

In-vivo (*Albino Mice*) and in-vitro Assimilation and Toxicity of Zinc Oxide Nanoparticles in Food Materials

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Purpose: Recent advances in nanotechnology have given rise to the potential utilization of nanoparticles as food, nano-medicine /biomedicines.

Patient: The study aimed to investigate the effects of nano-zinc oxide (nano-zinc) on the bio-assimilation of mineral (Zn) in mice, aged 3–6 weeks.

Methods: ZnO nanoparticles were added to the basal diet as a supplement at amounts of 0.07, 0.14 and 0.21 mg/kg. The synthesized material was characterized by Fourier transform infrared spectrophotometer, particle size, scanning electron microscope, Thermogravimetric Analysis Thermal, X-ray diffraction spectrophotometer and Zeta potential.

Results: In-vitro bioavailability of synthesized group ZnO (120 nm) was 43%, whereas for standard group ZnO (50 nm) was reported as 55%. In-vivo bioavailability of zinc oxide illustrated the maximum absorption level compared with the control. In-vivo toxicity was characterized as damage done to the liver and spleen tissues with a high dose of 0.21 mg/kg, while smaller doses indicated no toxic effects.

Conclusion: The study provided important insights on the toxicological effects of ZnO nanoparticles, depending on dose rate and bio-assimilation, as well as particles, under various conditions (in-vitro and in-vivo). These findings will motivate further detailed research on nano-based medicine for alleviating malnutrition conditions.

Keywords: bioavailability, toxicity, nanoparticles, nanomedicine, food

Introduction

Zinc is an essential mineral element that plays an important role in numerous physiological functions in the human body.^{1–3} Integrating nanoparticles in food fortification would improve people's efficacy in absorption of zinc, which is beneficial to health and productivity.⁴ Numerous scientific studies reported that potential application of ZnO nanoparticles as food fortifies supplement leading to an improvement in the growth of the human body,⁵ better zinc efficacy,^{6,8} and immunity to some diseases or conditions.^{7,9} Generally, zinc (ZnO) nanoparticles are synthesized by chemical processes, which improves the production rate and helps nanoparticles reproduce. For this reason, such methods are deemed to be unfavorable because of its high price, high energy intake and the more toxic and hazardous impacts that are evident.

Currently, researchers are showing a keen interest in the application of zinc oxide nanoparticles (ZnO NPs). The smallest pore size of these particles' is more important, due to their chemical reactivity. Hence, this ability of zinc oxide is expanded to a wider application in fields like electronics, biomedical sciences and agriculture.^{5,10} Zinc oxide is an inorganic element which contains certain properties including absorption, highly reactivity with good catalytic activity.^{11,12} Zinc oxide has been categorized by the United States Food and Drug Administration (FDA) as a [GRAS] generally

recommended as safe materials.¹² Nanoparticles exhibit unique physicochemical properties and can have many unknown biological outcomes.

In recent decades, nanoparticles have documented significant progress in numerous fields of nano-technology. Recently, the World Health Organization (WHO) findings, cancer is now the second most lethal disease globally,¹³ and about 10 million new cancer cases are reported annually.¹⁵ The current number of cancer cases will be doubled by the year 2030.¹⁴ Female breast cancer is the third most fatal disease of cancer following colorectal and lung cancer.¹⁶ So it is evident that much more efforts and strategies are required for treating cancer. Commonly, cancer chemotherapies often fail due to systemic toxicity inside the human body.¹⁷ Nanomedicine offers excellent potential as a safe mode for therapy,¹⁸ and ZnO NPs inhibit cytotoxicity in cancerous cells with a low dose rate in noncancerous cells. Antiproliferative activity of ZnO NPs as been reported for murine cancer cells (WEHI-3B, CT-26, CRL-1451).¹⁹

Anticancer studies reported ZnO NPs affect lung cells in an orthotopic animal model.⁸ A few scientific studies illustrated the toxic dangers of ZnO NPs in different organisms like bacteria, yeast marine and mice.^{20,21} Hence, anticancer application and biocompatibility of ZnO NPs have been analyzed recently.^{21,22} Graphene derivatives and nanomaterials such as nanomedicine (ZnO/RGO NCs) have indicated high anticancer effectiveness. Metal doping in ZnO/RGO NCs has illustrated the high potential of nanoparticles as anticancer substances as well as their biocompatibility. Biocompatibility of ZnO was evident in human breast (MCF10A) cells, and ZnO/Ag NCs revealed efficacy against cancer cells in cervical (HeLa) and ovarian (KOV-3) cells.²² ZnO-based NCs were developed and had various physical and chemical protocols like ultrasonic spray (pyrolysis,²³ hydrothermal,¹⁸ magnetron, etc.) whereas the green synthesis of NPs/NCs were reported as being more rapid, cost-effective, and eco-friendly biomedical applications.²⁴ Currently, the Mo-ZnO/RGO NCs composite form of NPs has anticancer potential and the best biocompatibility. Anticancer activity of NPs/NCs was assessed in human colorectal (HCT116)/breast cancer (MCF7) cells. Anticancer activity of ZnO NPs, Mo-ZnO NPs, Mo-ZnO/RGO NCs with various derivatives was assessed utilizing apoptosis and oxidative stress. Moreover, cytocompatibility for NPs was applied to the human colon (epithelial NCM460) and breast (epithelial MCF10A) cells. These cell lines were lethal cases of cancer globally.^{24,25}

Nanotoxicology is also emerging as a new discipline that has much potential efficacy in understanding the toxicity levels of NPs for better results, assessment of public health concerns and risks related to their application.^{26,27} Recently, various analytical techniques and methods have been devised to understand the maximum and minimum impact of nanomaterials on people, animals and the environment. The toxicity of nano materials has been assessed by both in-vitro and in-vivo studies, and in-vivo application has been reported for experimental animal and silicon (computational) models.²⁸ In both in-vitro and in-vivo studies on silicon models, these have good potential for providing important research data on the issue of toxicological effects. The in-vitro applications of nano materials are essentially the first phase of the toxicity assessment for nanoparticles.

