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Pathomorphological and Immunological Assessment of ZnO Nanoparticles Induced Hepatic Toxicity in Male Wistar Rats

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ABSTRACT

The present study entitled "Pathomorphological and Immunological Assessment of ZnO nanoparticles induced hepatic toxicity in male wistar rats" was undertaken. For this purpose, a total of 72 rats were randomly divided into four equal groups. The rats of group I were given normal basal feed and water as they served as control rats. The rats of Group II, III & IV were given aqueous solution of ZnO nanoparticles orally at the dose rate of 800 mg/kg BW, 400 mg/kg BW & 200 mg/kg BW, respectively. The histopathological alterations and immunological alterations were recorded at the intervals 15-, 30- and 45-days exposure of experimentation. Grossly, no marked changes were evident in liver of any treatment group upto 30 days of exposure period. Slight paleness was evident in liver at 45 days of exposure period. Histopathologically, normal histoarchitecture of hepatocytes was evident in all groups upto 15 days of exposure. Thereafter, cellular swelling, hydropic degeneration, focal necrosis and increased sinusoidal spaced were evident in liver of rats of groups II & III after 30 days. Hepatic IL-6 revealed a significant increase in group II after 30 days, while significant increase was evident in both groups II & III after 45 days of exposure. TNF-α increased significantly in group II in hepatic tissue after 30 days, while significant increase was evident in both groups II & III post 45 days of exposure. Based on the results of this study, it was concluded that ZnO nanoparticles caused sub-acute toxicity at doses above 200mg/kg.

HIGHLIGHTS

- The rising use of ZnO nanoparticles has raised concerns over its environmental accumulation.
- ZnO nanoparticles can cause sub-acute hepatic toxicity.
- Its toxic effects have been observed above the dose of 200 mg/kg orally.

Keywords: ZnO Nanoparticles, Hepatic toxicity, sub-acute exposure, Wistar rats

Nanotechnology is a branch of science that deals with the development, use and manipulation of materials of size ranging less than 100 nm. Applications of nanotechnology are wide ranging and include processes such as material science, agriculture, food industry, cosmetics, medical and diagnostic applications (Siddiqui *et al*., 2018). However, such exuberant use of nanoparticles has raised global concern with regard to their adverse impacts on human health, biodiversity and environment, largely

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due to their small dimension and unique physical and chemical properties. Since precise cellular mechanisms of interaction of nanoparticles with biological systems remain mostly unknown, a comprehensive nanotoxicology study demands assessments of interaction of nanoparticles with various biological systems based upon their routes of exposure. The widespread use and disposal of nanoparticles has become a profound environmental concern and has thus attracted a plethora of scientific studies to assess the toxicity potential of nanoparticles both in vitro and in vivo models (Ostrowski *et al.,* 2009).

One of the most widely synthesized engineered nanoparticles, Zinc oxide, has found tremendous application in various industries like rubber manufacturing, pigments, sunscreens, cosmetics, medicines, medical imaging, anti-cancer therapy, electronics owing to their antimicrobial potential, semiconducting and magnetic properties, UV light absorption and catalytic properties (Attia *et al.,* 2018). However, the risks associated with their widespread use cannot be overlooked as various studies with diverse animal models have suggested the potential of ZnO NP exposure to generate various toxic effects. Various studies have demonstrated that ZnO NPs, after systemic distribution, could reach various organs systems and exhibit toxic effects on lungs, liver, kidney, pancreas, spleen, stomach, testis, thymus, brain, heart, blood, etc. In addition to these, *in vitro* cytotoxicities have also been reported in many cells like epidermal cells, macrophages, etc (Wang *et al.,* 2017).

