Plant Archives Vol. 20, Supplement 2, 2020 pp. 76-80 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

TOXIC IMPACTS OF ZINC OXIDE NANOPARTICALS ON LIVER ENZYMES AND RENAL FUNCTION

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Abstract

Present study was conducted to find out the impacts of Znic oxide nanoparticles on liver enzymes, renal function and the antioxidant system in mice. the treatment groups were injected with 0.5 ml of ZNO nps 100 and 300 mg/kg for 15, 20 and 25 Successive days, the results showed a significant increase in Aspartate transaminase at 100 mg/kg after 25 days compared to the control group, in period 25 compared to period 15 and in period 25 compared to period 20, and there is also a significant increase in AST at 300 mg/kg for period 15,20 and 25 compared to the control group and for the 25 period compared to the period 15, there is a significant increase in the Alanine aminotransaminase at 100mg/kg for period 25 compared to the control and for the 25 period compared to the 15 period, and there is a significant increase in the ALT at 300mg/kg in period 15, 20 and 25 compared to the control, a significant increase in Alkaline phosphatase at both 100 and 300mg/kg at period 15, 20 and 25 compared to the control. Also there is a significant increase in urea at 100 mg/kg at period 20 compared to the control and 25 days compared to the control , in period 20, 25 compared to period 15, also there is a significant increase in urea at 300 mg/kg in period 15, 20 and 25 compared to the control, the creatinine level increased significantly at 100, 300 mg/kg for 15, 20 and 25 compared to the control group, as for creatinine at 300 mg /kg , there's a significant increased at period 25 compared to the period 15.There is a significant decrease in Superoxide dismutase at 100mg/kg after 25 days compared to control group and in period 25 compared to the period 20, a significant decrease in SOD at 300 mg/kg for periods15, 20 and 25 compared to the control and a significant lower in Catalase at 300 mg/kg in the 20 and 25 period compared to the control group.

Keywords: Zinc oxide, nanoparticles, Liver enzyme, renal function

Introduction

Nanoparticles are particles between 1 and 100 in size, they have an average diameter and dimensions approximately 9-10 nanometers. Because their size is very small, they possess distinct properties such as magnetic, chemical, physical, mechanical, and electrical properties (Soheili *et al*., 2013). Znic Oxide nanoparticles have a several important applications and this due to their unique properties, among these properties: optical, catalytic, ultraviolet light absorption, semiconducting, and antimicrobial properties (Kumari, 2010). Where's the Zno nps were used in the production of rubber, it's found in many electronic components, also a major component in dyes and paints, as they added to dyes because they possess semiconductor properties (Agmal *et al*., 2017). The uses of Zno nps have increased in medical disinfection as they work to prevent growth of microorganisms (Dwivedi *et al*., 2014). The ability to reflect ultraviolet radiation is another important characteristic of Zno nps, and this is why Zno nps is mainly used in sunscreens (Osmond & Mccall, 2010). On the other hand, there is a lot of evidence indicating that Zno nps Possibly toxic to mammalian cells and may cause many other harmful effects, like the stimulating the development of cancer and Metastatic tumor (Hakima *et al.*, 2015). Also, Zno nps are a highly toxic nanoparticle and are have ability to spread in blood, liver, renal, lungs, spleen, bone and another organ (Guan *et al.*, 2012). Zinc oxide nps caused O.S in the hepatic tissues which may impact on liver function Alarifi *et al*. (2013). and Lai *et al.* (2015). Proved that the ZnO nps stimulate the O.S and they have effects on the antioxidant enzyme. Where's some researches explained that ZnO nps stimulate O.S, generation of ROS and exhaustion of the antioxidant, O.S is caused by the excessive generate of ROS or because the low of antioxidant enzymes such as GSH, CAT and SOD, the antioxidant enzymes function are to protect cells from ROS (Abd El-Aziz *et al.,* 2015). Generally, cells try to faced oxidative stress by using a first line of defense such as an antioxidant enzymes system (SOD and CAT) (Hatice *et al.*, 2015).