The use of ZnO nanoparticles in everyday applications worldwide has been rising steadily, and nanoscale particles can enter the human body via various exposure pathways such as inhalation, ingestion and injection. Consequently, there are concerns about possible serious outcomes such as toxicity, cytotoxicity and genotoxicity.¹² Some studies have reported that ZnO nanoparticles exhibit low toxicity, while other research contends that ZnO nanoparticles at high doses of 1–5 g/kg may induce severe stress due to their impact on organs and systems, such as inflammation and altered heart rate. Humans are constantly exposed to nanoparticles in their daily activities, so assessing the toxicity of nanoparticles that can easily enter cells and induce oxidative stress^{28,29} is an emerging field of research.

Several studies have been conducted to assess acute toxicity of nano-ZnO (in-vitro and in-vivo analyses) in numerous cells lines, such as human cells,¹² the liver, retina, blood cells,²⁹ and Zn ion homeostasis. In-vivo exposure to nano-ZnO through inhalation revealed marked damage in liver and lung tissues within 3 days. Acute exposure with higher dose-rate ranges from 1 to 5 gram ZnO per kilo gram of body weight through oral feeding severely affected the vital organs of mice.²⁹ Oral use of nano-ZnO for 2 weeks in rats induced nephrotoxicity.¹⁸ It was noted that the toxicological effects of nanoparticles might occur due to the interference with Zn ion homeostasis by increasing the bioavailability and transport to the required tissue organs.³⁰

Zinc is an important but mostly deficient or absent mineral in the human body, so it is commonly added as a supplement diet. However, its concentration variations can contribute to adverse health or toxic effects.^{30,32} ZnO NPs were primarily reported in the ionic forms of tissues organs after oral feeding in animal models,^{27,30} while ZnO

nanoparticles were released in soluble form, causing the secretion of gastric juice up to 14% as documented in research.^{29,31} Of great importance here were their dissolution characteristics. Scientific studies showed that zinc oxide metal would be clearly dissolve in to Zn ionic forms with acidic bio-fluids.³² Zinc-supplemented foods were administered through oral feeding, whereas ZnO introduced into Zinc ionic form of the food matrices. Based on these reasons, it was preferable to apply ZnO nanoparticles as a source of Zn nutrient.

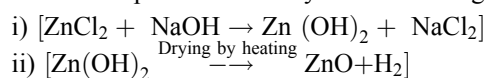
It should be noted that nanotechnology is still fairly recent (nano-based delivery systems) but can achieve the highest level of absorption of nutrients. It might be useful as a conventional source of micronutrients as efficacious food materials with the least toxic risk in targeted animal models. With this in mind, our current study investigated the effects of various doses of nano-ZnO in mice to evaluate both bioavailability and toxicity.

Materials and Methods

Studies were conducted at the Food Analysis Laboratory, Department of Food Science and Technology, University of Poonch, Rawalakot. Nano-ZnO served as a nutrient supplement for oral medication in mice. All chemicals were purchased from Sigma Aldrich, with reliable grades, and raw materials (zinc chloride and sodium hydroxide) were used in the form of powder to synthesize ZnO nanoparticles.

Synthesis of ZnO Nanoparticles

The ZnO nanoparticles were synthesized using the chemical precipitation method with zinc chloride,



According to a previously described procedure.^{33,34} Standard ZnO 50nm was purchased from Sigma-Aldrich in powder form, and the product number was 1314-13-2. Standard ZnO was used to assess the bio-assimilation of particles of various sizes. The synthesized nanoparticles were characterized in in-vitro and in-vivo studies utilizing Fourier-transform infrared spectroscopy (FT-IR; Agilent 660), scanning electron microscopy (SEM), thermogravimetric analysis (TGA/ DSA 1, Mettler Toledo) and X-ray diffraction spectroscopy (XRD-PANalytical Empyrean).

Formulation of ZnO Nano-Encapsulated Tablets

Nano-encapsulated tablets (500 mg) were formulated using a powder with nutrient content (ZnO as 0.07 mg/kg, 0.14 mg/kg and 0.21 mg/kg + starch 70% + avicel 2–4 mg/100 mg + magnesium citrate 8 mg/100 mg, 1% talcum powder + aerosol 15 mg/100 mg) for oral medication, according to methods of prior research.³⁴

In-vitro Bio-Accessibility Study

The ZnO nanoparticles 120 nm (primary size) for the synthesized group and ZnO 50 nm (primary size) nanoparticles for standard group were assessed via the dialysis bag method. This bag was used to separate the soluble zinc (Zn NPs) from ZnO and molecular cut off (pore size).³⁴ ZnO nanoparticles (0.1 mg) were dissolved in 1 mL of Phosphate Buffered Saline (PBS; pH 7) at 37°C, followed by horizontal shaking at fifty (50) rpm. The supernatant (10 µL) was diluted to 10 mL, and then analyzed using an Inductively Coupled Plasma–Optical Emission Spectrometry [ICP-OES] system²⁸ to determine the concentration of zinc.