Early investigations into the health hazards caused by ZnO NPs revealed liver as one of the main target organs of ZnO accumulation and consecutive damage. In another study in 2015, the distribution of ZnO NPs in the tissues of rats and mice administered through different routes were measured and it was found that ZnO NP mainly accumulated in liver, kidney and spleen (Chen *et al.,* 2016). Another study revealed that ZnO concentration in liver of 5-week-old rats increased in a dose dependant manner following 90 days of intragastric treatment with 500 mg/kg of ZnO NPs (Park *et al*., 2014). There is ample pathological evidence indicating that long term exposure to ZnO NPs could aggravate the signs of liver damage manifested as significant upregulation of pro-inflammatory cytokines such as IL-8 (Germain and Margulies, 1993) and hepatic injury markers such as Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) along with downregulation of drug metabolism enzyme genes such as cyp3a11, cyp2c29, ugt2b (Srivastav *et al*., 2019). It has also been documented that exposure to ZnO NPs has led to an increased accumulation of MDA with a consequent decrease in anti-oxidant enzymes such as glutathione, catalase, and significantly enhanced ROS production (Yousef *et al.,* 2019).

The objective of the current study was to evaluate the hepatotoxic effects of oral dosing of nano ZnO on male wistar rats by measuring the hepatic IL-6 & TNF-α as markers of immunological status, molecular assessment of HSP-70 gene as biomarker of oxidative stress and analysis of histopathological alterations in liver to develop a comprehensive understanding of the toxic effects induced by ZnO NPs.

MATERIALS AND METHODS

Place of investigation

Present study was carried out in the laboratories of the Department of Veterinary Pathology, Veterinary Pharmacology and Toxicology and Veterinary Parasitology of College of Veterinary Science and Animal Husbandry, DUVASU, Mathura.

Experimental animals

Seventy-two healthy male rats of Wistar strain, weighing 120-150 gram were procured from Small Animal House, Lala Lajpat Rai University of Veterinary and Animal sciences, LUVAS, Hissar. The rats were housed in smooth, impervious, poly-propylene cages thoroughly cleaned with disinfecting solution. Experimental protocol involving use of adult male rats was approved by IAEC approval no. (IAEC/22/2/10) vide letter no 138/IAEC/22 dated 28-12-2022 as per guidelines of CPSEA, Govt. of India.

Management and feeding

The rats were weighed and randomly grouped into four groups after an acclimatisation period of 15 days, as per the experimental protocol. Adequate lighting (12 hours light and 12 hour darkness), ventilation, temperature (21±2^{o}C), relative humidity (50±10 %) and hygienic conditions were maintained throughout the experiment. The rats

were kept in polypropylene cages lined with paddy husk as bedding material which was changed every alternate day to keep the moisture in check. Regular inspection was undertaken to keep a check on the health and management of rats. Recommendations of the IAEC (Institutional Animal Ethics Committee) as per the guidelines set forth by CPCSEA (Committee for the purpose of control and supervision of experiments on animals) were strictly followed throughout the period of study. Rats were fed with nutritionally adequate standard balanced pelleted feed (Ashirwad Feed Industry Chandigarh) & ad-libitum clean drinking water throughout the experimental period.

Zinc oxide nanoparticle

Zinc oxide nanoparticle (Sigma Aldrich) having particle size <100nm was used in present study. Zinc oxide nanoparticle was dissolved in distilled water to make the desired concentration as per requirement for the determination of LD-50 as well as for toxicological study. In the present study, $1/10^{th}$, $1/20^{th}$ & $1/40^{th}$ of LD-50 of Zinc oxide nanoparticle was given in the rats of different experimental group by oral gavaging.

Estimation of LD50 of Zinc oxide nanoparticle

For estimation of oral LD50 of ZnO nanoparticles, OECD guidelines, 2016 were followed. Female animals were selected for dosing. Rats were fasted overnight before dosing. As per the protocol of Limit test (guideline no 423, OECD, 2016), firstly one rat was dosed @ 2000 mg/kg ZnO nanoparticle orally and observed for a period of 72 hours for signs of toxicity and death. No signs of toxicity were noticed up to 72 hours. Three rats were again dosed @ 2000 mg/kg orally and observed for signs of toxicity and death upto 72 hours. No mortality occurred during the observation period. Further, three rats were dosed @5000 mg/kg (as per annexure 3 of the OECD guidelines) and observed for a period of 72 hours. The rats became dull however there was no mortality observed. Again, three rats were dosed @5000 mg/kg orally and observed upto 72 hours. Again, the rats became dull and only one mortality occurred during the observation period.

