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Garlic-mediated green synthesis of ZnO nanoparticles: Characterization, and comparative oral exposure study with bulk zno on body weight, food intake, and hemolytic activity of ZnO in male Albino Rats

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Abstract

Background: Zinc oxide (ZnO) is a widely used industrial material. When reduced to the nanoscale (ZnO NPs), its reactivity and functionality increase but safety concerns arise, particularly after repeated oral exposure. A greener synthesis uses garlic (Allium sativum) extract. Here, ZnO NPs were green-synthesized using garlic extract, characterized by FESEM and UV-Vis, and compared with bulk ZnO (at three dose levels) in male albino rats over 28 days. Body weight, food intake, and ex vivo hemolytic activity were assessed. A separate garlic-only group was included. Results showed that garlic extract neither altered nanoparticulate toxicity nor exhibited a standalone toxic effect.

Objective: This study comparatively evaluated toxicity of bulk ZnO and garlic-synthesized ZnO NPs

Methodology: ZnO NPs were green-synthesized using Allium sativum (garlic) extract and characterized by UV-Vis spectroscopy and FESEM. Male albino rats were allocated into eight groups: control, garlic extract alone, bulk ZnO at 400, 200, and 100 mg/kg, and ZnO NPs at 400, 200, and 100 mg/kg, administered daily via oral gavage for 28 days. Food intake was recorded daily, and body weight was measured weekly. At the end of the exposure period, ex vivo hemolytic activity of both ZnO forms was evaluated on isolated erythrocytes. Data were analyzed using one-way ANOVA followed by LSD post-hoc test ($P \leq 0.05$).

Results: Garlic extract administration (G2) did not produce any observable alteration in feed consumption or body weight. Weekly feed intake was reduced across all groups receiving either bulk or green-synthesized ZnO, with the most pronounced reduction recorded in the high-dose ZnO NPs group (G6), which also exhibited the lowest terminal body weight at week four. Body weight decline was delayed until the fourth week and was confined to groups G3, G6, and G7. In the hemolysis assay, ZnO NPs induced greater erythrocyte lysis than bulk ZnO at every tested concentration, reaching a maximum of 37% versus 26% at the 400 mg dose, with hemolysis demonstrating a clear concentration-dependent relationship.

Conclusion: The present findings demonstrate that repeated oral exposure to ZnO, particularly in its nanoform, induces dose-dependent reductions in feed intake, delayed body weight decline, and concentration-related erythrocyte hemolysis, with the nanoparticulate form eliciting markedly greater toxicity than its bulk counterpart.

Keywords: Zinc oxide nanoparticles; Bulk ZnO; Green synthesis; Allium sativum (Garlic); Body Weight; Food Intake; Hemolytic Activity of ZnO

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1. Introduction

Zinc oxide (ZnO) is an industrially important semiconductor owing to its high electrical conductivity, strong ultraviolet absorption, thermal stability, and moderate antimicrobial activity. These unique physicochemical properties have driven the emergence of ZnO-based materials in various optoelectronic and technological applications [1]. In the broader context of nanotechnology, nanomaterials as a whole exhibit distinctive features that have enabled their incorporation far beyond the pharmaceutical and medical sectors, extending into consumer products such as foods and cosmetics. This widespread use results in human exposure through multiple routes and raises potential health concerns. Notably, nanomaterials have drawn considerable attention for their tendency to accumulate in vital organs and induce adverse effects, with the liver being identified as a primary target of nanoparticle-mediated injury [2].Conventional physical and chemical methods for synthesizing zinc oxide nanoparticles (ZnO NPs) often rely on hazardous reagents or energy-intensive processes that can compromise the biocompatibility of the final product. In response, green synthesis has recently gained significant momentum as an eco-friendly, low-cost, and less toxic alternative. This approach preserves the desired nanoparticle properties while employing plant-derived biocompounds as reducing and stabilizing agents [3,4,5]. Several plant extracts have already been successfully utilized for the biogenic fabrication of ZnO NPs [6,7], demonstrating the versatility and potential of this strategy. In the present study, garlic (Allium sativum) extract was selected as the green synthesis medium, building on the broader evidence that plant extracts can effectively mediate the formation of stable ZnO NPs.

