Sohag University Sohag Medical Journal Faculty of Medicine

Biomedical Applications and Toxicological Aspects of Zinc Oxide Nanoparticles: A Review Article

Hend G. Aref[1], Ahmed M. Said[1], Eman K. Ahmed[2], Mai M. Abdelkader[1], Aya M. Elkady[1], Soheir A. Mohamed[1]

1- Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Sohag University, Egypt.
2- Department of Histology, Faculty of Medicine, Sohag University, Egypt.

Abstract:

Nanotechnology is one of the fastest-growing and most promising modern technologies. It deals with the manufacturing and use of nanoparticles. Nanoparticles are generally known as small-sized particles possessing a size range of 1 to 100 nm. Due to their distinctive physicochemical and mechanical characteristics, they are utilized in various fields like agriculture, material science, food industry, medical, diagnostic, and cosmetic applications. One of the nanoparticles that is most frequently used nowadays is Zinc oxide nanoparticles. They have been explored for numerous biomedical applications, such as drug delivery, anticancer, antibacterial, antidiabetic, and bioimaging. Researchers expect that usage of Zinc oxide nanoparticles will expand so; the human body's exposure to them will increase. Zinc oxide .nanoparticles released into the environment could lead to adverse human and animal health effects. Recent studies concluded that Zinc oxide nanoparticles could cross some blood vital organs barriers and cell membranes, generate free radicals, and exhibit oxidative stress, cytotoxicity, and genotoxicity. Therefore, great attention must be taken to assess the toxicological aspects of nanoparticles.

Keywords: Nanotechnology, Zinc oxide nanoparticles, Biomedical applications, Toxicity.

Introduction:

Nanotechnology is one of the fastest-growing and most promising modern technologies. It deals with the manufacturing and use of nanoparticles (NPs) that are generally known as small-sized particles possessing a size range of 1 to 100 nm.[1,2] Nanoparticles have distinctive physicochemical and mechanical properties as they have many reactive sites on the surface and a high surface area to volume ratio. These properties make them highly important to be applied in various fields like agriculture, material science, the food industry, and medical, diagnostic, and cosmetic applications.[3,4] Nanomedicine is the use of nanotechnology for biomedical purposes such as the control, prevention, monitoring, diagnosis, and treatment of diseases.[5]

One of the frequently utilized NPs today in consumer items is Zinc oxide nanoparticles (ZnO-NPs). They are found in cosmetics and sunscreens as they possess efficient ultraviolet absorption properties. Because of the antibacterial properties of ZnO-NPs, they are used in the food industry as

packaging and additives.[6,7] ZnO-NPs have been extensively reconnoitered for a variety of biomedical applications, such as antibacterial, anticancer, drug delivery, antidiabetic, and bioimaging[8].

Researchers expect that ZnO-NPs' usage will expand so, the human body's exposure to them will increase. Therefore, ZnO-NPs release into the environment could lead to adverse human and animal health effects that may be due to their cumulative effect [9,10].

1. Biomedical applications:

Antibacterial agent:

Because of their photocatalytic action and generation of reactive oxygen species (ROS), ZnO-NPs have antibacterial characteristics. Researchers discovered that ZnO-NPs have an impact on the cell membrane, leading to the generation of ROS. Zinc is absorbed by bacterial cells when they come into contact with ZnO-NPs, that limits the respiratory enzymes' activity, produces ROS, creates free radicals, and leading to oxidative stress. The Bacterial cell membrane, mitochondria, and DNA are irreversibly damaged by ROS leading to the death of bacterial cells [11]. Zinc oxide nanoparticles-based antibacterial agents have been used in daily-care products such as shampoos, diapers, and mouthwash and in dental composites.[12,13] **Sarwar et al.**[14] evaluated the usage of ZnO-NPs against Vibrio cholera and discovered that they resulted in protein leakage, altered cellular architecture, and enhanced depolarization and fluidity of the cell membrane. DNA damage and the production of ROS were also noted. The previous findings point to the synergistic effect of antibiotics and ZnO-NPs to treat bacterial diseases in a complementary manner.