Above experiment led to the conclusion that oral LD50 of ZnO in rats was above 5000 mg/kg. However, for the purpose of dosing and research, a LD50 dose of 8000 mg/kg was selected as per the previous research (Ahmed *et al.,* 2019).

Experimental design

Seventy-two male wistar rats were divided into four groups (18 rats each). The rats of group I was kept as control group and were fed with basal diet only. Group II, III and IV were treatment groups where the rats were administered an aqueous solution of ZnO nanoparticles through oral gavaging everyday upto the completion of the experimental period. Group II received ZnO NP @ 800 mg/kg BW (1/10 of LD50), Group III received ZnO NP @ 400 mg/kg BW (1/20 of LD50) and Group IV received ZnO NP @ 200 mg/kg BW (1/40 of LD50). Six rats from each group were humanely sacrificed by cervical dislocation at the intervals of 15^{th}, 30^{th} and 45^{th} day of experimentation.

The treatment given in different groups of the experiment is summarized as under:

Groups	Treatment	Distribution of Animals as per the Days of Collection of samples		
		15^{th} Day	30^{th} Day	45^{th} Day
I.	Control	6	6	6
II.	$1/10^{th}$ of LD50 ZnO NPs @ 800mg/kg	6	6	6
III.	$1/20^{th}$ of LD50 ZnO NPs @ 400mg/kg	6	6	6
IV.	$1/40^{th}$ of LD50 ZnO NPs @ 200mg/kg	6	6	6

Histopathological examinations

Six rats of each group were humanely sacrificed by cervical dislocation after 15^{th}, 30^{th} and 45^{th} day of oral gavaging with ZnO nanoparticles and were subjected to necropsy. Gross pathological examination of liver was carried out followed by its further processing for histopathological alterations. Gross pathological lesions were studied in liver tissues. After gross pathological examination, the tissues were fixed in 10 % neutral buffered formalin and were processed for paraffin embedding technique.

Immunological studies

Sample Preparation

Tissues samples were collected after washing in ice-cold PBS (pH = 7.4) to remove excess blood thoroughly. For the assay, tissues were minced and homogenised in chilled PBS (tissue wt. (g): PBS (ml) = 1:9) with a glass homogeniser on ice. To further break down the cells the samples were subjected to a sonicator. The homogenates were then centrifuged at 5000 rpm for 5 mins to get the supernatant.

Estimation of rat tumour necrosis factor α (TNF α)

Enzyme Linked Immuno Sorbent Assay (ELISA) kit was used for accurate quantitative estimation of Rat Tumor Necrosis Factor α in hepatic tissue samples. The plate has been pre-coated with Rat TNF-α antibody. Liver homogenate sample was added and TNF-α present in the sample binds to antibodies coated on the wells. And then biotinylated rat TNF-α Antibody was added and which binds to the TNF-α in the sample. Then streptavidin-HRP was added which binds to the biotinylated TNF-α antibody. After incubation, unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added and colour developed in proportion to the amount of rat TNF-α. The reaction was terminated by addition of acidic stop solution and absorbance was read at 450 nm.

Estimation of Rat Interleukin- 6 (IL-6)

Enzyme Linked Immuno Sorbent Assay (ELISA) kit was used for accurate quantitative estimation of Rat Interleukin-6 in hepatic tissue samples. The plate has been pre-coated with Rat IL-6 antibody. IL-6 present in the sample was added which binds to antibodies coated on the wells. And then biotinylated rat IL-6 Antibody was added that will bind to the IL-6 in the sample. Then streptavidin-HRP was added which binds to the biotinylated IL-6 antibody. After incubation, unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added and colour developed in proportion to the amount of rat IL-6. The reaction was terminated by addition of acidic stop solution and absorbance was measured at 450 nm.