For a reliable toxicological evaluation, body weight and food consumption serve as fundamental, integrative physiological indicators of an organism's overall health and metabolic status. Previous investigations have demonstrated that oral exposure to ZnO NPs can suppress both body weight gain and food intake, effects that are attributed to oxidative stress, systemic inflammation, and hepatic dysfunction [8,9]. In addition to these whole-animal endpoints, the in vitro hemolysis assay is widely accepted as a standard preliminary test for evaluating the cytotoxic potential of blood-contacting materials, providing a sensitive measure of erythrocyte membrane damage [10]. The present study was therefore designed to integrate these specific endpoints body weight, food consumption, and hemolytic activity within a comparative framework to assess the oral toxicity of green-synthesized ZnO NPs versus bulk ZnO in male albino rats.

2. Materials and Methods

2.1. Preparation of Garlic extract (*Allium sativum*)

Aqueous garlic (Allium sativum) extract was prepared following [11] with minor modifications. Fresh garlic bulbs were crushed, filtered through sterile gauze, and the clear filtrate was divided: one portion was used for green synthesis of ZnO nanoparticles, the other was administered orally to a separate rat group (G2). Both portions were stored at a low temperature until use.

2.2. Green Synthesis of Zinc Oxide Nanoparticles (ZnO NPs)

ZnO nanoparticles were green-synthesized using garlic (Allium sativum) extract as a reducing and stabilizing agent, following [12] with minor modifications. Zinc acetate (0.7, 1.5, and 3.0 g, corresponding to the 100, 200, and 400 mg/kg/day doses) was dissolved in 50 mL deionized water, heated to 50–60 °C, and garlic extract was added dropwise until a white precipitate appeared (~30 min). The temperature was then raised to 90–100 °C to reduce the volume to approximately 5 mL of colloidal nanosuspension, which was ultrasonicated for 15 min. The liquid nanosuspension was used for animal dosing, and a portion was oven-dried at 100 °C to obtain ZnO nanopowder for subsequent characterization.

2.3. Preparation of Bulk ZnO Suspension

Bulk ZnO powder (Fluka, Switzerland) was suspended in 5 mL deionized water at 0.250, 0.500, and 1 g (for the 100, 200, and 400 mg/kg/day doses, respectively) and administered orally.

2.4. Characterization of Zinc Oxide Nanoparticles

Synthesized ZnO NPs were characterized by UV-VIS and FESEM; samples were dispatched via the BPC Analysis Center (Baghdad) for analysis in Iran.

2.5. Animals and Experimental Design

Eighty male albino rats (2–3 months, 150–250 g) were housed under standard conditions with free access to water and pelleted diet. After acclimatization, rats were randomly divided into eight groups (n=10): Group 1 (untreated control); Group 2 (garlic extract); Groups 3–5 received bulk ZnO (400, 200, 100 mg/kg/day); Groups 6–8 received green-synthesized ZnO NPs at identical doses. All treatments were given daily by oral gavage for 28 days. Body weight was recorded weekly and food consumption daily..

2.6. Sample Collection

For hemolytic activity assessment, blood was collected via cardiac puncture from one untreated control rat humanely sacrificed under chloroform anesthesia and transferred into EDTA tubes.

2.7. The Hemolytic Activity of Zinc Oxide (ZnO)

Erythrocytes were washed twice with normal saline (10 min, 10,000 × g). A 2% suspension was prepared by adding 2 mL of packed cells to 100 mL of normal saline and was incubated at 37 °C for approximately 20 min. Equal aliquots of 4 mL were transferred into test tubes. The negative control tube received no further addition, while the remaining tubes were each supplemented with 1 mL of the test substance: Triton X-100 (positive control), green-synthesized ZnO NPs, or bulk ZnO, to obtain final concentrations of 100, 200, and 400 µg/mL. The tubes were then centrifuged (6000 × g, 10 min), and the absorbance of the supernatant was measured at 570 nm. The percentage of hemolysis was calculated using the equation: Hemolysis (%) = [(Abs sample – Abs negative) / (Abs positive – Abs negative)] × 100, adapted from [10] with minor modifications; Figure 1.