Anticancer therapy:

The Food and Drug Administration (FDA) has accepted ZnO-NPs as a novel and effective anticancer therapy [15]. By inducing ROS production and apoptosis, ZnO-NPs have anticancer properties. ZnO-NPs were found to have discriminatory toxicity to cancer cell in comparison to normal cell. Many published research articles have stated that ZnO-NPs exhibit more than (30-fold) selective cytotoxicity against cancer cell in comparison with healthy cell [6,16]. At physiological pH, ZnO-NPs possess a positive charge. Cancer cells have a great number of molecules that are negatively charged on their surface. So, the positively charged ZnO-NPs promote cytotoxicity selectively against the proliferation of cells at the negatively charged tumor sites.[17]

Drug delivery:

The possibility of using ZnO-NPs as a drug carrier is significant as they have a versatile surface chemistry. Zinc oxide nanoparticles have a large surface area, that offers more opportunities for the functionalization of the surface. The ZnO-NPs' small size contributes to longer drug retention times and lower drug loads [8,18]. ZnO-NPs function as an ideal drug delivery system, as they can overcome various drug resistance, according to **Liu et al.**[19] This research found that doxorubicin-loaded ZnO-NPs successfully bypassed P-glycoprotein and boosted the drug's molecule intracellular localization inside drug-resistant cells.

Antidiabetic:

Based on the knowledge that zinc is crucial for the synthesis, secretion, and storage of insulin, ZnO-NPs' antidiabetic activity was studied. **Nazarizadeh and Asri-Rezaie.**[20] investigated the antidiabetic activity of zinc sulfate

against that of ZnO-NPs in diabetic rats. When compared to zinc sulfate, ZnO-NPs showed a higher antidiabetic efficacy. This was evidenced by improvements in zinc status, insulin level, and glucose disposal.

Bio-imaging:

Zinc oxide nanoparticles have been recognized as a trustworthy choice for a convenient and cheap bio-imaging tool. They show intrinsic fluorescence characteristics with excitonic emission at near UV, green, and blue areas.[18] ZnO-NPs that have fluorescent characteristics are utilized for imaging cancer and bacteria cells.[21,22]

Biosensors:

The biosensor is an analytical device with a transducer and a biological recognition element. An enzyme, tissue, microorganism, or bioligand like nucleic acids and antibodies can serve as the biological recognition element.[23] Due to a variety of factors, including their ease of fabrication, optical characteristics, high electron mobility, and polymorphic nature, ZnO-NPs are one of the most commonly utilized NPs for biosensor applications.[24] Acetylcholinesterase,[25] glucose',[26] xanthine,[27] and urea [28] have all been shown to be detectable by ZnO-NPs biosensors.

2. Toxicological aspects :

Although Zinc oxide has got FDA approval as a generally recognized as safe (GRAS) substance, researchers found that NPs have unique physicchemical characteristics, and their toxicological profile differs from their large counterparts. Recent researches suggested that ZnO-NPs can iduce adverse effects on human health and environmental organisms [29,30].

1. Toxicity mechanisms of Zinc oxide nanoparticles:

Recently, many studies have been published that have shown the toxic effects of ZnO-NPs on cell lines and some organs. The toxic effects are ascribed to the high solubility feature of ZnO-NPs, causing oxidative stress, cytotoxicity, and mitochondrial dysfunction.[31] Three mechanisms have been proposed to explain ZnO-NPs' toxicity.[32]

a) Zinc ions release:

The probable dissolvability of ZnO-NPs into free zinc ions is an important cause for their toxicity. Zinc homeostasis disruption and ROS generation occur as a result of ZnO-NPs dissolution inside cells. In addition, the increase in zinc content leads to mitochondrial damage, enzyme inhibition, protein folding, lysosomal inactivation, oxidative stress, genotoxicity, and finally cell death.[34] The hydrated cellular zinc ion binds with unblemished ZnO-NPs in the lysosomal acidic environment promoting cellular zinc homeostasis disruption and mitochondrial dysfunction causing cell damage. [35]

b) Production of reactive oxygen species:

Zinc oxide nanoparticles enter cells by endocytosis or direct diffusion. They interact with the biological system and stimulate ROS production and oxidative stress- mediated damage. Cells possess inherent antioxidant mechanisms such tas antioxidant enzymes like, glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) to normalize the ROS generated during normal metabolisms[8]. When ROS production exceeds cellular antioxidant defenses, it induces the creation of potent incendiary cytokine leading to inflammation.[36] Inflammation exhibits mitochondrial disturbance,[37] damage to cellular components, cell membranes,[38] DNA, and an inc-

rease in lactate dehydrogenase (LDH) arrival which comes from necrosis and cell demise.[39]

c) Mechanical harm due to the direct interaction between Zinc oxide nanoparticles and the cells:

Many studies have revealed that Zn-ONPs could connect cell divider and produce mechanical damage as, malformations of membranes, altered cell morphology, leakage of intracellular structures, and mitochondrial damage. [40,41,42]

2. Toxicological hazards of Zinc oxide nanoparticles:

The toxic impacts of ZnO-NPs on certain organs and their biological functions depend on particle size, morphology, concentration, pH, biocompatibility, and also exposure time. ZnO-NPs can affect the liver, heart, kidney, lung, nervous system, hematological indices, and sex hormone levels.[43,44]

Pulmonary toxicity:

Since inhalation is the major route of ZnO-NPs' exposure in the workplace, the concern about pulmonary toxicity of ZnO-NPs is growing.[45] ZnO-NPs could induce cytotoxicity in human lung epithelial cell by promoting oxidative stress causing damage to DNA and apoptosis.[46] **Cho et al.** [47] detected that inhalation of ZnO-NPs induced inflammation and fibrosis in the tracheobronchial and alveolar tissues. The acidic fluid of the lung triggered dissolution of ZnO-NPs and then increased their concentration causing pulmonary toxicity.

Cardiotoxicity:

Baky et al. reported that inflamemation, DNA damage, and apoptosis in rat's heart were noticed following oral administration of ZnO-NPs in two doses (600 mg/kg and 1g/kg) for five days. Biochemical investigations in this study revealed elevations of myoglobin, creatine kinase myocardial band, and troponin T levels that are indicative of cardiac damage. [48]

Neurotoxicity:

Zinc oxide nanoparticles could damage major areas in the brain and induce losing of their function.[49] **Elshama et al.**[43] detected that prolonged use of ZnO-NPs through the intraperitoneal route prompted ultrastructural and histopathological changes in the rats' spinal cords and brains based on ROS generation**.**

Hematotoxicity:

Evaluation of the interaction of NPs with blood components is part of the preclinical risk assessment of newly manufactured materials.[50] **Yahya et al.**[51] conducted a study to detect the hematological changes caused by ZnO-NPs in albino rats. Rats were administrated ZnO-NPs orally in a dose of 10 mg/kg/day for four weeks. Results revealed a significant increase in the white blood cells count and a decrease in hemoglobin, red blood cells, and hematocrit counts and concentration. A Significant decrease in mean corpuscular hemoglobin values, increase in mean corpuscular volume, and decrease in blood platelet levels were also detected

Intestinal toxicity:

Abbasi-Oshaghi et al.[52] reported that oral treatment of normal rats and high fat diet fed rats with ZnO-NPs (50 and 100 mg/kg) for 28 days significantly induced intestinal injury via oxidative stress, inflammation, and apoptosis mechanisms**.**

Hepatotoxicity:

Experimental studies detected that ZnO-NPs administrated via the oral route revealed a special predilection towards hepatic accumulation and promote

oxidative stress and apoptosis in hepatocytes.[53,54] **Aboulhoda et al.**[55] stated that orally administrated ZnO-NPs in three doses (100, 200, 300 mg/kg/day) to rats for two weeks affected the liver functionally and histopathologically. ZnO-NPs disrupted liver architecture, increased the serum level of aspartate transaminase, alkaline phosphatase, and alanine transaminase enzymes. In addition, it produced a dose-dependent decrease in the antioxidant enzymes activity GPx, CAT, and SOD with an elevation in the lipid- peroxidation product malondialdehyde (MDA) level.