Material and Methods

Animals and experimental groups

Laboratory mice *Mus musculus* L were used throughout this study. These mice were reared in the animal house of the biology Department/College of Education for Pure Sciences/Basra University under controlled conditions by temperature 20-25 Co and 12-hour light/dark cycle throughout the year. The mice were placed in plastic cages measuring (30x 12 x11) cm North Kent plastic Kent U.K. The floor of the cages was sprayed with sawdust, which changed weekly and the food and water was available, male mice were used to study the effect of ZNO nps at 100 and 300 mg/kg for 15, 20 and 25 days on liver enzymes, kidney functions, and antioxidant enzymes (CAT and SOD). Eighty-one males were used, age ranges (10-12) weeks, with weight (25 - 27) g. The mice were divided into 3 groups, 27 mice for each group: (1- control group, 2- treatment group treated with concentration 100 mg/kg 3-third group treated with 300 mg/kg). Control group injected with 0.5 ml of physiological solution 0.9%. While the treated groups were i.p injected with 0.5 ml of ZNO nps (100 and 300 mg/kg) for period 15, 20 and 25 successive days.

Preparation of samples

On 15th, 20th and 25th day after the treatments, the mice of the treated group and the mice of the control group were sacrificed, and blood was collected directly from the heart after the anesthesia using chloroform by using a 1 ml syringe, blood samples were placed in gel tubes, left it for 5-10 minutes after that the centrifugation of the sample was conducted at a rate of 3500 cycles/minute (rpm) for 15 minutes to obtain the serum. Then the serum was divided and

distributed into Eppendorf to avoid repetition of melting, as the samples were saved at -20 C^o until the tests were performed.

Biochemical tests

The blood serum was used for chromatic method determination of liver enzymes (AST and ALT) by using kits from Randox the United Kingdom company (Reitman and Frankel, 1957). And ALP by using kits from Biolabo the French company (Tietz, 1999). Serum creatinine level was measured by using kit Biolabo from the French company (Tietz, 1999). While the urea was measured by use a test kit supplied by Spectrum an Egyptian company (Tietz, 1990). On the other hand, SOD was estimated by adopting the Elisa technology, type competitive by using analysis kit from Elabscience -USA company, Catalase (CAT) Activity was measured by the decreasing in the absorbance due to H_2O_2 consumption.

Statistical Analysis

The statistical program Statically Package for the Social Sciences (SPSS) version 22 was used to statistically analyze the data, as the ANOVA test was used to compare the mean of control samples and treatment samples at probability level $P \leq 0.05$.

Results

Our results of the current study confirmed the presence of a significant increase in liver enzymes (AST) in treated male mice after injection with ZnO nps (100 mg / kg) after 25 days compared to the control group at $p \leq 0.05$, for the period 25 compared to the period 15 and the period 25 compared to the period 20 and there is also a significant increase in AST enzyme at (300 mg / kg of ZnO nps) for the period 15 compared to the control group at $p \leq 0.05$, for the period 20 compared to the control group at $p \leq 0.05$ and for the period 25 compared to the control group at $p \leq 0.05$ and for period 25 compared to the period 15.

Table 1 : The impact of ZnO nps on liver enzymes (AST)

No.	AST (U/L)	5 days 1		20 days		days25	
	Conc.	mean	SD±	mean	SD±	mean	SD±
1	100 mg/kg	23.200	3.900	24.333	6.110	36.233[a,c,d]	4.801
2	300 mg/kg	27.233 [a]	3.901	34.666 [a]	7.094	40.667[a,c]	15.502
3	Control	12.966	1.761	1.233	0.907	13.566	1.457

(a) Significant different between period and control, (b) Significant different between Period 15 and 20, (c) Significant different between period 15 and 25, (d) Significant different between period 20 and 25.

There is a significant increase in the ALT enzyme with at (100 mg/kg ZnO nps) for the period 25 compared to the control group at $p \leq 0.05$ and for the period 25 as compared to the period 15, also there is a significant increase in the ALT enzyme at concentration of (300 mg/kg ZnO nps) in period 15 as compared to the control group at $p \leq 0.05$, period 20 as compared to the control group at $p \leq 0.05$ and period 25 as compared to the control group at $p \leq 0.05$.