Figure 1 Hemolytic activity of zinc oxide

2.8. Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS, 2019). One-way analysis of variance (ANOVA) and the least significant difference (LSD) test were applied to compare group means. In the data tables, means within the same column not sharing a common superscript letter are significantly different. For food consumption, significance was set at **P ≤ 0.01, while for body weight, significance was set at *P ≤ 0.05.

3. Results and discussion

3.1. Characterization of Zinc Oxide Nanoparticles

3.1.1. Field Emission Scanning Electron Microscopy (FESEM)

Morphological characterization of the green-synthesized ZnO nanoparticles was performed using a field emission scanning electron microscope FESEM (ZEISS, Germany). Images were acquired at magnifications of A -1.00 k× and B-50.00 k× to examine particle size and surface morphology, as shown in Figure 2.

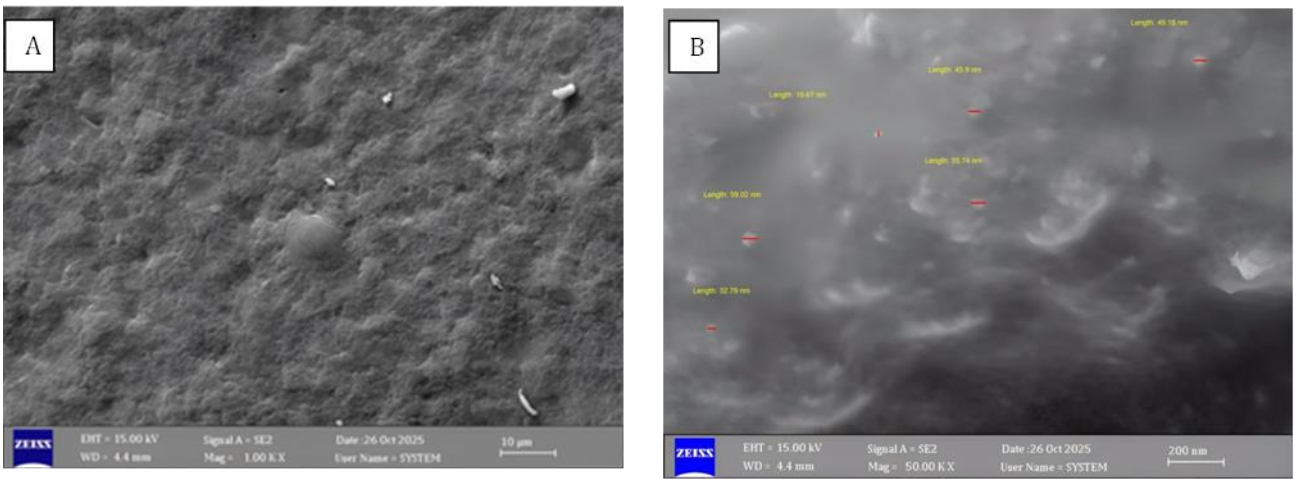

Figure 2 FESEM micrograph of green synthesized ZnO NPs at (1.00 KX and 50.00 KX) magnification

The FESEM images of the biosynthesized ZnO nanoparticles showed a rough, granular surface with agglomerates at low magnification (1.00 KX, scale bar 10 µm), attributed to van der Waals forces and high surface energy (13). At high magnification (50,000×, scale bar 200 nm), quasi-spheroidal nanoparticles within the nanoscale were observed, forming clusters. FESEM confirmed the quasi-spheroidal shape, consistent with the role of phytochemicals as reducing and stabilizing agents. SEM also revealed an irregular surface morphology, in agreement with previous reports (14).