Nephrotoxicity:

Recent studies concluded that the nephrotoxicity of ZnONPs may be attributed to DNA degradation and induction of both lipid peroxidation and systemic inflammation.[56]

Heidai-Moghadam et al.[57] reported that oral treatment of rats with 50 mg/kg/day ZnO-NPs for 14 days induced renal injury. Investigations of this study revealed a significant increase in blood levels of creatinine, blood urea nitrogen (BUN), and uric acid. Renal injury was explained by promoting oxidative stress in kidney via enhancing the contents of MDA and decreasing GPx and SOD enzymes activity.

Regarding histopathological effects **Chien et al.**[58] evaluated the renal toxicity of ZnO-NPs in rats exposed to the relevant occupational levels (1.1 and 4.9 mg/m^3 ZnO-NPs) through inhalation for two weeks. Examinations of rats' kidney by light microscope revealed interstitial lymphocytic infiltration, a significant increase in inflammatory foci with pronounced periglomerular inflammation, and tubulitis with lymphocytic infiltrate in the tubular epithelium.

Lin et al. [59] stated that increased BUN, creatinine concentrations, and decreased creatinine clearance in rats injected intraperitoneally with varying concentrations of ZnO-NPs indicating kidney dysfunction

Testicular toxicity:

The toxic effects of ZnO-NPs on the reproductive system have been progressively reported in both in vivo and in vitro studies. Recent researches revealed that ZnO-NPs could cross blood-testes barrier and accumulate in testis and epididymis. [60-61] The NPs accumulation leads to harmful testicular effects, as perm malformations, testicular lesions, alterations in sex hormone serum level, and testis-specific gene expressions [62-63]. **Tang et al.** [60] studied the effect of orally administrated 50, 150, and 450 mg/kg ZnO-NPs for 14 days to adult male mice. Results of this study revealed that testosterone hormone concentration in serum and the sperms number in epididymis were decreased with increasing ZnO-NPs dose. Histopathological examination of testis showed atrophy, detachment, and vacuolization of germ cells.

Hussein et al. [64] reported that orally treated rats with 100 and 400 mg/kg/day ZnO-NPs for 12 weeks revealed a significant decrease in motility of sperm, sperm cell count, normal and live sperms, and testosterone hormone level. There was a significant elevation in the MDA content and a reduce in antioxidant enzymes activities, CAT, SOD, GPx, and reduced glutathione level. In addition, histopathological testicular damage was detected.

Conclusion:

Zinc oxide nanoparticles are important metal oxide nanoparticles. They are commonly used in many fields because of their unique physical and chemical properties. They have been widely utilized in biomedical applications, food industry, electronics, and co-

smetics. Biomedical applications of Zinc oxide nanoparticles are increasing day by day in many processes as anticancer, antidiabetic, antibacterial, biosensors, drug delivery, and bioimaging. The widespread usage of zinc oxide nanoparticles increases the potential exposure of consumers, workers, and scientists to them, so more attention must be taken regarding their potential harmful effects on different body organs.

References:

1. **Siddiqi KS, Husen A, Rao RAK.** A review on biosynthesis of silver nanoparticles and their biocidal properties. Journal of Nanobiotechnology. 2018; 16(1):1-28.

2. **Bayda S, Adeel M, Tuccinardi T, Cordani M, Rizzolio F.** The history of nanoscience and nanotechnology: From chemical-physical applications applications to nanomedicine. Molecules. 2020; 25(1): 112-126.

3. **Husen A, Siddiqi KS.** Phytosynthesis of nanoparticles: concept, controversy and application. Nanoscale Research Letters.2014; 9(1):1-24.