Table 2 : The impact of ZnO nps on liver enzymes (ALT)

No	ALT (U/L)	1 5 days		20 days		25 days	
	Conc.	Mean	SD±	Mean	SD±	Mean	SD±
1	100 mg/kg	16.866	4.409	21.200	3.800	[a,c]29.600	4.357
2	mg/kg300	[a]31.100	7.308	[a]9.966 2	11.437	[a]31.600	14.304
3	Control	[a]12.223	0.550	[a]13.600	5.992	13.833	2.830

There is a significant increase in the ALP at (100 and 300 mg/kg ZnO nps) for the period 15 compared to the control group at $p \leq 0.05$, for the period 20 compared to the control group at $p \leq 0.05$ and for period 25 compared to the control group at $p \leq 0.05$.

Table 3 : The impact of ZnO nps on liver enzymes (ALP)

No	ALP (U/L)	15 days		20 days		25 days	
	Conc.	Mean	SD±	Mean	SD±	Mean	SD±
1	100 mg/kg	[a]4.939	0.986	[a]5.470	2.783	[a]6.688	1.641
2	300 mg/kg	[a]6.954	6.954	[a]7.574	0.543	[a]7.973	0.598
3	Control	2.138	1.001	2.276	1.002	2.401	0.531

The Creatinine level increased significantly at concentration (100 mg / kg of ZNO nps) for the 15 compared with control group at $p \leq 0.05$, 20 period compared to the control group at $p \leq 0.05$ and for a 25-period compared with control group at $p \leq 0.05$, and there is also a significant increase in Creatinine at the concentration (300 mg / kg of ZNO nps) for the 15 period compared to the control group at $p \leq 0.05$, 20 period compared to the control group at $p \leq 0.05$ and for the 25 period compared with control group at $p \leq 0.05$ as well as for the 25 period compared to the period 15.

Table 4 : The impact of ZnO nps on renal function (Creatinine)

No.	Creatinine (mg/dl)	15 days		20 days		25 days	
	Conc.	Mean	SD±	Mean	SD±	Mean	SD±
1	100 mg/kg	[a]1.4496	0.155	**1.636** [a]	0.267	[a]1.653	0.244
2	300 mg/kg	[a]1.701	0.192	[a]2.027	0.076	[a,c]2.484	0.438
3	Control	0.788	0.493	0.989	0.394	1.011	0.440

The results of current study showed that ZnO-NPs caused significantly increase in serum urea in treated male mice with dose (100 mg/kg of ZNO nps) after 20 days of injection as compared with control group at p≤ 0.05 and 25 days as compared with control group at p≤ 0.05, period 2o compared with period 15 and period 25 compared to the period 15, there was also a significant increase in urea at the concentration of (300 mg / kg of ZNO nps) in period 15 compared with control group at p≤ 0.05, period 20 compared with control group at p≤ 0.05 and period 25 compared with control group at p≤ 0.05.

Table 5 : The impact of ZnO nps on renal function (Urea)

No	Urea mg/dl	15 days		20 days		25 days	
	Conc.	Mean	SD±	Mean	SD±	Mean	SD±
1	100 mg/kg	32.222	5.791	[a,b]42.103	5.861	[a,c]44.285	7.835
2	300 mg/kg	[a]45.119	1.556	[a]44.722	10.776	[a]50.228	5.289
3	Control	26.702	1.362	36.529	3.482	27.023	2.738

The results of current study showed that ZnO-NPs caused significant decrease in the activities of SOD in treated male mice with dose (100 mg/ kg of Zno nps) after 25 days of injection as compared with control group at p≤ 0.05 and period 25 compared to the 20 period, as well as there is significant decrease in SOD at concentration (300 mg/ kg of Zno nps) for15 period compared with control group at p≤ 0.05, 20 period compared with control group at p≤ 0.05 and 25 compared with control group at p≤ 0.05.

Table 6 : The impact of ZnO nps on Antioxidant enzymes (SOD).