Statistical analysis

Results are expressed as mean ± SEM. Statistical analysis of data was performed using GraphPad Prism 8.0.2 (263) by two-way ANOVA followed by tukey's-b multiple comparison tests. A value of $p<0.05$ was considered as statistically significant.

RESULTS

Gross and histopathological alterations

Six rats of each group (I, II, III and IV) were humanely sacrificed by cervical dislocation after 15th, 30th and 45th day of oral gavaging with ZnO nanoparticles and were subjected to necropsy. The morbid lesions in toxicity groups (II and III) were almost alike with little differences in the magnitude of lesions. Grossly, no marked changes were evident in liver of rats of any treatment groups (II, III and IV) after exposure to ZnO NP upto the 30 days of experimentation. After 45 days of experimental period, slight paleness was observed in liver of the rats of group II that were exposed to highest dose of ZnO NPs throughout the experimentation. Microscopically, no noticeable changes were observed in histoarchitecture of hepatocytes in the all the treatment groups (II, III and IV) after first 15 days of dosage (Fig. 1B,1C,1D) as compared to control group (Fig. 1A). Mild congestion in central vein and increase in sinusoidal space was observed after 30 days of exposure. Mild hydropic degeneration was evident in hepatocytes in rats of group II (Fig. 2B) that were exposed to ZnO NP orally at the dose rate of 800 mg/kg. The hepatocytes showed degenerative changes ranging from cellular swelling to mild to moderate vacuolization with infiltration of few mononuclear cells in the portal area of liver in the rats of group II (Fig. 3B) after 45 days of exposure to oral ZnO nanoparticles.

Liver plays a central role in detoxification, metabolism and excretion of various drugs in the body which makes it an important target organ of ZnO NPs. Several degenerative changes affecting both the cytoplasm and nucleus of hepatocytes were evident in this study. These changes typically represented vacuolated cytoplasm and ballooning degeneration of hepatocytes. One possible reason of such degenerative changes could be disturbance in hepatocyte membrane functions which could possibly result in massive influx of water and Nations.

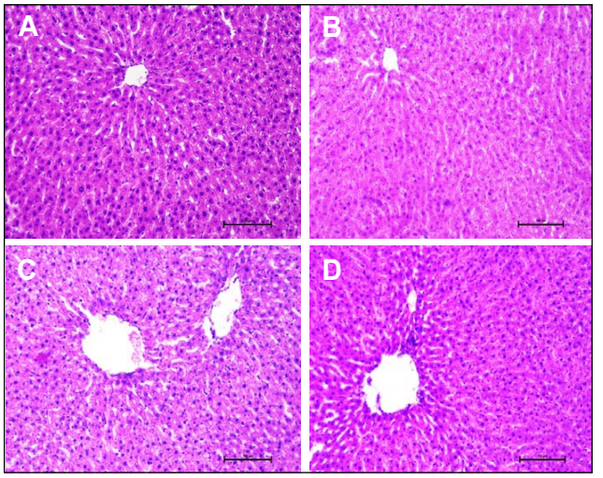

Fig. 1: Images showing normal histoarchitecture of hepatic tissues in rats of different treatment groups after 15 days of oral dosing. **(A)** Group- I (Control) Rat liver tissue section showing normal histoarchitecture of hepatocytes. (H&Ex200x); **(B)** Group II- Rat liver tissue section showing normal arrangement of hepatocytes. (H&Ex200x); **(C)** Group III- Rat liver tissue section showing normal arrangement of hepatocytes. (H&Ex200x); **(D)** Group IV- Rat liver tissue section showing normal arrangement of hepatocytes (H&Ex200x)