In-vivo Bio-Accessibility Study

Animals

In-vivo examination of 21 albino white female mice was conducted at the University of Veterinary and Animal Sciences, Lahore (UVAS). The animals were purchased from the animal department of the Theriogenology Laboratory, UVAS. Studies were conducted in accordance with the Pakistan Animals Scientific Procedures Act 1986³⁸ and the University of Poonch, Rawalakot, Ethical Act (Registration No. 2013-AGRI-235). Animals were housed under standard husbandry conditions in individual cages. Oral medication (tablets of ZnO for animal groups with increased dose rate 0.07 mg/kg, 0.14 mg/kg and 0.21 mg/kg) was administered to the animals once a day, along with a control group, and blood was collected weekly.

Sample (Blood) Collection and Preparation

Blood samples were collected weekly, after fasting for 12 hours, without the use of anaesthesia or euthanasia. Blood (1 mL per mouse) was obtained with a syringe via cardiac puncture and placed in sterile glass test tubes containing EDTA (ethylenediamine tetra acetic acid) as anticoagulant solution. Test tubes were kept in a slanting position at 25°C for 6 hours, and then stored at -20°C, as follows.²⁸ The blood metal (Zn) profile was conducted using an atomic absorption spectrometry (AAS) device (Hitachi Model No. Z-8230 Polarized Zeeman) for zinc, as used by Sastry.³⁶ Once the blood collection was completed, mice from each group were sacrificed and dissected to procure their organs for histopathological examination.

Estimation of Mineral (Zinc) in Blood

Samples were collected from blood for the Estimation of zinc concentration by Atomic Absorption Spectrometry (AAS). Blood (1 mL) was placed in a 50 mL clean flask, and 5 mL of an acid mixture (HNO₃, H₂SO₄ and HClO₄; 4:2:1) was added. The mixture was heated on a hot plate at 150°C for 30 minutes until perchloric acid smoke was no longer emitted. The remaining contents in the flask were allowed to cool, and the volume was made up to 25 mL with double distilled water. The prepared blood samples (mineral element zinc was first assimilated in blood and then distributed to the other body organs - this explains why we selected the blood for estimating the amount of Zn) were estimated for zinc concentration using AAS.

In-vivo Toxicological Study

Selected tissue organs including brain, heart, kidney, liver and spleen were removed, sectioned into suitable segments, and fixed in 10% neutral buffered formalin. The samples from each organ tissue were subjected to further analysis stages including dehydration, cleaning and infiltration. Then, all samples from each organ were embedded in blocks, and 0.4 µm thick tissue sections of the organs were cut and finally analyzed for their histopathological characteristics. It was done using an Olympus CX-41 binocular microscope at the operational lab, UVAS, following prior research methods.³⁸⁻⁴⁰

Results and Discussion

Characterization of ZnO NPs

Fourier Transform Infrared [FTIR] Spectroscopy

ZnO nanoparticles were characterized by Fourier transform infrared (FTIR), as illustrated in Figure 1. Absorption bands in the region <1000 cm⁻¹ represent interatomic vibrations. Figure 1 confirms that the ZnO nanoparticles had absorption peaks at 3438, 2918, 2367, 1634, 1446, 1033, 876 and 701 cm⁻¹. The peaks at around 1033 cm⁻¹ were characteristic peaks (absorption) of the Zn-O bonding. Absorption peaks at 1634 and 1446 cm⁻¹ are due to stretching vibration of C=C in the capping agent.²³ Meanwhile, peaks at 1033, 876 and 701 cm⁻¹ confirm the presence of Zn-O in the sample.⁴¹ The absorption peaks at around 1446 cm⁻¹ represent C=C stretching, while the peak at 3600 cm⁻¹ indicates the stretching vibrations of OH. The presence of C-O is indicated by a peak at 2367 cm⁻¹. Absorption peaks can also be observed at 3428 cm⁻¹, which then diminish gradually.^{10,41} The interference pattern illustrated in the FTIR spectra clearly demonstrates the absorption peaks of Zn-O at 1033 cm⁻¹, which authenticated the presence of ZnO.

Particle Size Analyser

The particle size of the ZnO synthesized sample was measured using a particle size analyser and zeta sizer, NanoPlus HD Common Model 601916 instrument. The reported particle size was 120 nm. Figure 2 illustrates the particle size for ZnO. Diameter and specific surface area (SSA) and PDI was measured by BT-90 NANO PSA BetterSize. Diameter was 21.4 nm and surface area for the ZnO nanoparticles amounted to SSA: 103.54 m²/g, whereas [distribution light intensity was Poly 0.39410, and poly distribution light intensity was PDI: 0.158, respectively].⁴²

Scanning Electron Microscopy

Images for the analysis of the ZnO samples were obtained using SEM (JEOL-JSM5800). The micrographs (Figure 3) clearly indicate agglomeration, which means that the formation of irregular spherical ZnO nanoparticles had taken place.