3.2. UV–Vis spectrum of Zinc Oxide Nanoparticles

The UV-Vis spectrum of the green-synthesized ZnO nanoparticles (250–1100 nm) showed a distinct absorption peak at 250 nm, followed by a decrease at 275 nm, then a small secondary peak around 300 nm. Beyond 300 nm, the absorbance gradually declined up to 400 nm, with a low absorbance at 600 nm and a near-flat baseline from 900 nm to 1100 nm as shown in Figure 3.

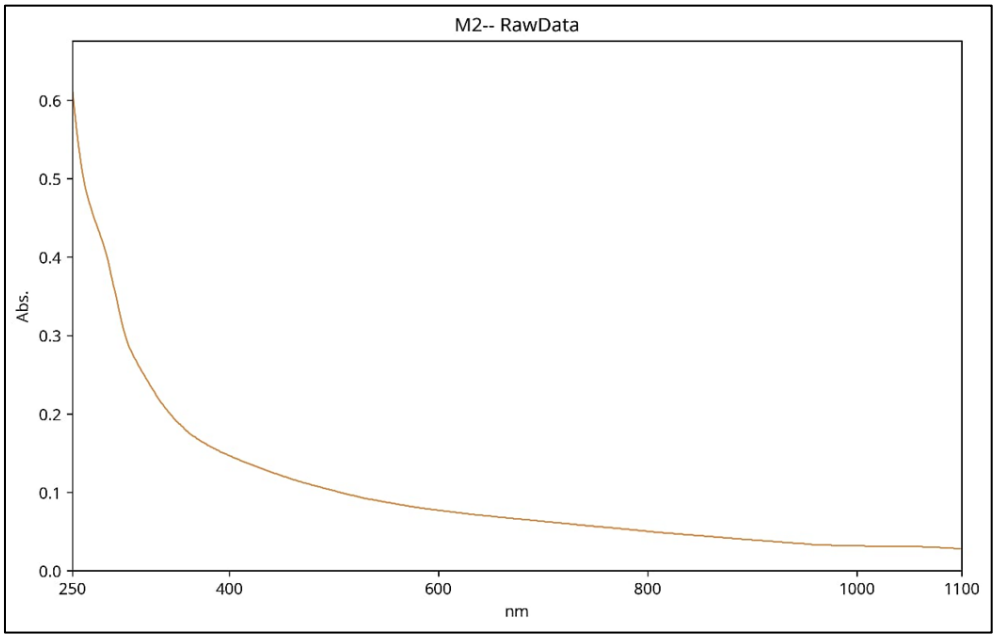

Figure 3 UV–vis spectrum of ZnO NPs biosynthesized from garlic extract.

The peak at 250 nm is attributed to the excitonic transition of very small ZnO nanoparticles, which is blue-shifted due to quantum confinement (15). The small peak around 300 nm falls within the typical absorption range of ZnO nanoparticles (300–380 nm) and confirms the presence of a nanoscale phase (16). The gradual decline in absorbance

beyond 400 nm and the flat baseline indicate good dispersion without aggregation (17). These features are consistent with green-synthesized ultrasmall ZnO nanoparticles (18).

3.3. The Hemolytic Activity of Zinc Oxide (ZnO)

As shown in Table 1, both materials exhibited a concentration-dependent increase in hemolytic activity. At 100 µg/mL, bulk ZnO caused 10% erythrocyte lysis, while ZnO nanoparticles induced 19% lysis. At 200 µg/mL, hemolysis reached 15% for bulk ZnO and 25% for ZnO NPs. At the highest concentration (400 µg/mL), bulk ZnO produced 26% lysis, whereas ZnO NPs resulted in 37% lysis.