No	SOD(ng/ml)	1 5 days		20 days		25 days	
	Conc.	Mean	SD±	Mean	SD±	Mean	SD±
1	100 mg/kg	3.133	0.585	3.833	1.001	[a,d]2.233	0.351
2	300 mg/kg	[a]1.766	0.416	[a]1.700	0.100	[a]1.334	0.602
3	Control	3.933	0.230	3.766	0.737	4.667	0.577

The results of current study showed a significant decrease in Catalase activity level at concentration (300 mg / kg of ZNO nps) at period 20 as compared with control group at p≤ 0.05 and 25 period as compared with control group at p≤ 0.05.

Table 7 : The impact of ZnO nps on Antioxidant enzymes (CAT).

No	CAT (U/ml)	15 days		20 days		25 days	
	Conc.	Mean	SD±	Mean	SD±	Mean	SD±
1	100 mg/kg	2.210	0.135	1.899	0.486	1.383	0.529
2	300 mg/kg	1.031	0.023	[a]0.844	0.969	[a]0.536	0.307
3	Control	2.148	0.849	2.282	0.848	2.428	0.848

Discussion

The results of the current study confirmed a significant increase in liver enzymes (AST, ALT and ALP) at (100 and 300 mg / kg) of ZnO nps and these results are consistent with the study of Hua-Qiao *et al.* (2016). They explained the impact of ZNO nps on liver function in rats when were they feed with ZnO nanoparticles at 100, 300 and 600 mg/kg, for 1 week Where noticed the increased the liver enzymes. Imen. (2015) Noticed increased the liver enzymes (AST, ALT and ALP) after giving Wistar rats zno nps in distilled water 10 mg/ kg by oral gavage for period 5 consecutive days. Mansouri *et al.* (2015) confirmed increased serum ALT, AST and ALP through oral gavage to male Wistar rats with zno nps at dose of 5, 50 and 300 for 14 days. The liver is the organ that detoxifies the body and is therefore vulnerable to damage caused by foreign organisms, The level of AST and ALT enzymes in the blood is an indication of liver damage so a increased of AST and ALT enzymes in all mice exposed to ZnO nps are evidence that these particles caused liver damage (Babadi *et al.*, 2013). The significant increase of AST, ALP, and ALT In all treated groups perhaps a reasult of the hepatic damage that caused by ZnO nps for this reason these enzymes are released to the bloodstream. (Ali *et al.*,2015) the levels of ALT and AST enzymes are indicators of Liver cell damage, in spite of both enzymes are widely distributed in other tissues of the body, the ALT level out of the liver is low, for this reason, the ALT level is a sensitive indicator for liver cell injury (Shipra *et al.*, 2016). The increased of liver enzymes perhaps are the result of damage of the hepatocyte and cellular membrane, where increased permeability of hepatocyte membranes results in release of liver enzymes in the bloodstream. (Kubrak *et al.*, 2013 and Xue *et al.*, 2014). Where the oxidative stress and release of ROS lead to breakdown and necrosis of liver cells, which leads to leakage ALT and AST into the bloodstream (Rahbani, 1999).

Renal function effects

The results of current study Proved that ZnO-NPs caused significantly increase in serum urea and creatinine in treated male mice with dose (100 and mg / kg of ZNO nanoparticles) this result are a consistent with study of Banafsheh *et al.* (2017). Where they noted that Zno nps caused increased creatinine and urea, in Waster mice after injected into the peritoneum at a dose (200 mg/kg), after 15 days. But the results of the current study contradict with that of Iman *et al.* (2015). These researchers found that there was no effect of Zno nanoparticles on the renal function of rats that treated orally at dose of Zno nps (10 mg / kg) for 5 consecutive days. Mokhtar *et al.* (2019) nanoparticles have toxic effects such as increase of serum urea and creatinine, which means the possibility of kidney damage there are many mechanisms by which the nanoparticles make a toxic effects to cells including release of (ROS), lipid peroxidation, O.S, stimulating inflammatory pathways and genotoxicity (Mokhtar *et al.*, 2019). A simple test can be done to detect