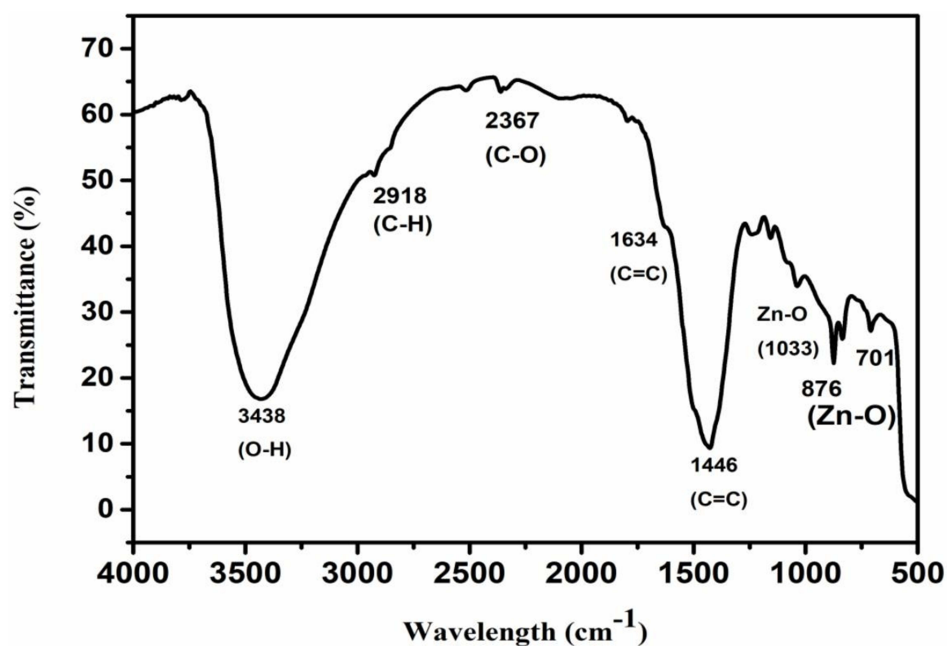


Figure 1 FTIR spectra of ZnO nanoparticles.

Abbreviations: ZnO, zinc oxide; FTIR, Fourier transform infrared spectrophotometer.

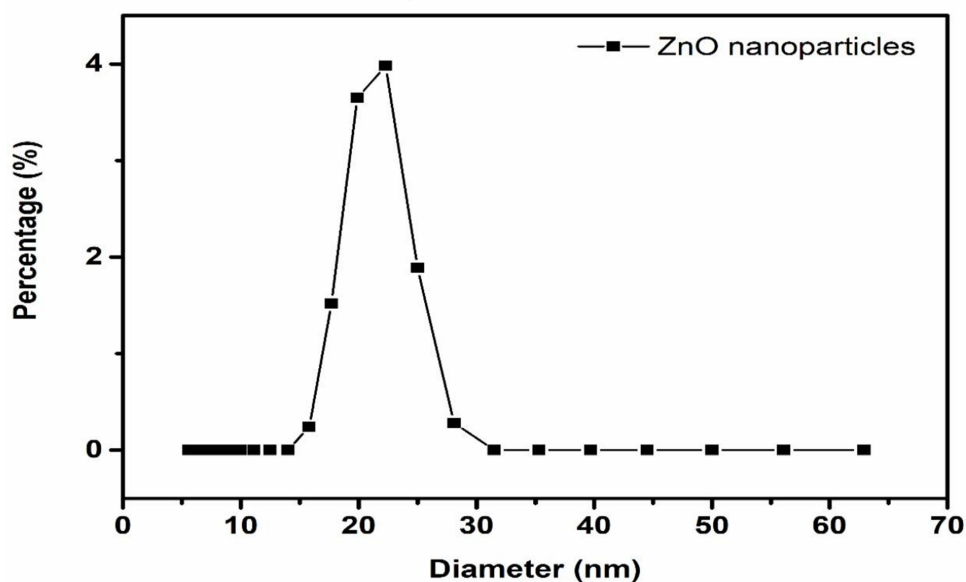


Figure 2 Particle size for ZnO nanoparticles.

Abbreviation: ZnO, zinc oxide.

Thermogravimetric Analysis

TGA of the ZnO samples was conducted using a TGA/DAS STAR system (METTLER, Model No. 3760), applying an increase in heat at 10°C per minute. A TGA graphical presentation of the ZnO sample is depicted in Figure 3, and it illustrates a gradual and continuous weight loss up to 300°C, after which no significant loss was recorded. TGA analysis indicates that when increasing the temperature, the weight decreases percentage.⁴³ First weight loss in the sample is observed at around 123 °C which continues till 241°C and this results in a loss of 15% followed by 04% loss till 368°C is reached, and 5.2% till 610°C is reached. The loss of weight is justified by a change in heat flow which is visible in

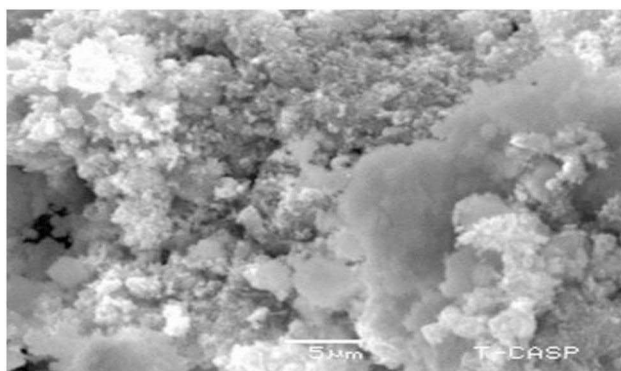


Figure 3 SEM image of ZnO NPs.

Abbreviations: ZnO, zinc oxide; NPs, nanoparticle size; SEM, scanning electron microscope.