Table 1 Hemolytic Activity of Zinc Oxide by bulk and green-synthesized ZnO NPs**.**

Concentration (µg/mL)	Bulk ZnO (%)	ZnO NPs(%)
100	10	19
200	15	25
400	26	37

The concentration-dependent hemolytic activity observed for both bulk and nano ZnO is consistent with the established role of nanoparticle size in membrane disruption (19). The consistently higher lysis induced by the nano-formulation across all tested doses confirms that nanoscale dimensions enhance interaction with the erythrocyte membrane, leading to increased fragility and hemoglobin release. This finding agrees with a comparative study on human red blood cells, which reported that ZnO nanoparticles exhibit greater hemotoxic potential than bulk ZnO (20). Importantly, despite the green synthesis using garlic extract (which contains surface-capping phytochemicals), the ZnO nanoparticles retained significant hemolytic activity, similar to other plant-mediated ZnO nanoparticles (21)(22). Overall, the hemolysis assay serves as a reliable indicator of nanoparticle hemocompatibility (23), and the present results confirm that the green-synthesized ZnO nanoparticles are less hemocompatible than their bulk counterpart, which correlates with their higher in vivo systemic toxicity.

3.4. Body Weight Changes

No significant differences in body weight were observed among all groups during the first three weeks. At Week 4, a significant decrease was observed in G3, G6, and G7 (174.87 ± 1.90, 159.00 ± 5.34, and 180.63 ± 5.87 gm respectively) compared to the control group (212.25 ± 16.22 gm), while no significant differences were noted in G2, G4, G5, and G8 (210.75 ± 11.51, 191.43 ± 7.51, 190.13 ± 6.61, and 187.50 ± 8.03 gm respectively) compared to the control group as shown in Table 2

Table 2 Comparison between difference groups in weekly body weight

Group	Mean ±SE of Body weight (gm)			
	Week1	Week2	Week3	Week4
G1: Control	180.63 ±15.85	191.37 ±15.90	193.87 ±15.24	212.25 ±16.22 a
G2: Garlic ext.	180.63 ±10.78	191.13 ±10.71	193.63 ±11.81	210.75 ±11.51 a
G3: ZNO 400	180.37 ±12.14	189.25 ±15.31	180.00 ±11.71	174.87 ±1.90 bc
G4: ZNO 200	180.25 ±12.44	190.50 ±11.71	183.13 ±5.56	191.43 ±7.51 ab
G5: ZNO 100	180.75 ±6.31	191.25 ±5.68	190.88 ±7.60	190.13 ±6.61 ab
G6: ZNONPS 400	180.50 ±4.87	175.28 ±4.10	164.00 ±4.54	159.00 ±5.34 c
G7: ZNONPS 200	180.37 ±6.37	186.87 ±5.58	194.28 ±6.70	180.63 ±5.87 bc
G8: ZNONPS 100	180.75 ±14.23	190.87 ±13.42	188.50 ±11.54	187.50 ±8.03 abc
L.S.D.	31.298 NS	32.185 NS	30.246 NS	29.455 *
P-value	0.9896	0.9812	0.6269	0.0190
Means having with the different letters in same column differed significantly. * (P≤0.05), NS: Non-Significant.				

Garlic extract (G2) showed no body weight change, confirming its safety (24)(25). At the highest dose, both G3 and G6 reduced weight, with a more pronounced effect in G6, indicating higher nano-toxicity (8)(26)(27). At the intermediate dose, G7 caused significant weight loss, while G4 had no effect (28). At the lowest dose, neither G5 nor G8 produced noticeable changes, suggesting a threshold below which growth is unaffected (29).

3.5. Food Consumption

No significant differences in feed intake were observed during Week 1 except for G6 and G7 (361.14 ± 6.04 and 362.43 ± 4.41 gm, respectively) which showed a significant decrease compared to control (379.00 ± 3.26 gm). No significant changes were noted in G2, G3, G4, G5, and G8 (377.28 ± 1.82, 376.14 ± 2.54, 377.86 ± 3.19, 378.14 ± 3.72, and 375.43 ± 3.17 gm) (Table 3)

In Week 2, a significant decrease was observed in G3, G4, G6, G7, and G8 (362.43 ± 1.25, 376.00 ± 0.82, 331.57 ± 1.56, 368.14 ± 0.91, and 374.29 ± 1.79 gm, respectively) compared to control (379.57 ± 0.65 gm), while G2 and G5 showed no significant change (380.43 ± 0.78 and 377.71 ± 0.68 gm) compared to control

