intraperitoneal injection of zinc oxide NPs for 7 and 14 days in mice caused behavior changes such as introversion, loss of appetite for food, lethargy and hunched back

The alteration in the stool colour and watery consistence of treated animals with different doses of ZrO2 NPs that observed in this study could be attributed to the toxicity of these materials to liver or to cause disturbance the function of digestive tract. A study by Abdel Kareem (2017) found that exposure male of albino rat to nanomaterial (Copper nanoparticles) caused change of their stool color to a greenish -brown due to causes a disturbance in function of the liver as a way to get the required nutrients

Weight changes: Comparing with the control groups, the average body weight of mice showed statistically significant decrease after 28 days of exposure to 400 and 500 mg /kg of ZrO2 NPs, with time effects (Table 1).

Table (1): showed alteration in the body weight of mice treated with 400 and 500 mg/kg of ZrO_2 NPs for 14 and 28 days compared to control

Treatment	Period of exposure/day	Body weight(gm)
Control	-	29.52±2.28 a
400 mg/kg ZrO_2NPs	14	28.49±0.65 a
500 mg/kg ZrO_2NPs	14	26.11±0.87 a
400 mg/kg ZrO_2NPs	28	22.08±0.80 b*
500 mg/kg ZrO_2NPs	28	20.99±1.01 b*

Data represent average means ±S.E., N= 5. Similar letters refer to no statistically significant difference (P≥0.05). The different letters refer to statistically significant difference (P≤0.05). * Refer to significant different between time of the same concentration

The reduction in the animals body weight could be related to the accumulation of these materials in the digestive system resulting disturbance in the function of intestinal system or impairment of the intestinal physical barriers. The current result is lined with a study by Mehdikhani et al. (2020) that found decline in the mean body weight after 30 days of exposure rats to 400 ppm of zirconium oxide nanoparticles compared to the control group. Whereas, the intravenously injection of 5 and 10 mg/kg of ZrO_2 NPs in mice for 9 and 21 days caused decrease in the mean body weight of treated animals, with concentration and time dependents (Al-Bairuty et al., 2023). The intravenous injection of ZrO2 NPs at 500 mg/kg in the mice caused decrease the weight of animal body over 10% after 24 hrs injection (Yang et al. 2019).

The current finding is agreed with Quanzhong et al. (2023) study that found the oral exposure to Ag NPs caused decline in the body weight compared to control due to the diarrhea and disorder of the digestive system. Whereas, study by Radhi and Al-Bairuty (2019) recognized that exposure to ZnO NPs led to reduced animal body weight due to disruptions in the gastrointestinal tract.

MDA Changes: The level of MDA concentration in the liver tissue of treated animals with 400 and 500 mg/kg bw. of ZrO_2 NPs for 14 and 28 days showed statistically significant elevated (P≤0.05) compared to control group, with concentration and time effect (Fig. 3). Whereas in blood serum, the level of MDA concentration at day 14 revealed a significant increased with 400 mg/kg of ZrO_2 NPs and with both concentrations of ZrO2 NPs at day 28 compared to control, with concentration and time effect (Fig.3). In the liver tissue of treated animals with both concentrations of ZrO_2 NPs, the level of MDA concentration was higher than in the blood. This could indicate that the liver is the primary target organs after the entrance of ZrO2 NPs to the body through the causing of oxidative stress to this organ. Yang et al (2019) found that ZrO2 NPs can generate oxidative stress to the liver, and they suggest that

once these materials were administrated intravenously, the liver may have been one of their main targets.

Oxidative stress is produced when imbalance occur between the defense of antioxidant system and the generation of ROS. The ROS have been revealed to be harmful due to the causing damage to the cellular membranes and DNA. Worries have been elevated, particularly concerning the part that oxidative stress plays a role in the liver damage brought on by nanomaterials. In vitro studies, exposure to ZrO2 NPs occasioned in cytotoxicity in Hepg2 cells in a dependent effect of dose- and time, and were demonstrated to generate oxidative stress, accumulation of lipid, cell cycle inhibit, and cell death (apoptosis) to Hepg2 cells (Sun et al., 2020).

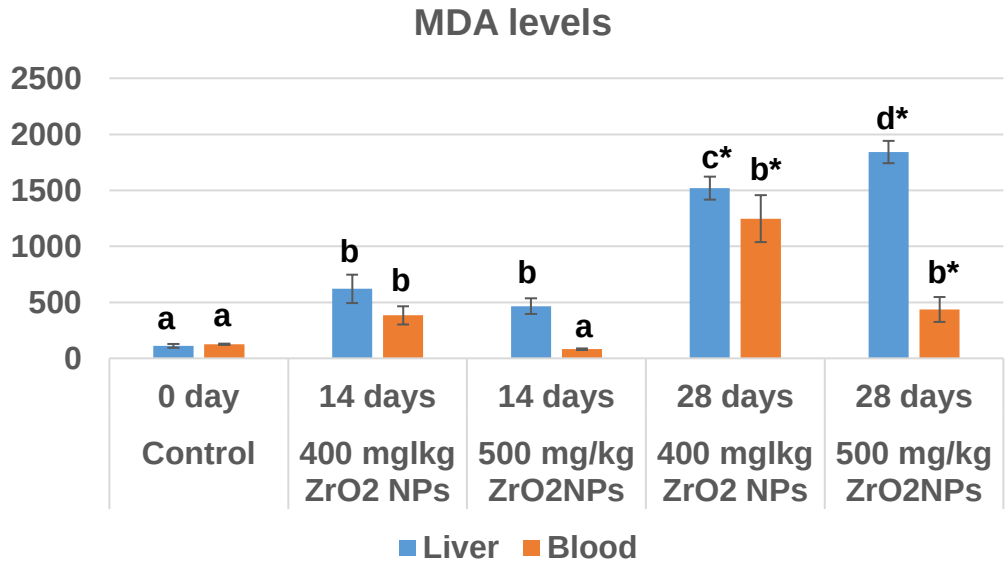

Figure (3): Showed alteration in the MDA level in the liver and blood of mice treated with 400 and 500 mg/kg of ZrO2 NPs for 14 and 28 days compared to control. Similar letters refer to no statistically significant difference (P≥0.05). different letters refer to statistically significant difference (P≤0.05). * Refer to significant different between time of the same concentration

Conclusion

The results of this work suggest that ZrO2 NPs of 40 nm diameter induce significant lipid peroxidation in the liver and blood due to the production of oxidative stress that have negative impact on DNA, cell membrane and cell life.

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