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Assessment of renal toxicity induced by sub-chronic zinc nanoparticle administration

Abhivyakti Pathak[1]*, Seema Agarwal[1] and S.P. Singh[2]

[1]*Department of Veterinary Pathology, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand*
[2]*Department of Veterinary Pharmacology and Toxicology, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand*

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ABSTRACT

Zinc nanoparticles (ZnNPs) are increasingly utilized in biomedical, agricultural, and industrial applications due to their favorable physicochemical properties like melting point, density, boiling point, viscosity, and solubility. However, their potential for inducing organ-specific toxicity warrants comprehensive evaluation. This study aimed to assess the renal toxicity following sub-chronic exposure to ZnNPs in rats. Thirty-five albino rats were grouped into control (G1) and treated (G2) groups, with the treated group receiving zinc oxide nanoparticles at the NOAEL dose (31.25 mg/kg body weight/day) for 90 days. Renal biomarkers, including blood urea nitrogen (BUN), creatinine, and total protein, were evaluated at 30, 60, and 90 days post-treatment (DPT). Significant increases (p<0.05) in creatinine, BUN, and total protein levels were observed in the treatment group compared to controls at all time points. Histopathological examination of kidney tissues revealed interstitial hemorrhage, necrosis of tubular epithelium, leukocytic infiltration, and detachment of tubular epithelial cells in treated rats, while control animals showed no lesions. These findings indicate that sub-chronic administration of ZnNPs induces progressive renal damage, likely mediated by oxidative stress and inflammatory responses. The study underscores the need for regulatory evaluation and risk assessment of ZnNP exposure to ensure their safe application in consumer and therapeutic products.

1. Introduction

Nano zinc, a form of zinc engineered at the nanoscale, has emerged as a multipurpose biomaterial with significant applications in medicine, agriculture, and industry (Gulab et al., 2024). Nano zinc, owing to their specialized physicochemical properties, has significant attention across various industrial and biomedical applications (Lebaka et al., 2025). Their physical properties include small size and increased surface area contribute to enhanced reactivity and potential biological interactions, raising concerns about their

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* **Corresponding author E-mail addresses:** abhivyaktipathak@gmail.com (Abhivyakti Pathak)

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safety profiles, particularly regarding organ systems like the kidneys (Goswami et al., 2024). Renal toxicity assessment is crucial due to the kidneys' role in filtration, excretion, and maintenance of fluid and electrolyte balance (Caiati et al., 2025). Understanding the impact of zinc nanoparticles on renal function requires meticulous investigation, considering both acute and chronic exposure scenarios (Nag et al., 2024). While acute exposure studies provide insights into immediate effects, subchronic exposure studies, spanning weeks to months, are essential for assessing prolonged exposure effects that may manifest gradually over time (Havelikar et al., 2024). Now a day, the rapid expansion in nanotechnology has spurred advancements in many fields, including biomedicine, electronics, and environmental science (Emeihe et al., 2024). However, alongside these advancements, concerns about the potential adverse health effects of nanoparticles have intensified (Tiwari et al., 2024). The kidneys, as primary filtration organs, are particularly susceptible to nanoparticle accumulation and subsequent toxicity (Meng et al., 2025). Therefore, elucidating the mechanisms and extent of renal failure caused by zinc nanoparticles is pivotal for ensuring their safe use and mitigating potential health risks (Shang et al., 2024). Previous research indicates that nanoparticles can translocate through tissue barriers and deposit in vital body organs, like liver and kidneys, altering cellular functions and inducing oxidative stress (Umair et al., 2024). Oxidative stress occurs by excessive production of reactive oxygen species (ROS) and failure of antioxidant defenses mechanism, is a common pathway through which nanoparticles exert toxicity in renal tissues (Patel et al., 2024). Physicochemical properties of Zn nanoparticles, including surface charge, coating, and size, alter their interaction with renal cells and tissues, modulating their toxicological outcomes (Adil et al., 2025). The current study aims to contribute to existing knowledge by comprehensively evaluating the renal effects of zinc nanoparticles following subchronic exposure. Through a systematic experimental approach, incorporating biochemical assays and histopathological examinations, this research seeks to identify biomarkers of renal injury and elucidate underlying mechanisms of nanoparticle-induced nephrotoxicity. Such insights are critical for establishing safe exposure limits, designing effective protective strategies, and guiding regulatory policies concerning nanoparticle applications.