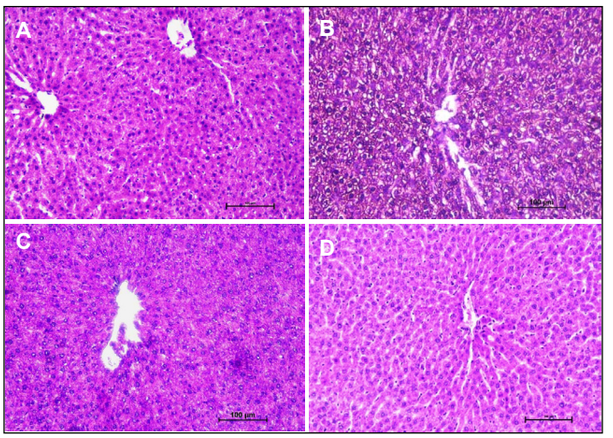

Fig. 2: Images showing histoarchitecture of hepatic tissues in rats after 30 days of oral exposure to ZnO NPs. Note the changes in hepatocytes of group II characterised by cellular swelling and hydropic degeneration. **(A)** Group- I (Control) Rat liver tissue section showing normal histoarchitecture of hepatocytes (H&Ex200x); **(B)** Group II- Rat liver tissue section showing prominent cellular swelling in hepatocytes (H&Ex200x); **(C)** Group III- Rat liver tissue section showing normal arrangement of hepatocytes. (H&Ex200x); **(D)** Group IV- Rat liver tissue section showing clear normal cord like arrangement of hepatocytes. (H&Ex200x)

Ballooning degeneration of hepatocytes may also result from leakage of lysosomal hydrolytic enzymes leading to cytoplasmic degenerations (Tang *et al.,* 2016). Other than this, congestion in central vein was a noteworthy feature of treated groups.

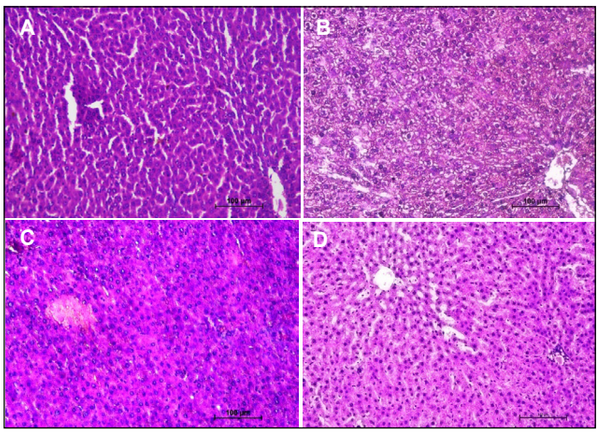

Fig. 3: Images showing histoarchitecture of hepatic tissues of after 45 days of oral exposure to ZnO NPs. **(A)** Group I- (Control) Liver section showing normal histoarchitecture of hepatocytes (H&Ex200x); **(B)** Group II- liver tissue section showing cellular swelling to hydropic degeneration in hepatocytes (H&Ex200x); **(C)** Group III- Liver tissue section showing areas of focal necrosis (H&Ex200x); **(D)** Group IV- Liver tissue section showing normal histoarchitecture of hepatocytes with mild increase in sinusoidal spaces (H&Ex200x)

Immunological parameters

The statistical data depicted a non-significant increase in the mean value of TNF-α in the rats of all treatment groups (II, III and IV) as compared to rats of control group (I) at the intervals of 15 days of experimentation. A significant rise in the level of TNF-α was observed in the rats of group II as compared to the rats of other groups (I, III and IV) after 30 days of oral exposure of ZnO nanoparticles.

Thereafter, a significant rise in the levels of TNF-α was evident in the rats of groups (II and III) post 45 days of oral exposure as compared to the rats of groups (I and IV). There was also a significant variation in the mean value of TNF-α in the rats of group II as compared to the rats of group III at 45 days of interval of exposure period.