4. **Eastlake A, Zumwalde R, Geraci C.** Can control banding be useful for the safe handling of nanomaterials? A systematic review. Journal of Nanoparticle Research. 2016; 18(6):1-24.

5. **Tinkle S, McNeil SE, Mühlebach S, Bawa R, Borchard G, Barenholz Y, et al.** Nanomedicines: addressing the scientific and regulatory gap. Annals of the New York Academy of Sciences. 2014; 1313(1: 35-56.

6. **Rasmussen JW, Martinez E, Louka P, Wingett DG.** Zinc oxide nanoparticles for selective destruction of tumor cells and potential for drug delivery applications. Expert Opinion on Drug Delivery.2010; 7(9): 1063-1077.

7. **Kumar SS, Venkateswarlu P, Rao VR, Rao GN.** Synthesis, characterization and optical properties of zinc oxide nanoparticles. International Nano Letters. 2013; 3(1):1-6.

8. **Sruthi S, Ashtami J, Mohanan PV.** Biomedical application and hidden toxicity of Zinc oxide nanoparticles. Materials Today Chemistry. 2018; 10: 175-186.

9. **Saptarshi SR, Duschl A, Lopata AL.** Biological reactivity of zinc oxide nanoparticles with mammalian test systems: An overview. Nanomedicine. 2015; 10: 2075–2092.

10. **Almansour MI, Alferah MA, Shraideh ZA, Jarrar BM.** Zinc oxide nanoparticles hepatotoxicity: histological and histochemical study. Environmental Toxicology and Pharmacology. 2017; 51: 124-130.

11. **Dwivedi S, Wahab R, Khan F, Mishra YK, Musarrat J, Al-Khedhairy, A A.** Reactive oxygen species mediated bacterial biofilm inhibition via zinc oxide nanoparticles and their statistical determination. PloS One. 2014; 9(11):1-9

12. **Hernández-Sierra JF, Ruiz F, Pena DCC, Martínez-Gutiérrez F, Martínez AE, Guillén, ADJP, et al.(2008):** The antimicrobial sensitivity of Streptococcus mutans to nanoparticles of silver, zinc oxide,andgold. Nanomedicine: Nanotechnology, Biology and Medicine.2008; 4(3): 237-240.

13. **Aydin Sevinç B, Hanley L.** Antibacterial activity of dental composites containing zinc oxide nanoparticles. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2010; 94(1): 22-31

14. **Sarwar S, Chakraborti S, Bera S, Sheikh IA, Hoque KM, Chakrabarti P.** The antimicrobial activity of ZnO nanoparticles against Vibrio cholerae: variation in response depends on biotype. Nanomedicine: Nanotechnology, Biology and Medicine. 2016; 12(6): 1499-1509.

15. **Shen C, James SA, de Jonge M D, Turney TW, Wright PF, Feltis B N.** Relating cytotoxicity, zinc ions, and reactive oxygen in ZnO nanoparticle-exposed human immune cells. Toxicological Sciences. 2013; 136(1): 120-130.

16. **Taccola L, Raffa V, Riggio C, Vittorio O, Iorio MC, Vanacore R, et al.** Zinc oxide nanoparticles as selective killers of proliferating cells. International Journal of Nanomedicine.2011; 6:1129-1140.

17. **Hanley C, Layne J, Punnoose A, Reddy KÁ, Coombs I, Coombs A, et al.** Preferential killing of cancer cells and activated human T cells using ZnO nanoparticles.Nanotechnology. 2008; 19(29): 295103-295113.

18. **Wu Y L, Fu S, Tok A I Y, Zeng XT, Lim CS, Kwek L C et al.** A dual-colored bio-marker made of doped ZnOnanocrystals. Nanotechnology. 2008; 19(34): 1-9.

19. **Liu J, Ma X, Jin S, Xue X, Zhang C, Wei T, et al.** Zinc oxide nanoparticles as adjuvant to facilitate doxorubicin intracellular accumulation and visualize pH-responsive release for overcoming drug resistance. Molecular Pharmaceutics. 2016; 13(5): 1723-1730.