2. Materials and methods

2.1. Ethical committee clearance

The experimental work was approved by the Institutional Animal Ethics Committee, Vide No. "IAEC/CVASc/VPT/307 dated February 25, 2015 and conducted according to guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.2. Experimental animals

Thirty-five albino rats, six weeks aged, and weighing between 150 to 200 grams comprising both males and females sex were procured from the Indian Veterinary Research Institute (IVRI), situated in Bareilly, India. Animals were acclimatized in plastic cages for two weeks before the experiment in the laboratory animal house of the institution. Throughout the trial, rats were given fresh water and standard feed.

2.3. Experimental design

The animals were randomly grouped into two groups: control group (G1) and treatment group (G2), G1 containing of 20 rats and G2 containing of 15 rats. In G1 group giving water, while Group 2 administers nano zinc particles (procured from Sisco Research Laboratories Pvt. Ltd) at the No Observable Adverse Effect Level (NOAEL) dosage of 31.25 mg/kg body weight /day. This treatment was administered from the first day of the experiment and continued for 90 days.

2.4. Samples collection

Blood was collected during morning hours from five animals from each group at 0^{th} (only from control group), 30^{th}, 60^{th} and 90^{th} DPT and after that separate serum for biochemical studies.

2.5. Serum enzyme profile

Serum separated from the collected blood samples was utilized for the analysis of serum enzymes. Commercial diagnostic kits from Erba Diagnostics Mannheim Ltd., Baddi, District Solan (Himachal Pradesh), India, were employed to estimate serum total protein, blood urea nitrogen, and creatinine, following the instructions provided in the kit protocol.

2.6. Gross and microscopic histopathological examination

After completion of study period all animals were examine for external clinical examination and sacrificed by following standard ethical guideline. All the rats were

subjected to detailed post mortem examination and the gross lesions were recorded on 0th, 30th, 60th and 90th DPT. For histopathological studies, representative samples from kidney was collected in Neutral Buffered Formalin (NBF) and processed according to Joseph et al. (2006).

2.7. Statistical Analysis

The experimental data were statistically analyzed using standard procedures outlined by Snedecor and Cochran (1994), with the assistance of SPSS software version 23.0. The data were evaluated using one-way Analysis of variance (ANOVA) and the two-sample t-test.

3. Results

The data regarding renal toxicity effect on various enzymes of zinc nanoparticle treatments after 30th 60th and 90th day in rats are presented in Table 1. Experimental rat's shows mean serum BUN values in different groups at different intervals. There was significant difference in mean serum BUN at 30th 60th and 90th DPT. The mean serum BUN values of treated group at 30th 60th and 90th DPT were 29.90, 30.81 % and 20.82 %, respectively higher than the control group animals. A significant (p<0.05) increase in the activity of creatinine in zinc nanoparticle treated rats as compared to control group was found at different intervals. There was significant difference in mean serum creatinine at 30th 60th and 90th DPT. The mean serum creatinine value of treated group at 30th 60th and 90th DPT was 0.621, 0.851 and 1.081, respectively higher than the control group animals. A significant (p<0.05) increase in the activity of total serum protein in zinc nanoparticle treated rats as compared to control group was found at different time intervals. There was significant difference in mean total serum protein at 30th 60th and 90th DPT. The mean total serum protein value of treated group at 30th 60th and 90th DPT was 7.07, 12.85 and 9.81, respectively greater than the animals of control group.

Table 1: The toxicological effect of zinc nanoparticles on blood urea nitrogen (BUN)

Blood Urea Nitrogen (mg/dl)		
Day Post Treatment	**I Control**	**II Treated**
0	25.45±2.5^{c}	25.45±2.5^{c}
30	27.15±4.41^{a}	29.90±3.49^{b}
60	16.31±2.36^{b}	30.81±2.65^{a}
90	11.07±3.15^{b}	20.82±3.82^{a}

Values are expressed as Mean ± SE, different superscript letters (a, b, and c) within the same row indicate a statistically significant difference (p<0.05) when compared horizontally.