Figure 4, due to phase transformation and the observed sudden rise in heat flow. After a sudden decrease in weight, it is observed that the product remains stable until 610°C.^{43,44}

X-Ray Diffraction

The XRD pattern of ZnO nanoparticles is shown in **Figure 5**. XRD peaks are observed at 31.76°, 34.44°, 36.22°, 47.52°, 56.22° and 62.88°. X-ray diffraction pattern of ZnO in **Figure 5**, indicates nanoparticles with strong diffraction peaks of 31.67°, 34.28°, 36.05°, 47.4°, 56.34°, 62.49°, 66.02°, 67.7° and 68.8°. These correspond to the HKL values of 100, 100, 002, 101, 102, 111, 110, 103, 200, 112 and 201. The strong peaks correlate with the JCPDS No. 008, 82–1042 and 5–0664, indicating the presence of crystallite structure hexagonal wurtzite.²¹ The Debye and Scherrer equation³ was used to determine the crystallite size, which was approximately 5.36 nm, corresponding to the peak with an HKL value of 100. The Williamson-Hall equation²⁷ calculated the average size which was found to be 74.12 nm. A similar pattern for XRD peaks at the same angles has been reported in the literature.^{17,22} The findings of other research are similar to our findings.⁴⁵

Zeta Sizer

Zeta potential was measured using a NanoPlus HD Common Model No. 601916 instrument. Image for the ZnO is depicted in **Figure 6**. Zeta potential for ZnO was analyzed with a particle size analyser and its results correlated with

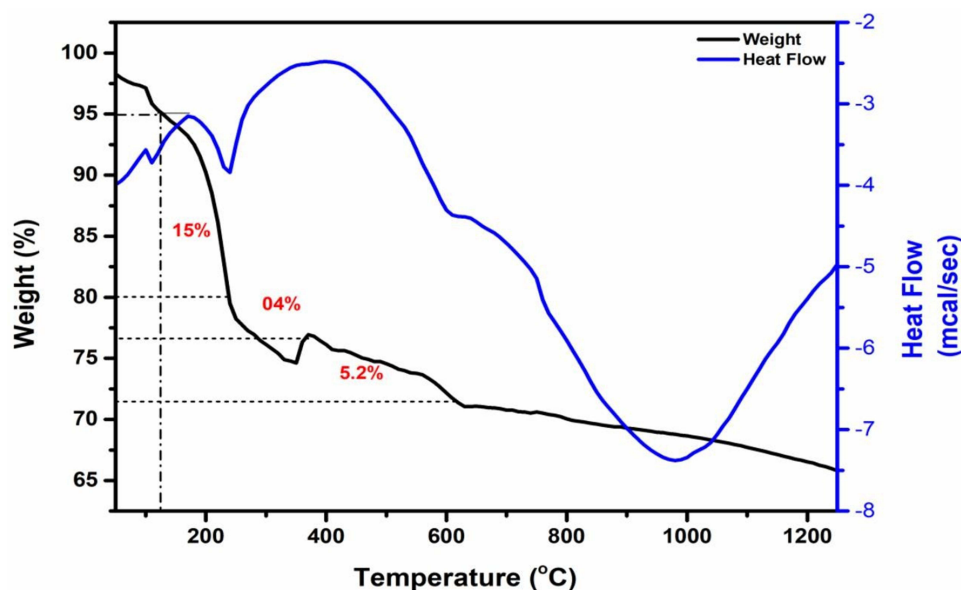


Figure 4 TGA curve for ZnO NPs.

Abbreviations: ZnO, zinc oxide; NPs, nanoparticle size.

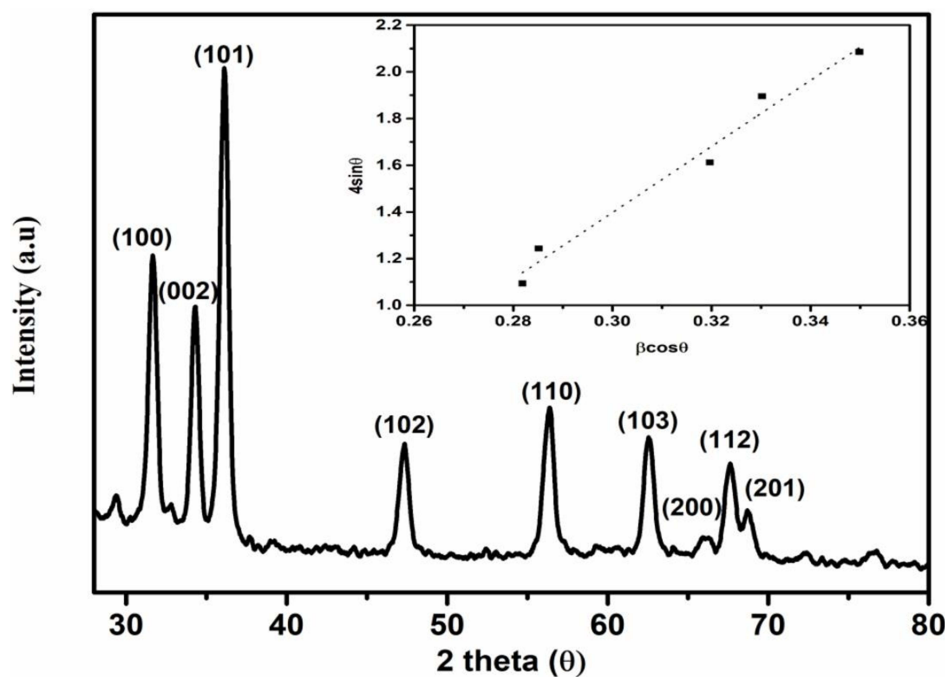


Figure 5 XRD patterns of ZnO nanoparticles.

Abbreviation: ZnO, zinc oxide.