20. **Nazarizadeh A, Asri-Rezaie S.** Comparative study of antidiabetic activity and oxidative stress induced by zinc oxide nanoparticles and zinc sulfate in diabetic rats. American Association of Pharmaceutical Scientists (AAPS) PharmSciTech,. 2016;7(4): 834-843.

21. **Wang H, Wingett D, Engelhard MH, Feris K, Reddy KM, Turner P, et al.** Fluorescent dye encapsulated ZnO particles with cell-specific toxicity for potential use in biomedical applications. Journal of Materials Science: Materials in Medicine. 2009; 20(1):11-22.

22. **Sudhagar S, Sathya S, Pandian K, Lakshmi BS.** Targeting and sensing cancer cells with ZnO nanoprobes in vitro. Biotechnology Letters. 2011; 33(9): 1891-1896.

23. **Scognamiglio V.** Nanotechnology in glucose monitoring: Advances and challenges in the last 10 years. Biosensors and Bioelectronics. 2013; 47: 12-25.

24. **Xu C, Yang C, Gu B, Fang S.** Nanostructured ZnO for biosensing applications. Chinese Science Bulletin. 2013; 58(21): 2563-2566.

25. **Wang G, Tan X, Zhou Q, Liu Y, Wang M, Yang L.** Synthesis of highly dispersed zinc oxide nanoparticles on carboxylic graphene for development a sensitive acetylcholinesterase biosensor. Sensors and Actuators B: Chemical. 2014; 190: 730-736.

26. **Hwa K Y, Subramani B.** Synthesis of zinc oxide nanoparticles on grapheme-carbon nanotube hybrid for glucose biosensor applications. Biosensors and Bioelectronics, 2014; 62: 127-133.

27. **Devi R, Yadav S, Pundir C S.** Amperometric determination of xanthine in fish meat by zinc oxide nanoparticle/chitosan/multiwalled carbon nanotube/polyaniline composite film bound xanthine oxidase. Analyst. 2012; 137(3): 754-759.

28. **Ali A, Ansari AA, Kaushik A, Solanki P R, Barik A, Pandey M K, et al.** Nanostructured zinc oxide film for urea sensor. Materials Letters. 2009; 63(28): 2473-2475.

29. **Morris A S, Salem A K.** Nanotoxicity. Milane, L.S. and Amiji, M.M. (editors). In: Nanomedicine for Inflammatory Diseases, 1st edition, CRC press, 2017; chapter 3, 67-79.

30. **Mohd Yusof H, Mohamad R, Zaidan U H.** Microbial synthesis of zinc oxide nanoparticles and their potential application as an antimicrobial agent and a feed supplement in animal industry: a review. Journal of Animal Science and Biotechnology. 2019; 10(1): 1-22.

31. **Condello M, De Berardis B, Ammendolia M G, Barone F, Condello G, Degan P, et al.** ZnO nanoparticle tracking from uptake to genotoxic damage in human colon carcinoma cells. Toxicology in Vitro. 2016; 35: 169-179.

32. **Singh S.** Zinc oxide nanoparticles impacts: cytotoxicity, genotoxicity, developmental toxicity, and neurotoxicity. Toxicology Mechanisms and Methods. 2019; 29(4): 300-311.

33. **Yan G, Huang Y, Bu Q, Lv L, Deng P, Zhou J, et al.** Zinc oxide nanoparticles cause nephrotoxicity and kidney metabolism alterations in rats. Journal of Environmental Science and Health, Part A. 2012; 47(4):577-588.

34. **Wilhelmi V, Fischer U, Weighardt H, Schulze-Osthoff K, Nickel C, Stahlmecke, B, et al.** Zinc oxide nanoparticles induce necrosis and apoptosis in macrophages in a p47phox-and Nrf2-independent manner. PloS One. 2013; 8(6): 65704-65718.

35. **Song W, Zhang J, Guo J, Zhang J, Ding F, Li L, et al.** Role of the dissolved zinc ion and reactive oxygen species in cytotoxicity of ZnO nanoparticles. Toxicology Letters. 2010; 199(3): 389-397.

