Plant Archives Volume 20 No. 2, 2020 pp. 9462-9466 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

NEPHROTOXIC EFFECTS OF ZINC NANOPARTICLES IN MALE MICE

Shatha Q. AL Tamimi[1], Azhar Abdul Jabbar Ameen[2] and Hanaa Salman Kadhum[2]

[1]College of Dentistry, Branch of Basic Sciences, Basra University, Iraq.
[2]College of Sciences, Department of Biology, Basra University, Iraq.

Abstract

The ZnO NPS uses in many various aspects of life and has the ability to penetrate physiological barriers and moving with blood circulation to different organs, which affects the health of the organism, so aimed the present studying to know the impact of ZnO-NPs of renal of male mice. Materials and Methods were used eighteen of Mus musculus male mice, the treated group injected (i.p) with ZnO NPS (300 mg/kg) at a period 30 day, while a group of control injected by 0.5 ml of 0.9 physiological solution. Urea, creatinine and uric acid were measured as a biomarker indicates to renal function, Malondialdehyde was measured in the renal tissues to evaluated oxidative stress, apoptosis was also evaluated in renal tissues by measured the gene expression of caspase 8 and caspases 9. Results : ZNO NPS resulted in significant increase in renal function (Creatinine, Urea and uric acid), a significant increase in Malondialdehyde, a significant decrease in caspase 9 in the renal while there's no significant difference in caspase 8. Conclusion : ZNO NPS was caused nephrotoxic and It has an inhibiting effect of the intrinsic pathway of apoptosis of male mice.

Key words: ZNO NPS, Malondialdehyde, caspase**,** nephrotoxic effects.

Introduction

ZnO NPS has various shapes and structures may be spherical, rod or irregular shapes and as Grouped or agglomerated forms of amorphous or crystalline or organic, inorganic matters (Dan and Wan-Xi, 2016). Since ZnO NPS possesses many unique properties, so it is used in many important applications and commercial products, it added to dyes because they possess semiconductor properties (Alferah, 2018), is also using in medical disinfection because it works to prevent the growth of microorganisms (Ali *et al.,* 2015). and is used in sunscreens because it has the ability to reflect ultraviolet radiation (Ali *et al.,* 2014), In addition to that, it is used in the production of rubber and is added to dyes and paints, it is also found in many electronic materials (Alferah, 2018). The ZnO NPs released to the environment as a result of its increased uses, the effect toxic of NPs on living organisms has become a source of anxiety for people (Ya-Nan *et al.,* 2012). However, ZnO NPS Possibly toxic and perhaps cause many other harmful effects for living organisms such as stimulating cancer and Metastatic tumor (Ana, 2010). Once the living organism is exposed to metallic nanoparticles by any known method such as intravenous or intraperitoneal injection, inhalation or by instillation, oral administration, the NPS can be transmitted to the blood and hence distribute to the secondary organs, like the liver, kidneys, lungs brain and spleen (Bin *et al.,* 2016) As NPS enters the bloodstream in the form of ions, it spreads to different organs of the body for 3 days regardless of the charge and size of the particles (Abdelmonem *et al.,* 2018) Many previous studies confirmed that ZnO NPS is a cell toxic compound, it causes oxidative stress, stimulating the release of ROS and cause dysfunction in mitochondria, then follows by DNA damage (Karlsson *et al.,* 2014) which at the end leads to apoptosis by stimulating caspase and p53 pathways (Ali *et al.*, 2014) and (Hua-Qiao *et al.,* 2016).

Materials and Methods

In the current experiment was used laboratory male mice *Mus musculus* L., were raised at 20-25^{0}C and a cycle of light/darkness for a 12-hour throughout the year. We used eighteen males, age between (10-12) weeks and their weights (25-27)g mice partitioned into two groups: (control, the treatment group the control group consists of (9) male and the treatment groups include (9) males. Control group injected with half ml of 0.9% physiological solution injected the half ml of Zinc oxide NPS (300 mg/kg) to the treated group was with for 30

successive days, on the 30th day the males of control and treatment groups were killed, the kidneys were removed and isolated for the qPCR experiment and were frozen in a deep freeze until an experiment performed to assess the level of creatinine in the blood serum, it was used kit Biolabo of the French company (Tietz, 1999). Blood urea nitrogen and uric acid were assessed through using a kit of Egyptian company Spectrum (Tietz, 1990).