During Week 3, feed intake significantly decreased in G3, G6, and G7 (348.57 ± 1.56, 224.86 ± 5.24, and 346.00 ± 1.15 gm, respectively) relative to control (380.43 ± 1.78 gm), with no significant change in G2, G4, G5, and G8 (380.57 ± 0.99, 373.14 ± 0.91, 375.57 ± 3.82, and 374.14 ± 2.80 gm) compared to control

In Week 4, all treated groups except G2 showed a significant decrease: G3, G4, G5, G6, G7, and G8 (338.57 ± 1.56, 371.14 ± 0.91, 374.86 ± 5.27, 224.00 ± 1.20, 343.14 ± 1.10, and 373.85 ± 2.30 gm, respectively) compared to control (384.43 ± 3.03 gm). G2 (384.00 ± 0.72 gm) did not differ significantly compared to control (Table 3).

Table 3 Comparison between difference groups in weekly feed intake

Group	**Mean ±SE of Feed intake (gm)**				
	Week1	Week2	Week3	Week4	Total
G1: Control	379.00 ±3.26 a	3790.57 ±0.65 a	380.43 ±1.78 a	384.43 ±3.03 a	1623.44 ±6.60 a
G2: Garlic ext.	377.28 ±1.82 a	380.43 ±0.78 a	380.57 ±0.99 a	384.00 ±0.72 a	1522.29 ±2.76 a
G3: ZNO 400	376.14 ±2.54 a	362.43 ±1.25 e	348.57 ±1.56 b	338.57 ±1.56 c	1425.71 ±5.57 c
G4: ZNO 200	377.86 ±3.19 a	376.00 ±0.82 bc	373.14 ±0.91 a	371.14 ±0.91 b	1498.14 ±4.84 b
G5: ZNO 100	378.14 ±3.72 a	377.71 ±0.68 ab	375.57 ±3.82 a	374.86 ±5.27 b	1506.29 ±7.38 ab
G6: ZNONPS 400	361.14 ±6.04 b	331.57 ±1.56 f	224.86 ±5.24 c	224.00 ±1.20 d	1141.57 ±8.82 d
G7: ZNONPS 200	362.43 ±4.41 b	368.14 ±0.91 d	346.00 ±1.15 b	343.14 ±1.10 c	1419.71 ±6.21 c
G8: ZNONPS 100	375.43 ±3.17 a	374.29 ±1.79 c	374.14 ±2.80 a	373.85 ±2.30 b	1497.71 ±9.12 b
L.S.D.	10.565 **	3.211 **	7.702 **	7.018 **	18.89 **
P-value	0.0022	0.0001	0.0001	0.0001	0.0001
Means having with the different letters in same column differed significantly. ** (P≤0.01).					

Garlic extract (G2) did not affect feed intake, confirming its safety (24). At the highest dose, G3 and G6 reduced feed intake, with G6 causing a more severe and earlier decrease (30)(8) (27). At the intermediate dose, G7 consistently reduced intake from week 2 to week 4, whereas G4 only decreased at week 4 (28)(26). At the lowest dose, G5 had no effect, while G8 caused intermittent reductions at weeks 2 and 4 (29).

4. Conclusion

The green-synthesized ZnO nanoparticles were successfully produced using garlic extract, as confirmed by UV-Vis and FESEM analyses, showing ultrasmall, quasi-spheroidal particles with good dispersion. In rats, the nano-form caused significantly greater reductions in body weight and feed intake compared to bulk ZnO, especially at higher doses. The hemolytic activity assay also showed that ZnO nanoparticles induce stronger concentration-dependent erythrocyte

lysis. These results confirm that the nanoscale size and surface reactivity are the main drivers of toxicity, while garlic extract alone is safe and non-toxic.

Recommendations

- Apply strict dose control when using ZnO nanoparticles in biomedical or food-related applications.
- Conduct long-term toxicity studies to assess chronic effects on organs and blood.
- Investigate surface coating strategies to reduce hemolytic activity.
- Perform risk-benefit analyses before using ZnO nanoparticles in products intended for oral exposure.

Compliance with ethical standards

Disclosure of conflict of interest

This study without any conflict of interest.

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