36. **Khanna P, Ong C, Bay BH, Baeg GH.** Nanotoxicity: an interplay of oxidative stress, inflammation and cell death. Nanomaterials. 2015; 5(3): 1163-1180.

37. **Lai X, Wei Y, Zhao H, Chen S, Bu X, Lu F, et al.** The effect of Fe_2O_3 and ZnO nanoparticles on cytotoxicity and glucose metabolism in lung epithelial cells. Journal of Applied Toxicology. 2015; 35(6):651-664.

38. **Babu EP, Subastri A, Suyavaran A, Premkumar K, Sujatha V, Aristatile B, et al.** Size dependent uptake and hemolytic effect of zinc oxide nanoparticles on erythrocytes and biomedical potential of ZnO-ferulic acid conjugates. Scientific Reports. 2017; 7(1):1-12.

39. **Ghosh M, Jana A, Sinha S, Jothiramajayam M, Nag A, Chakraborty A, et al.** Effects of ZnO nanoparticles in plants: cytotoxicity, genotoxicity, deregulation of antioxidant defenses, and cell-cycle arrest. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2016; 807: 25-32.

40. **Hu C W, Li M, Cui Y B Li D S, Chen J, Yang L Y.** Toxicological effects of TiO2 and ZnO nanoparticles in soil on earthworm Eisenia fetida. Soil Biology and Biochemistry. 2010; 42(4): 586-591.

41. **Zhang W, Bao S, Fang T.** The neglected nano-specific toxicity of ZnO nanoparticles in the yeast Saccharomyces cerevisiae. Scientific Reports. 2016; 6(1): 1-11.

42. **Babele PK, Thakre PK, Kumawat R, Tomar RS.** Zinc oxide nanoparticles induce toxicity by affecting cell wall integrity pathway, mitochondrial function and lipid homeostasis in Saccharomyces cerevisiae. Chemosphere. 2018; 213:65-75.

43. **Elshama SS, Abdallah ME Abdel-Karim R I.** Zinc oxide nanoparticles: therapeutic benefits and toxicological hazards. The Open

NanomedicineJournal. 2018; 5(1):16-22

44. **Vimercati L, Cavone D, Caputi A, De Maria L, Tria M, Prato E, et al.** Nanoparticles: An experimental study of zinc nanoparticles toxicity on marine Crustaceans. General overview on the health implications in humans. Frontiers in Public Health. 2020; 8: 192-210.

45. **Chong C L, Fang C M, Pung SY, Ong CE, Pung Y F, Kong C, et al.** Current updates on the in vivo assessment of zinc oxide nanoparticles toxicity using animal models, BioNanoScience. 2021; 11(2): 590-620.

46. **Sahu D, Kannan GM Vijayaraghavan R, Anand T, Khanum F.** Nanosized zinc oxide induces toxicity in human lung cells. International Scholarly Research Notices. 2013; 1-8.

47. **Cho WS, Duffin R, Howie SE, Scotton CJ, Wallace WA, MacNee W**, **et al.** Progressive severe lung injury by zinc oxide nanoparticles; the role of Zn^{+2} dissolution inside lysosomes. Particle and Fibre toxicology.2011; 8(1):1-16.

48. **Baky NAA, Faddah LM, Al-Rasheed NM, Fatani AJ.** Induction of inflammation, DNA damage and apoptosis in rat heart after oral exposure to zinc oxide nanoparticles and the cardioprotective role of α-lipoic acid and vitamin E. Drug Research. 2013; 63(5): 228-236.

49. **Yaqub A, Faheem I, Anjum K M, Ditta SA, Yousaf MZ, Tanvir F**, **et al.** Neurotoxicity of ZnO nanoparticles and associated motor function deficits in mice. Applied Nanoscience. 2020; 10(1): 177-185.