Real Time PCR

Using a Promega Kit was isolated the Total cellular RNA from the tissue of treated and untreated, by a Nanodrop 2000c spectrophotometer were evaluated the quality and quantity of isolated RNA, was transcribed the reverse RNA into cDNA and consider as the template to amplification of PCR a final volume of the PCR is 25 µL in the reaction system that has 0.5 µL of every primer, SYBR green reagent 12.5 µL, the cDNA template 5 µL and nuclease-free water 6.5 µL. The first denaturation step was at $95C^0$ for 2 mins then at 45 cycles at $95C^0$ a period 30 sec, $60C^0$ for a period 30 sec. and $72C^0$ for period 30 seconds and final extension $72C^0$ for a period 10 min. The reference gene is GAPDH.

Table 1: The sequence of primers used in RT-PCR.

Name	Sequences (5➤ 3´) of Primers
Caspase 8	TGC TTG GAC TAC ATC CCA CAC GTT GCA GTC TAG GAA GTT GAC C
Caspase 9	TCC TGG TAC ATC GAC ACC TTG AAG TCC CTT TCG CAG AAA CAG
GAPDH	GAC GGC CGC ATC TTC TTG TGC TGC CAC TGCAATGG CAG CC

Statistical Analysis

To statistically analyze data, a statistical program (SPSS) was used version 22, T-test was used to analyze independent samples at $P \leq 0.05$ (Weinberg and Abramowitz, 2015).

Result

The effects of ZnO NPS and biochemical tests biomarkers

The result showed increased significantly in Urea, Creatinine and uric acid at (300 mg/kg ZnO NPS during 30 days) of treated mice at $p \leq 0.05$ Fig. 1.

The effects of ZnO NPS and MDA level

The results showed a significantly increased MDA of renal of treated mice with ZnO NPS at 30 days comparison with the control group at $p \leq 0.05$. Fig. 2.

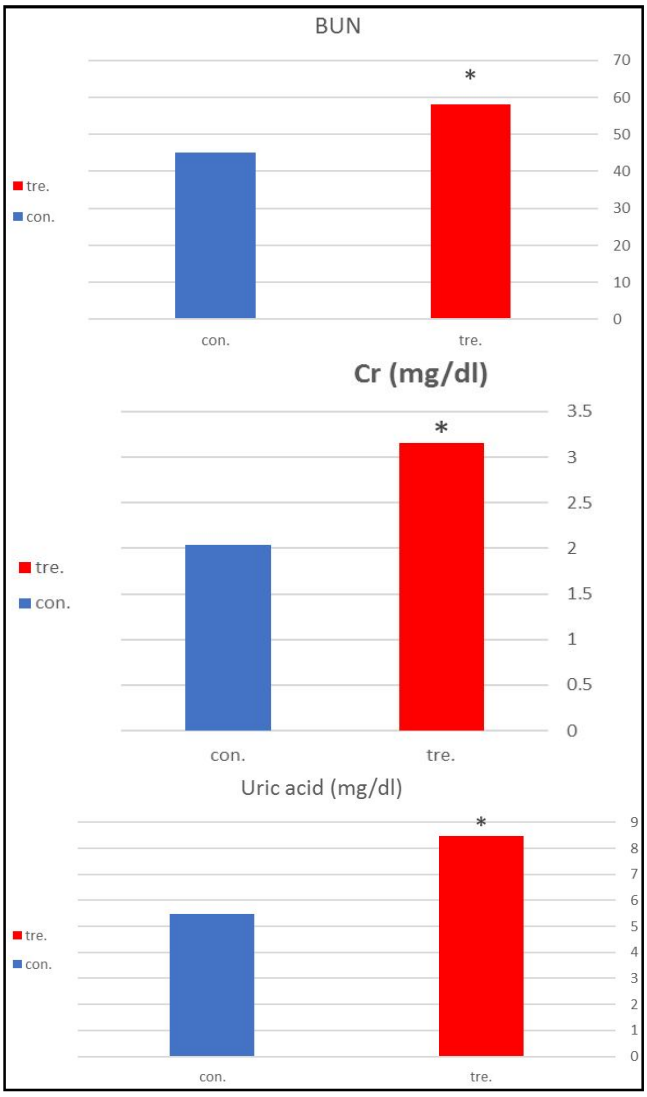

Fig. 1: Biochemical test of control and treated groups at $p \leq 0.05$.
*indicate a difference significantly between control and treatment groups.