The statistical analysis of the mean values of TNF-α within the group indicated a non-significant variation in the rats of group (I, III and IV) at all the intervals of exposure

Table 1: Effect of oral administration of ZnO NP (800 mg/kg BW, 400 mg/kg BW and 200 mg/kg BW) on hepatic TNF-α levels (ng/L) in rats of different treatment groups

INTERVAL	GROUP I (Control)	GROUP II (800 mg/kg)	GROUP III (400 mg/kg)	GROUP IV (200 mg/kg)
15 DAYS	230.38±1.77^{Aa}	238.78±2.01^{Aa}	235.02±2.54^{Aa}	233.14±2.16^{Aa}
30 DAYS	235.89±3.76^{Aa}	254.52±2.24^{Bb}	236.60±2.07^{Aa}	236.60± 1.83^{Aa}
45 DAYS	238.48±2.73^{Aa}	264.85±1.60^{Bb}	254.71±2.39^{Cb}	240.79±2.69^{Aa}

(Values (mean ± SEM: n=6) bearing different superscripts in the same column and same row differed significantly ($P < 0.05$) using two-way ANOVA).

Table 2: Effect of oral administration of ZnO NP (800 mg/kg BW, 400 mg/kg BW and 200 mg/kg BW) on hepatic IL-6 levels (ng/L) in rats of different treatment groups

INTERVAL	GROUP I (Control)	GROUP II (800 mg/kg)	GROUP III (400 mg/kg)	GROUP IV (200 mg/kg)
15 DAYS	152.26±3.44^{Aa}	171.04±4.80^{Aa}	170.61±3.55^{Aa}	154.97±3.69^{Aa}
30 DAYS	157.59±3.67^{Aa}	192.01±4.09^{Ba}	178.45±1.76^{Aa}	160.55±5.59^{Aa}
45 DAYS	163.51±4.75^{Aa}	200.00±3.61^{Bb}	181.84±2.39^{Ca}	164.58±1.07^{Aa}

(Values (mean ± SEM: n=6) bearing different superscripts in the same column and same row differed significantly ($P < 0.05$) using two-way ANOVA).

period. However, a significant variation in the mean value TNF-α was seen in the rats of group II at 45 days interval of experimentation.

DISCUSSION

Nanotechnology has become a research hotspot in modern material science**.** Nanoparticles may be defined as controlled or manipulated particles at the atomic level (1–100 nm) and they show size-related properties which are significantly different from bulk materials. Due to their small size, NPs have large surface area in comparison with their micro sized counterparts. This distinctive property of NPs allows their possible applications in many fields such as biosensors, nanomedicine, and bio nanotechnology (Fan *et al*., 2013; Keller *et al*., 2014).

The global consumption of ZnO NPs has been increasing recently due to its application in diverse areas such as cosmetics, paints and coatings, rubber, agriculture, textiles, electronics, and food industries. In recent years, the applications of ZnO NPs have been extended to the field of biomedical imaging and diagnostics. In accordance with a recent report published by Allied Market research, the global nano zinc oxide market is anticipated to reach $7677 million by 2022 (Lynn *et al.,* 2021).

With the increasing use of ZnO NPs, its consequent release into freshwater, marine ecosystems, and even surface water bodies are inevitable, in addition to the direct exposure to human bodies. Incidentally, only in the US, the release of nano-ZnO is estimated to be 1,800–2,100 metric tons/year into the environment or landfills from the use of personal care products (Sirelkhatim *et al.*, 2015). Their continuous release undoubtedly leads to accumulation in the environment and unpredictable risk to living organisms, which has been confirmed by numerous studies concerning the toxicity of nano-ZnO (Hou *et al*., 2018).

The present work was undertaken to elucidate the hepatic alterations in male wistar rats, following oral administration of ZnO NP @ 800 mg/kg BW (group II), 400 mg/kg BW (group III) and 200 mg/kg BW (group IV) at the intervals of 15th, 30th and 45th days of experimental period.