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Intensity Distribution

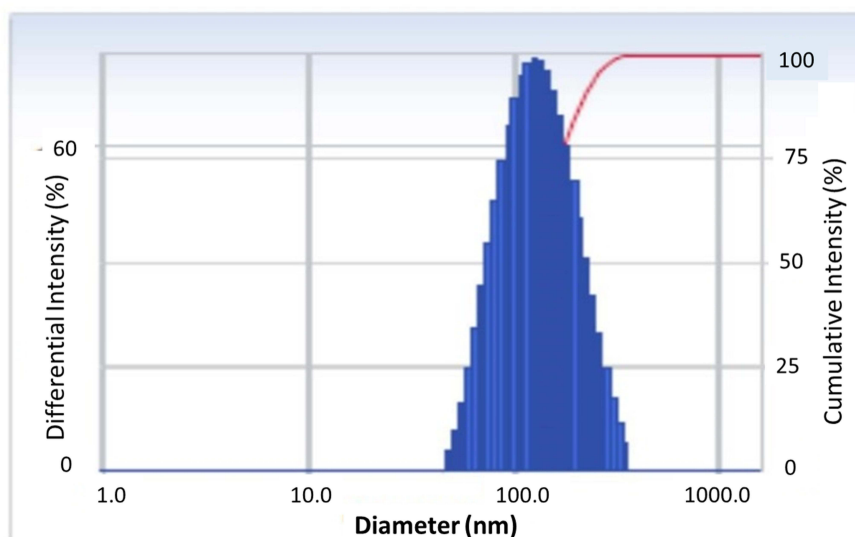


Figure 6 Zeta potential of ZnO nanoparticles.

Abbreviation: ZnO, zinc oxide.

DLS-PSA and reported as -44.95 mV with the mobility $-3.505e^{-0.04}$ cm²/vs. Conversely, its conductivity was 1.4143 mS/cm at a frequency base of 126.4. The data shown are similar to findings in other research.^{44,45}

In-vitro Bio-Accessibility of Nanoparticles

After being suspended in a phosphate buffer saline (PBS) at 0.1 mg/mL, the ZnO particles were fully assimilated in 48 hours. The particle size of nano materials also affected the absorption (cellular uptake) of NPs. The permeability of the

nutrient ZnO NPs passing through the intestine fluid waned with increasing particle size, reaching a cellular cut-off at 500 nm. The nutrient contents varied for zinc nanoparticles from 50 nm to 120 nm, which is a more favorable range for particle size in the intestinal uptake of nano materials. Assimilation results for the release profile of ZnO nanoparticles (Figure 7) were completed in 48 hours for both the standard and synthesized groups. The release profiles for both were, respectively, 43% and 55%. The release profiles of the nutrients were assessed by ICP-OES. The ZnO calculation formula for Zn was measured with the percentage method including all four phases with time intervals of 12, 24, 36, and 48 hrs. Then, the data were reported to be micro gram per liter ($\mu\text{g/L}$).⁴⁶

Absorption Mechanism

The absorption rate rose as the particle size shrank. This is attributed to the larger surface area as the particle size decreases. This would contribute to the highest assimilation or bioavailability differences between the samples, as reported in the data (Figure S1). The subsequent release profile of nano-encapsulated nutrients will increase the bioavailability of nutrient content absorption for in-vitro conditions which depend on particle size. Consequently, nano approaches may be ideal for the least use of nutrient content to attain the best bioavailability as far as in-vitro conditions are concerned.^{46,48}

In-vivo Bioavailability of Metal-Oxide in Blood

The absorption level of ZnO was 4.1 mg/kg compared with the control level of 0.46 mg/kg. Results revealed that nano-encapsulated ZnO exhibited a greater absorption rate than the control sample material with the same treatment (0.07 mg/kg). ZnO at a dose rate of 0.14 mg/kg showed a zinc absorption level in blood of 4.2 mg/kg, whereas at 0.21 mg/kg, an absorption of 4.1 mg/kg was reported. The assimilation level of ZnO with the medium dose rate for the same treatment illustrated a slightly increased response in absorption compared with the low dose rate. Statistical analysis of the data revealed that the coefficient of variance (CV) value was 10.0. The effect of ZnO at low, medium and high dose rates (0.07 mg/kg, T1D1, 0.14 mg/kg, T2D2; and 0.21 mg/kg, T3D3, respectively) illustrated that T1D3 outperformed the other experimental treatments, followed by T1D1. T2D3 and T2D1 were statistically non-significant relative to T1D1, while T3D3 was inferior to the other treatments in the experimental study.^{46,47}

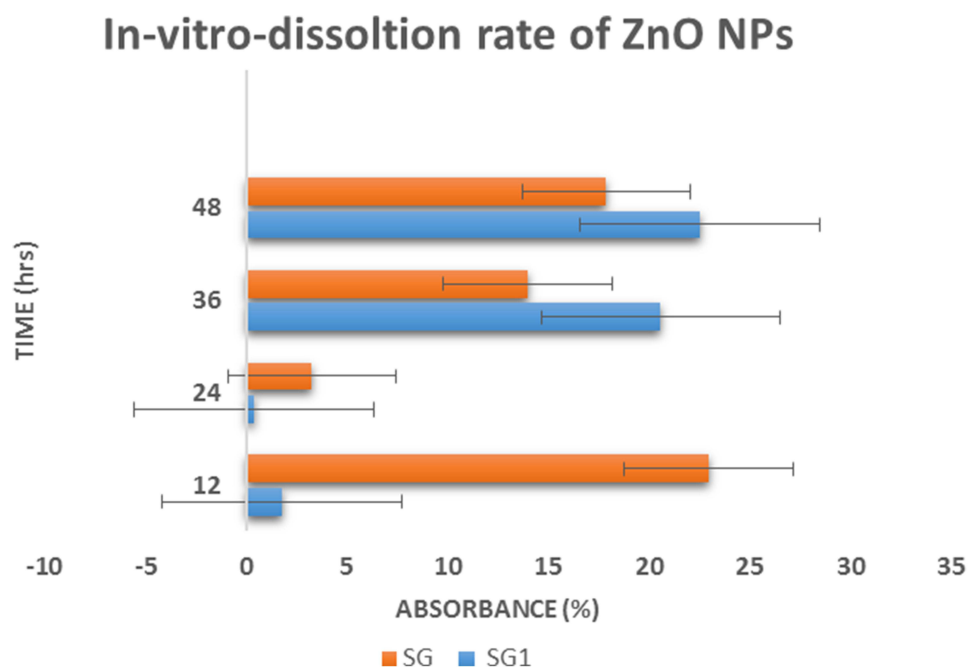


Figure 7 Graphical illustration for ZnO NPs in vitro bioavailability.