50. **Koohi MK, Hejazy M, Najafi D, Sajadi SM.** Investigation of hematotoxic effect of nano ZnO, nano Fe_3O_4 and nano SiO_2 in vitro. Nanomedicine Research Journal. 2017; 2(2): 93-99.

51. **Yahya RA, Attia AM, El-Banna SG, El-Trass EE, Azab AE, Jbireal JM, et al.** Hematotoxicity induced by copper oxide and/or zinc oxide nanoparticles in male albino rats. Journal of Biotechnology and Bioengineering. 2019; 3(4): 1-7.

52. **Abbasi-Oshaghi E, Mirzaei F, Mirzaei A.** Effects of ZnO nanoparticles on intestinal function and structure in normal/high fat diet-fed rats and Caco-2 cells. Nanomedicine. 2018; 13: 2791-2816.

53. **Osmond MJ, McCall MJ.** Zinc oxide nanoparticles in modern sunscreens: an analysis of potential exposure and hazard. Nanotoxicology. 2010; 4:15-41.

54. **Rani V, Verma Y, Rana K, Rana SVS.** Zinc oxide nanoparticles inhibit dimethylnitrosamine induced liver injury in rat. Chemico-Biological Interactions. 2018; 295: 84-92.

55. **Aboulhoda BE, Abdeltawab DA, Rashed LA, Abd Alla MF, Yassa H D.** Hepatotoxic effect of oral zinc oxide nanoparticles and the ameliorating role of selenium in rats: a histological, immunohistochemical and molecular study. Tissue and Cell. 2020; 67: 101441-101453.

56. **Yousef MI, Mutar TF, Kamel MAEN.** Hepato-renal toxicity of oral sub-chronic exposure to aluminum oxide and/or zinc oxide nanoparticles in rats. Toxicology Reports.2019: 6: 336-346.

57. **Heidai-Moghadam A, Khorsandi L, Jozi Z.** Curcumin attenuates nephrotoxicity induced by zinc oxide nanoparticles in rats. Environmental Science and Pollution Research. 2019; 26(1): 179-187.

58. **Chien C C, Yan YH, Juan HT, Cheng TJ, Liao JB, Lee HP. et al.**

Sustained renal inflammation following 2 weeks of inhalation of occupationally relevant levels of zinc oxide nanoparticles in Sprague Dawley rats. Journal of Toxicologic Pathology. 2017; 30(4): 307-314.

59. **Lin YF, Chiu IJ, Cheng FY, Lee YH, Wang YJ, Hsu YH. et al.** The role of hypoxia-inducible factor-1α in zinc oxide nanoparticle-induced nephrotoxicity in vitro and in vivo. Particle and Fibre Toxicology. 2015; 13(1): 1-14.

60. **Tang Y, Chen B, Hong W, Chen L, Yao L, Zhao Y**, **et al.** ZnO nanoparticles induced male reproductive toxicity based on the effects on the endoplasmic reticulum stress signaling pathway. International Journal of Nanomedicine.2019;14 : 9563-9576.

61. **Kong T, Zhang SH, Zhang C, Zhang JL, Yang F, Wang GY et al.** The effects of 50 nmunmodified nano-ZnO on lipid metabolism and semen quality in male mice. Biological Trace Element Research. 2020; 194(2): 432-442

62. **Gao G, Ze Y, Zhao X, Sang X, Zheng L, Ze X, et al.** Titanium dioxide nanoparticle-induced testicular damage, spermatogenesis suppression, and gene expression alterations in male mice. Journal of Hazardous Materials. 2013; 258: 133-143.

63. **Hong F, Zhao X, Si W, Ze Y, Wang L, Zhou Y**, **et al.** Decreased spermatogenesis led to alterations of testis-specific gene expression in male mice following nano-TiO_2 exposure. Journal of Hazardous Materials. 2015; 300:718-728.

64. **Hussein MM, Ali HA, Saadeldin IM, Ahmed MM.** Querectin alleviates zinc oxide nanoreprotoxicity in male albino rats. Journal of Biochemical and Molecular Toxicology. 2016; 30(10): 489-496.