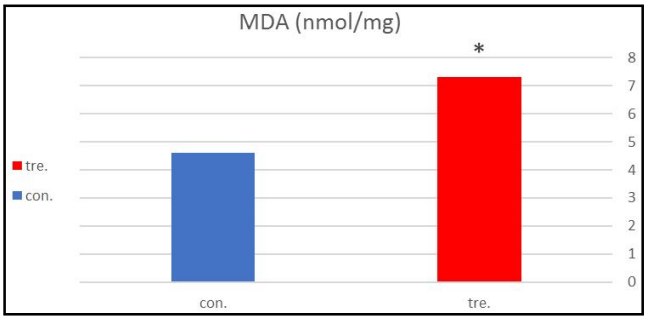

Fig. 2: MDA level of control and treated groups at $p \leq 0.05$.
*indicate a difference significantly between control and treatment groups.

The impacts of ZnO NPS and apoptosis

Gene expression results indicated there's no difference significantly in caspase 8 at renal of treated mice at 300 mg/kg of Zinc oxide NPS through 30 days

comparison with the control group Fig. 4.

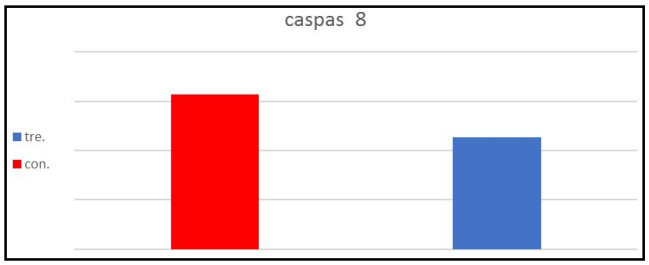

Fig. 4: Gene expression of caspase 8 of control and treated groups at $p \leq 0.05$.

Gene expression results indicated to present a significant decrease in caspase 9 in renal of treated mice with Zinc oxide NPS 300 mg/kg at a period of 30 days Fig. 5.

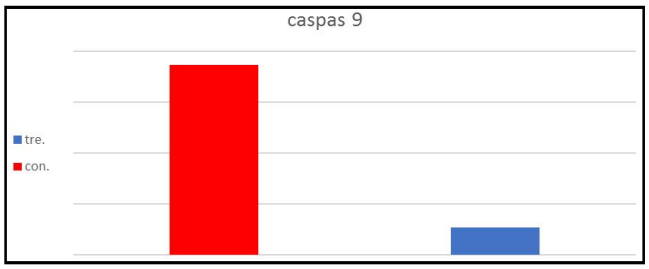

Fig. 5: Gene expression of caspase 9 of control and treated groups at $p \leq 0.05$.

*indicate a difference significantly between control and treatment group.