Abbreviations: ZnO, zinc oxide; NPs, nanoparticle size.

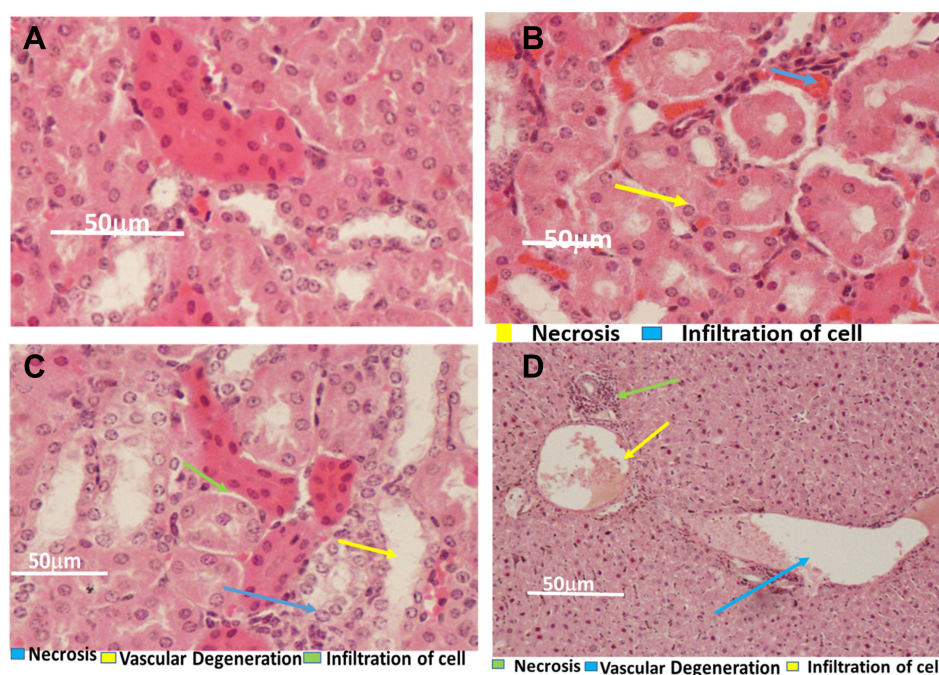


Figure 8 Image by Olympus CX-41, binocular microscope. Liver tissues showing different levels of toxicity with (A) control, (B) treated with ZnO at the dose of 0.07 mg/kg, (C) treated with ZnO at the dose of 0.14 mg/kg, (D) treated with ZnO at the dose of 0.21 mg/kg. Changes were noted in the form of mononuclear cellular infiltration, mild vascular degeneration and necrosis in all treated groups.

Abbreviations: ZnO, zinc oxide; mg, mille gam; Kg, kilo gram.

In-vivo Toxicological Evaluation of Tissues

The microscopic findings of the animals' liver and spleen tissues, for the same mineral treatment applied at the same dose rate of 0.07 mg/kg over 21 days via oral intake, revealed no significant changes in the tissues (Figure S2). In the case of brain and heart tissues, following a similar mineral treatment with dose rates of 0.14 and 0.21 mg/kg, no toxic effects were observed in the tissues compared with the control group. Microscopic findings for the liver and kidney tissues treated with a similar mineral 0.14 mg/kg dose rate at the same conditions were similar to those for the 0.07 mg/kg dose rate. However, microscopic observations of spleen tissue with the same treatment and dose rate showed mild but negligible pathological changes (Figure 8). Kidney tissues treated with zinc oxide (ZnO) at a dose rate of 0.21 mg/kg under the same conditions indicated mild inflammation in the form of tubular swelling (data presented in Table 1). In contrast, the sections of liver and spleen at a 0.21 mg/kg dose rate suggested moderate vascular degeneration and sub-capsular degeneration of cytoplasm. Remarkable changes were only noted in the liver and spleen, as depicted in Figuresakhyar100@gmail.com 8D 9.^{47,48}

Table 1 Levels of Toxicity in the Organs at Different Dose Rates (mg/kg)

Sr. NO	Organ	Weight (g)	0.07 mg/kg	0.14 mg/kg	0.21 mg/kg
1	Brain	0.45	No significant changes	No significant changes	No significant changes
2	Heart	0.152	No significant changes	No notable changes	No significant changes
3	Liver	1.355	No inflammatory response	No inflammatory response	Infiltration of cells, mild vascular degeneration
4	Kidney	0.176	No significant changes in morphology	No significant changes in morphology	No significant changes
5	Spleen	0.08446	No significant changes	No significant changes	Moderate vascular degeneration, sub capsular degeneration of cytoplasm