kidney damage, in which some of the kidney enzymes are measured in the blood (Najafzadeh *et al.*, 2013). The most sensitive markers for evaluating kidney function are Urea and Creatinine because they mainly excreted by the kidneys (Asmaa *et al.*, 2018). But creatinine is a more suitable indicator for kidney function (Price & Finney, 2000). Also, decrease of the Kidney function led to an increase of Creatinine level, where the increase of Creatinine level that caused by renal dysfunction is most likely due to the management of Zno nps (Najafzadeh *et al.*, 2013).The increase of Urea and Creatinine that caused by Zno nps treatment may indicate to renal insufficiency and tubular injury Najafzadeh *et al.* (2013). The glomerular filtration rate is proportional to the Creatinine level in blood, Urea also plays a role in the metabolism of substances containing protein within the animal's body (Sarkar *et al.*, 2006). Urea and Creatinine that secreted by the kidneys are products of dietary protein metabolism, the increased of Urea and Creatinine are an indicator of kidney harmful, in any case, the exposure method and concentration have an important role in zinc toxicity (Ali *et al.*, 2014).

Antioxidant effects of ZNO nanoparticles

The results of current study explained that the ZnO-NPs caused significant decrease in SOD in treated male mice with dose (100 mg/ kg of Zno nps), the inhibition of SOD after ZnO-nps injection agree with Nel *et al.* (2009), Xia *et al.* (2008), Alarifi *et al.* (2013) and Lai *et al.* (2015). These authors reported that ZnO nps induce the oxidative stress and decreased of the antioxidant. Other research however have proved that ZnO nps caused an increase in SOD Other research however have proved that ZnO nps caused an increase in SOD as was the case in the study of Abd El-Aziz *et al.* (2015), Khurram *et al.* (2019) and Fathi *et al.* (2016). The reason of the decrease of SOD enzyme may be its consumption in the break-down of the superoxide radicals as a result increased release of ROS, thus the detoxification mechanism will be useless (Ruhollah *et al.*, 2017). Some research explained that ZnO NPs stimulate O.S, ROS generation and depletion of the antioxidant ,the cause of oxidative stress is due to the increased ROS product and or decrease of antioxidant enzymes, which include GSH, SOD and CAT, where these enzymes function is Protect the cell against the toxicity of reactive oxygen species. (Abd El-Aziz *et al.*, 2015). The cells try to faced oxidative stress through using first line defense such as enzymes of radical-scavenging (SOD and CAT) (Hatice *et al.*, 2015). The superoxide dismutase induce the segmentation of superoxide into hydrogen peroxide and oxygen least harmful to cells, it keeps the superoxide at a low concentration, thus it play a key role in facing O.S. Samir *et al.* (2016). Hydrogen peroxide that created by SOD is still as a danger for the cell, thus is substrate for another enzyme (catalase) (Ana ^ipak *et al.*, 2010,). The results of this study also explained a significant decrease in Catalase activity level at concentration (300 mg / kg of ZNO nps). Hence the results of this study fully agree with the (Hao & Chen, 2012), (Abd El-Aziz *et al.*, 2015) and (Dalal *et al.*, 2015). Catalase is contained in the peroxisomes, and it broken down the hydrogen peroxide to oxygen and water (Samir *et al.*, 2016). A lower catalase concentration may be an indication of an increase level of hydrogen peroxide (Rahbani-Nobar, 1999). Zinc oxide nanoparticles can inhibit the CAT activity, and this indicate to H_2O_2 that product by SOD cannot be disposed of directly and completely by CAT and may cause accumulation of ROS Inside the cells (Sabah *et al.*, 2018). (Sabah *et al.*, 2018). Excessive accumulation of free radicals may exceed the ability to be removed by antioxidant enzymes (Hao *et al.*, 2012). Dalal *et al.* (2015). Has shown that Large amount of ROS can be production when small amounts of ZNO nps are enter into cells, when the ZNO nps enters the cell perhaps stimulate O.S inside the cells by disturbing the balance between oxidant and anti-oxidant processes. Hence the reason for the low activity of CAT and SOD is that the antioxidant defense system cannot faced the O.S after exposure to ZNO nps (Aysun *et al.*, 2007).

Conclusion

The results showed that the higher the ZNO nps concentration and the greater the period, the effect of ZNO nps on the liver and kidney will increase, which caused an increase in liver enzyme and renal function, and also caused a reduction in both SOD and CAT through the oxidative stress.

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