Table 2: The toxicological effect of zinc nanoparticles on creatinine

Creatinine m(g/dl)		
Day Post Treatment	**Group I**	**Group II**
0	0.213±0.02^{c}	0.213±0.02^{c}
30	0.301±0.02^{a}	0.621±0.02^{b}
60	0.318±0.02^{b}	0.851±0.06^{a}
90	0.343±0.02^{b}	1.081±0.08^{a}

Values are expressed as Mean ± SE, different superscript letters (a, b, and c) within the same row indicate a statistically significant difference (p<0.05) when compared horizontally.

Table 3: The toxicological effect of zinc nanoparticles on serum total protein

Total serum protein (g/dl)		
Day Post Treatment	**Group I**	**Group II**
0	5.59±0.98^{c}	5.59±0.98^{c}
30	6.39±1.04^{a}	7.07±0.82^{b}
60	5.01±0.31^{b}	12.85±0.89^{a}
90	7.22±0.56^{b}	9.81±0.71^{a}

Values are expressed as Mean ± SE, different superscript letters (a, b, and c) within the same row indicate a statistically significant difference (p<0.05) when compared horizontally.

Gross pathological examination of rat kidneys from different groups was conducted at various time intervals, and the observed pathological changes were documented. In the zinc nanoparticle-treated group, kidneys exhibited interstitial hemorrhage, necrosis of tubular epithelium, and leukocytic infiltration in both the glomeruli and interstitial spaces (Fig. 1). In contrast, no histopathological alterations were noted in the control group. By the 60th day post-treatment (DPT), kidney sections from the nano zinc treated group showed necrotic alterations in tubular epithelium, interstitial hemorrhage, leukocytic infiltration in interstitial spaces and glomeruli, along with detachment of the tubular epithelium from the basement membrane (Fig. 2), while the control group remained free of such lesions. Similarly, at 90th DPT, the treated group continued to display interstitial hemorrhage, necrosis in tubular epithelium, leukocytic infiltration, and epithelial detachment from the basement membrane (Fig. 3), however, no histopathological alteration were showed in the control group animals.

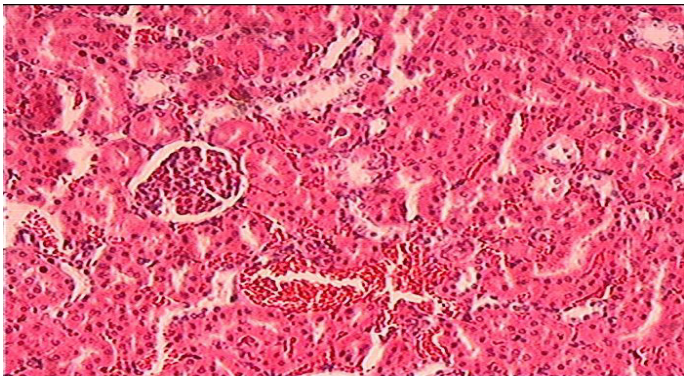

Fig. 1: Photomicrograph of kidney at 30th DPT showing necrosis in tubular epithelium, interstitial hemorrhage, infiltration of leucocyte in the interstitial spaces and glomeruli (Group II, Magnification 100×)

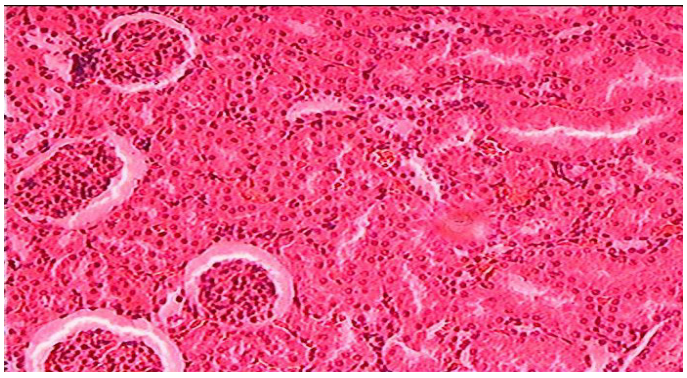

Fig. 2: Photomicrograph of kidney at 60th DPT showing necrosis in tubular epithelium, hemorrhages in interstitial, leucocyte infiltration in interstitial spaces and glomeruli and detachment of tubular epithelium from basement membrane (Group II, Magnification 100×)