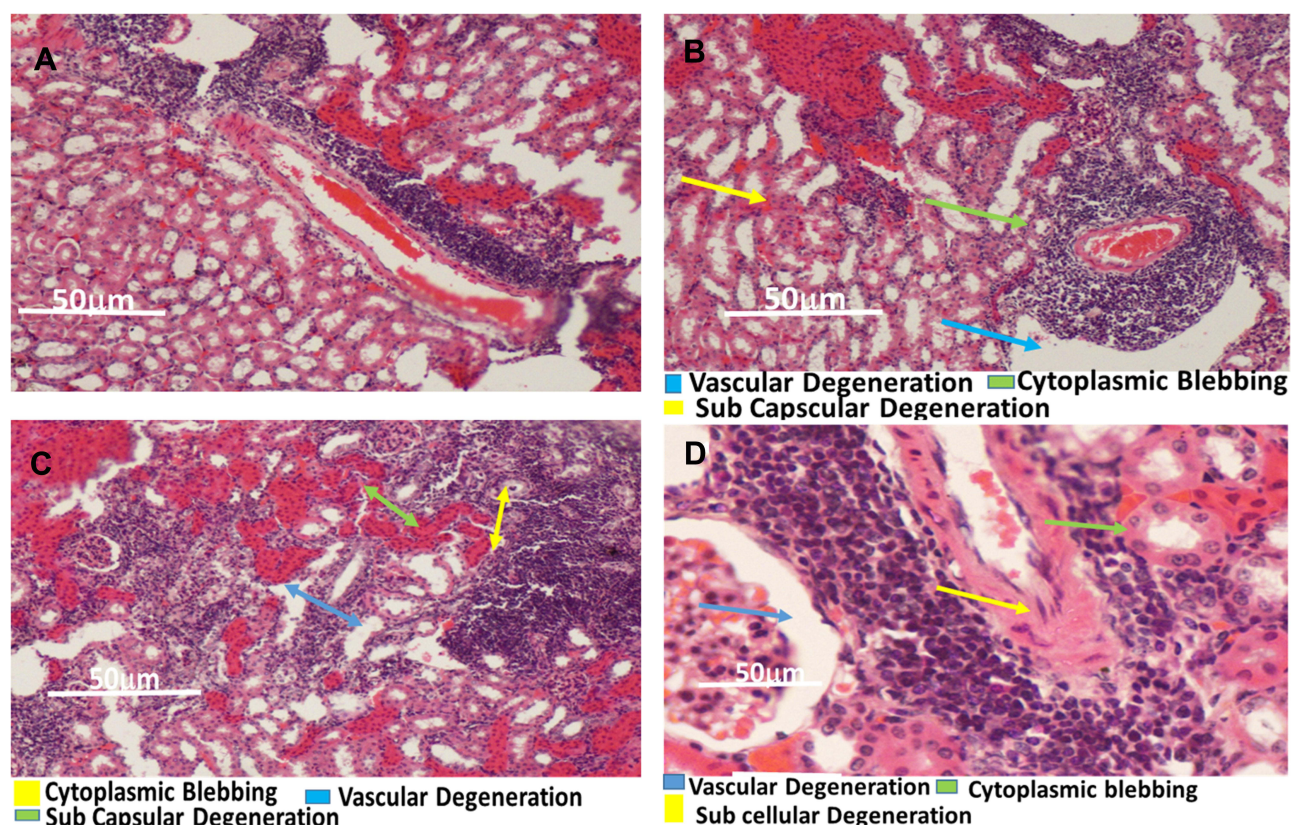


Figure 9 Spleen tissues showing effects at different levels of toxicity with (A) control, (B) treated with ZnO at the dose of 0.07 mg/kg, (C) treated with ZnO at the dose of 0.14 mg/kg, (D) treated with ZnO at the dose of 0.21 mg/kg. Changes were noted in the form of moderate vascular degeneration, cytoplasmic blebbing and sub-capsular degeneration of cytoplasm in all treated groups (H&E, X40 and 5µm section).

Abbreviations: ZnO, zinc oxide; mg, mille gam; Kg, kilo gram.

Conclusion

ZnO is commonly used in cosmetics and medicine, but it is not widely used as a dietary supplement. Zn supplementation from ZnO is a new approach that can achieve the best bio-assimilation. ZnO has the potential to dissolve as much as 14% of Zn in ionic form. Very few studies with ZnO as conventional source have been published, and that is why we chose ZnO as a conventional source to assess in-vitro and in-vivo applications in one study, with the same conditions to generate data for future studies to build on. It was concluded here that in-vitro absorption level of ZnO did vary, and it was further found that with a change of particle size, the assimilation level was 55% for the standard group and 43% for the synthesized group. The difference in assimilation between the two groups was due to the difference in particle size. It was clearly seen that a smaller particle showed more efficacy in terms of absorption in in-vitro conditions. However, in in-vivo assimilation of mineral oxide (ZnO) in blood similar results for low and medium doses emerged, whereas, in-vivo toxicity had some differences for medium and high doses. The toxicity level among animal tissues affected the liver, spleen and kidney the most. Larger doses led to histopathological degeneration in the tissues of the liver when compared to the control. Spleen and kidney tissues micrograph illustrated similar degenerative changes, and a mild level of toxicity was observed. Based on the experimental data the in-vitro study for both standard and synthesized groups and then the in-vivo study, it can be stated here that ZnO supplementation with smaller doses of nutrients containing ZnO could function as a suitable therapeutic application.

Institutional Review Board Statement

The Animal Ethics Committee granted approval to graduate student (Miss Saiqa Bashir) for this study involving animals from the University of Poonch. This study was conducted under the guidelines of Pakistan Animal Scientific Act 1986, which is followed by UVAS animal lab facility at Lahore, Pakistan.

Data Sharing Statement

The data that support the results documented here can be found in a thesis submitted by Saiqa Bashir (Reg. No. 2013-AGRI-235) in the Department of Food Science and Technology, Faculty of Agriculture, University of Poonch Rawalakot Azad Jammu and Kashmir with the title “Synthesis, characterization and application of nano encapsulated metal oxides in apt food materials”.

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Disclosure

The authors declare that they have no conflicts of interest related to this study.

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