Discussion

Our results explained that ZnO-NPS caused a significant increase in creatinine, blood urea nitrogen and uric acid in the treated group at a concentration (300 mg/kg) for 30 consecutive days, results of our study are agreement with the results of Banafsheh *et al.,* (2017). they demonstrated an increase in the biomarkers of the kidney after injected the Waster mice with ZnO NPS (200, mg/kg) into the peritoneum for 15 days. ZnO NPS caused a significant increase of creatinine, blood urea nitrogen and uric acid, the level of the renal markers in plasma are changed under the influence of kidney disorders, the renal markers (that present inside the proximal cells of nephrons) are released in the blood when the kidney damages, therefore the increased concentration of them indicates cell damage (Layasadat *et al.,* 2018). ZnO NPS caused toxic effects of the kidney resulting to the potential of kidney damage, thus increased the biomarkers of the kidney (urea and creatinine), there are several mechanisms by which the nanoparticles can cause the cell toxicity, including the production of (ROS), O.S, genotoxicity, lipid peroxidation and stimulation the pathway of inflammatory (Mokhtar *et al.,* 2019) dependent on dose the ZnO NPS can stimulate renal toxicity (Layasadat *et al.,* 2018) generation O.S). The previous studies indicated that a low dose of ZnO NPS can stimulate more renal toxicity, but the mechanism of this result is unclear (Layasadat *et al.,* 2018). The most important markers that used to evaluate kidney function, are BUN and Cr because it's mainly released from the kidneys, however, creatinine is a more sensitive indicator of kidney function (Shivaraj *et al.,* 2010). Also, impaired kidney function leads to a high level of creatinine in the bloodstream, the creatinine level increased is the result of impaired kidney function, which is mostly produced from treatment with ZnO NPS (Najafzadeh, *et al.,* 2013). The levels increased of BUN and Cr in the blood results from the management of ZnO NPS may indicate renal insufficiency, Routes of exposure and dosage have a major role in ZnO NPS toxicity (Najafzadeh *et al.,* 2013). Recent studies indicated that uric acid may indicate to the kidney disease development, it has not been determined if the uric acid is a hazard factor independent or not, a slightly increased in the uric acid level was linked to an approximately doubled risk of kidney disease (Rudolf *et al.,* 2008). Uric acid is surlily linked with chronic renal disease development and maybe a bad predictive agent to renal failure progression (Christin *et al.,* 2015). The Zinc oxide NPS stimulates renal toxicity via the production O.S and this agrees with the study of (Layasadat *et al.,* 2018; Sharma *et al.,* 2011. and Sabah *et al.,* 2018). Malondialdehyde (MDA) is can define as a biomarker of O.S, ROS production, lipid peroxidation in the living organization, MDA is one of the end products of oxidation of unsaturated fatty acids in cells, on another hand, the free radicals increase the generation of MDA (Negre *et al.,* 2008). The increased concentration of MDA of renal tissues indicate to the oxidative stress can stimulate lipid oxidation by ZnO NPS (Layasadat *et al.,* 2018). Verena *et al.,* (2013) explained that ZnO NPs caused O.S in cells, lead to lipid oxidation, the membrane of cell damage and at the end, apoptosis occurs.Mostly the cell death results from O.S, by the apoptosis signaling or by the necrosis signaling according to their severity (Caixia *et al.,* 2015). Xiao *et al*., (2016) indicated that the treated rat's liver with 3 mg/kg ZnO NPS a period of 5 days caused an increase of MDA and reduced the SOD enzymes activity. The MDA levels elevation and reduced antioxidant enzymes activity in tissues promote lipid peroxidation formation, indicates insufficient protection of antioxidants against excessive production of free radicals (Layasadat *et al.,* 2016). Apoptotic pathways provide an important defense mechanism that reduces the cell's sensitivity to harmful events and promotes the right developmental in multicellular organisms, a key medium of apoptosis is a family of proteinase called

caspases (Tapan and Smruti, 2015). The apoptosis is too complicated and includes a series of energy-dependent events, including three pathways: 1- The pathway of extrinsic 2- The pathway of intrinsic or mitochondrial 3- The pathway of perforin (Tapan *et al.,* 2015). the initiator caspases (caspases 8 and 9) activate by the extrinsic and intrinsic pathways, thus killing the cell by damaging proteins randomly (Böhm & Schild, 2003). The pathway of apoptosis is started by the permeabilization of the mitochondrial membrane increased, this path activates by O.S and ROS (Shih *et al.,* 2020). Caspase 9 has a key role at a mitochondrial pathway or intrinsic pathway, caspase 9 inactivation resulting in disorders and disease including cancer (Ping *et al.,* 2017). The decrease of Caspase 9 gene expression may indicate that the ZnO NPS inhibits the intrinsic pathway of apoptosis so that is probably the cells enter the carcinogenic stage. On the other hand, gene expression results demonstrated there's no difference significantly in caspase 8 in the kidney tissue.

Conclusion

The ZnO NPS has a nephrotoxic effect of mice by induced oxidative stress and it has a potential carcinogenic effect through inhibiting the intrinsic pathway of apoptosis.

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