In this study, we noted marked cellular swelling and hydropic degeneration in hepatocytes of rats of group II after 30 and 45 days of oral dosing. These degenerative changes in hepatocytes could be a result of membrane damage due to ZnO nanoparticles. No significant gross changes were observed in liver in any of the treatment groups as compared to control group. Only slight paleness was observed in liver in rats of group II after 30 days of oral exposure. Our findings are in accordance with Srivastava *et al.,* (2016), who also did not observe any significant change in gross morphology of liver in rats treated with ZnO NP.

No marked histopathological alteration was noticed in liver in rats of any experimental group after first 15 days of exposure. In later period of study, slight cellular swelling was evident in hepatocytes in liver of rats of group II. Areas of focal necrosis, increased sinusoidal spaces and cellular swelling progressing towards hydropic degeneration was evident in hepatocytes in liver of rats in group II & III. Our findings are in accordance with those of Tang *et al.* (2016), who reported marked cellular swelling and vacuolisation in hepatocytes of rats exposed to ZnO NP.

Kavaz *et al.* (2021), also reported significant cellular swelling and cytoplasmic vacuolisation in hepatocytes of rats exposed to biosynthesised ZnO NP.

Ramadan *et al*. (2022), in their study on effect of sub-chronic exposure of ZnO NP in wistar rats, also reported increased sinusoidal spaces and hydropic degeneration of hepatocytes.

In this study, it was observed that there was an increment in the values of hepatic IL-6 and TNF-α in rats of treatment groups (II, III, IV). A significant rise in hepatic IL-6 and TNF-α levels was evident in group II after 30 days of oral exposure to ZnO NP. Further, a rise in values of hepatic IL-6 and TNF-α was observed in rats of both groups II & III after 45 days of oral exposure to ZnO NP.

These findings are in accordance with those of Tang *et al*. (2016), who reported significant increase in cytokine levels (IL-6, TNF-α and IFN-γ) in hepatic tissues of rats exposed to ZnO nanoparticles orally at the doses of 300 mg/kg and 600 mg/kg BW.

The higher circulatory levels of cytokine as well as enhanced cytokine levels in tissues gave evidence about the inflammatory status of rats exposed to ZnO NP. Also, these nanoparticles are encountered by immune cells once they enter the circulation and this could be possibly responsible for the altered cytokine profile of the rats exposed to ZnO NP (Yousef *et al*., 2019)

CONCLUSION

The present study was undertaken to study the deleterious effects of nano ZnO when given through oral route. This study found that ZnO nanoparticles can induce pathological changes in the body when given at doses higher than 200mg/kg BW. Also, ZnO nanoparticles did not produce acute toxicity in the body but produced pathological lesions only after sub-acute exposure. ZnO nanoparticles also incited inflammation at higher doses leading to increased expression of inflammatory cytokines.

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REFERENCES

Ahmed, S.M., Mesallam, D.A., Deraz, R.H. and Abdel, S.M. 2019. Toxicity of subacute oral zinc oxide nanoparticles on testes and prostate of adult albino rats and role of recovery. *J. Histol Histopathol.*, **6**: 255-291.

Attia, H., Nounou, H. and Shalaby, M. 2018. Zinc oxide nanoparticles induced oxidative DNA damage, inflammation and apoptosis in rat's brain after oral exposure. *Toxics.*, **6**: 11-29.

Chen, A., Feng, X., Sun, T., Zhang, Y., An, S. and Shao, L. 2016. Evaluation of the effect of time on the distribution of zinc oxide nanoparticles in tissues of rats and mice: A systematic review. *IET Nanobiotechnol.*, **10**: 97–106.

Fan, W., Li, Q., Yang, X. and Zhang, L. 2013. Zn subcellular distribution in liver of goldfish (*Carassius auratus*) with exposure to zinc oxide nanoparticles and mechanism of hepatic detoxification. *PLoS One*, **8**: 78-123.

Germain, R.N. and Margulies, D.H. 1993. The biochemistry and cell biology of antigen processing and presentation. *Annu. Rev. Immunol.,* **11**: 403–450.