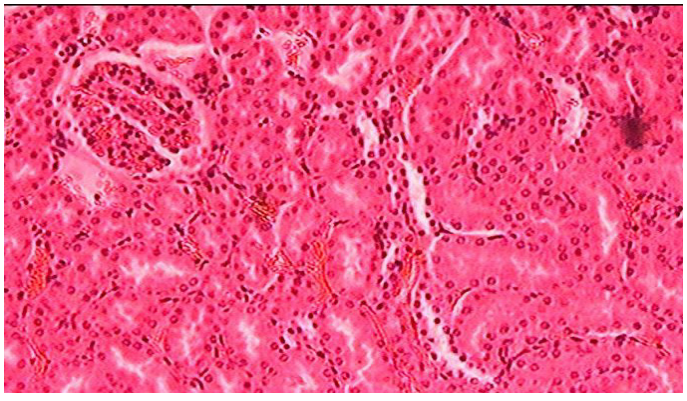

Fig. 3: Photomicrograph of kidney at 90th DPT showing necrosis in tubular epithelium, interstitial hemorrhage, infiltration of leucocytes in the interstitial spaces and glomeruli and detachment of tubular epithelium from basement membrane (Group II, Magnification 100×)

4. Discussion

Nano zinc oxides damaged the organ such as lung, liver and pancreas and decrease their relative weight in rats (Ramadan et al., 2022; Pathak et al., 2022). The current experiment revealed renal toxicity by the significant alteration of various biochemical parameters like increase in BUN, creatinine and total protein value in nano zinc treatment group. Our results is similar with Alqahtani et al. (2024) studied zinc oxide treatment in rats and found rats increased level of liver enzymes named BUN, creatinine and total protein in nano zinc oxide treated group as compare to control group. Histopathological examination confirms lesions in kidneys of treatment group. Kidney showed detachment of tubular epithelium, hemorrhage in the interstitial space, necrosis in tubular epithelium, and infiltration of leucocytes in interstitial spaces and glomeruli. Our results are similar with Faddah et al. (2012) studied zinc oxide treatment in rats and found necrosis, degeneration and epithelial desquamation. Severe congestion was found in the renal interstitial space. Renal tubules also showed some casts in the lumen. The biodistribution analysis demonstrated widespread distribution of ZnNPs in various organs, with highest accumulation observed in the liver and kidneys (El-Shenawy et al., 2023). This accumulation pattern is consistent with the observed organ-specific toxicity and highlights the importance of nanoparticle size and surface characteristics in determining their biological fate (Jin et al., 2025). Furthermore, the inflammatory response triggered by ZnNPs, evidenced by elevated cytokine levels (e.g., TNF-alpha, IL-6), implicates immune system activation in nanoparticle-induced toxicity pathways (Sau et al., 2024). Such immune activation may exacerbate tissue damage and contribute to the overall toxicological profile observed (Min et al., 2023).

5. Conclusion

The results of the present experiment clearly demonstrate that sub-chronic exposure to zinc oxide nanoparticles at the NOAEL dose induces significant renal toxicity in Wistar rats. Elevated levels of serum BUN, creatinine, and total protein indicate impaired renal function, while histopathological alterations such as interstitial hemorrhage, tubular necrosis, leukocytic infiltration, and epithelial detachment confirm structural kidney damage. These results suggest that even at doses considered non-toxic by conventional standards, prolonged exposure to ZnNPs can lead to progressive nephrotoxicity. The observed renal lesions highlight the potential risk linked with the widespread application of ZnNPs in industrial and biomedical applications. Therefore, a thorough evaluation of their long-term toxicological effects is essential. This study provides critical data to support the development of regulatory guidelines for the safe use of zinc nanoparticles and emphasizes the importance of continued research into nanoparticle-induced organ-specific toxicity.

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Conflicts of interest and financial disclosures

The authors state that there are no conflicts of interest to disclose.

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