Hou, J., Wu, Y., Li, X., Wei, B., Li, S. And Wang, X. 2018. Toxic effects of different types of zinc oxide nanoparticles on algae, plants, invertebrates, vertebrates and microorganisms. *Chemosphere.,* **19**: 852–860.

Kavaz, D., Abubakar, A.L., Rizaner, N. and Umar, H. 2021. Biosynthesized ZnO nanoparticles using albizia lebbeck extract induced biochemical and morphological alterations in wistar rats. *Molecules,* **26**: 38-64.

Keller, A.A., Vosti, W., Wang, H. and Lazareva, A. 2014. Release of engineered nanomaterials from personal care products throughout their life cycle. *J. Nanopart. Res.,* **16**: 24-89.

Lynn, C., Chee, M.F., Pung, S.Y., Chin, E.O., Pung, Y.F., Kong, C. and Pan, Y. 2021. Current Updates on the In vivo assessment of zinc oxide nanoparticles toxicity using animal models. *Bio. Nanosci.*, **10**: 1882-1892.

Ostrowski, A.D., Martin, T., Conti, J., Hurt, I. and Hartthron, B.H. 2009. Nanotoxicology: characterizing the scientific literature, 2000–2007. *J. Nanopart. Res.,* **11**: 251–257.

Park, H.S., Shin, S., Meang, E.H., Hong, J.S., Park, J.I., Kim, S.H., Koh, S.B., Lee, S.Y., Jang, D.H. and Lee, J.Y. 2014. A 90-day study of subchronic oral toxicity of 20 nm, negatively charged zinc oxide nanoparticles in Sprague Dawley rats. *Int. J. Nanomed*., **9**: 79–92.

Ramadan, A.G., Yassein, A.M., Eissa, A.E. and Mohammad, M.S. 2022. Biochemical and histopathological alterations induced by subchronic exposure to zinc oxide nanoparticle in male rats and assessment of its genotoxicicty. *J. Umm Al Qura Univ. Appl. Sci*., **8**: 41–49.

Siddiqui, K.S., Rahman, A. and Tajuddin, H.A. 2018. Acute toxicological effects of zinc oxide nanoparticles in mice after intratracheal instillation. *Nanoscale research letters.,* **13**: 40-148.

Sirelkhatim, A., Mahmud, S., Seeni, A., Kaus, N.H.M., Ann, L.C., Bakhori, S.K.M., Hasan, H., and Mohamad D. 2015. Review on Zinc Oxide Nanoparticles: Antibacterial Activity and Toxicity Mechanism. *Nano-micro Lett.*, **3**: 219–242.

Srivastav, A.K., Dhiman, N., Tiwari, R., Arjaria, N., Prakash, J., Jagdale, P., Ayanur, A., Singh, D., Patnaik, S., and Kumar, M. 2019. Sub-acute oral exposure of zinc oxide nanoparticles causes alteration in iron homeostasis through acute phase response: A protective effect by surface modification. *J. Trace Elem. Med. Biol.,* **52**: 270–287.

Srivastav, A.K., Kumar, M. and Ansari, N.G. 2016. A comprehensive toxicity study of zinc oxide nanoparticles versus their bulk in Wistar rats: Toxicity study of zinc oxide nanoparticles. *Human Exp. Toxicol.*, **12**: 1286-1304.

Tang, H.Q., Xu, M., Rong, Q., Jin, R.W., Liu, Q.J. and Li, Y.L. 2016. The effect of ZnO nanoparticles on liver function in rats. *Int. J. Nanomed.*, **11**: 4275–4285.

Wang, D., Li, H., Liu, Z., Zhou, J. and Zhang T. 2017. Acute toxicological effects of zinc oxide nanoparticles in mice after intratracheal instillation. *International J. Occupation. Environ. Health,* **23**(1): 11–19.

Yousef, M.I., Mutar, T.F. and Kamel, M.A.E. 2019. Hepato-renal toxicity of oral sub-chronic exposure to aluminum oxide and/or zinc oxide nanoparticles in rats. *Toxicol. Rep.,* **6**: 336–346.