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Effect of orally administered silver nanoparticles on histological alterations in Wistar rats

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ABSTRACT

The present study aimed to evaluate the histopathological effects of orally administered silver nanoparticles (AgNPs) at the No-Observed-Adverse-Effect Level (NOAEL) in Wistar rats over a 90 days period. Thirty-five healthy, six-weeks-old Wistar rats of both sexes were randomly divided into two groups: Group I (control, n=20) and Group II (treatment, n=15). Rats in Group II received AgNPs suspended in distilled water at a NOAEL dose of 30 mg/kg body weight/day via oral gavage for 90 consecutive days. Five rats from each group were sacrificed at 0 (only from Group I), 30th, 60th, and 90th days post-treatment (DPT), and tissue samples were collected for gross and histopathological examination. No lesions were observed in any organs of the control group throughout the study. In contrast, treated rats exhibited gross lesions such as red patches on the liver, discoloration in the lungs, and mild cardiac congestion. Histologically, the liver showed congestion, thrombus formation, and hepatocellular degeneration; lungs revealed thickened interalveolar walls, emphysema, congestion, and increased mononuclear cell infiltration. Kidneys demonstrated congestion, interstitial hemorrhages, and coagulative necrosis of tubular epithelial cells. Other organs including the spleen, uterus, and testis did not show any significant lesions. These findings indicate that chronic oral exposure to nanosilver, even at NOAEL levels, can induce adverse histopathological changes in vital organs of Wistar rats.

1. Introduction

Nanoparticles have become increasingly valuable in medicine because of their distinctive physical and chemical characteristics, making them extremely valuable in the development of novel drugs and therapeutic applications (Zhang and Saltzman, 2013). The rapid expansion of nanotechnology in daily life, driven by its numerous benefits, has simultaneously raised significant concerns regarding its potential health hazards (Ema et al., 2010). The toxicological implications of nanoparticles are not limited to industrial or medical settings; environmental pathways such as water, soil, and air can also serve as routes of nanoparticle exposure, facilitating their entry into animal and human systems.

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Humans and animals are routinely and often unknowingly exposed to both naturally occurring and synthetic nanoparticles present in their surroundings. Studies have demonstrated that nanoparticles can be absorbed through various routes, where they may exert toxic effects. Among metallic nanoparticles (MNPs), AgNPs are extensively used across multiple sectors including medicine, food processing, healthcare, consumer products, and industry due to their strong antimicrobial activity and desirable physicochemical characteristics (Natsuki et al., 2015). Silver, particularly in its ionic or soluble compound form, has long been known for its antimicrobial efficacy (Drake and Hazelwood, 2005). However, exposure to silver especially at higher levels has also been associated with toxicity in humans and animals, with adverse effects on various organs (Skalska and Strużyńska, 2015). Individuals working in silver-related industries such as mining, manufacturing, and packaging are particularly at risk of such toxicities (Al Gurabi et al., 2015). AgNPs exert toxicity primarily through oxidative stress, mitochondrial dysfunction, and disruption of cellular integrity. They generate reactive oxygen species, causing lipid peroxidation, DNA damage, and protein denaturation (Choudhary et al., 2022). AgNPs also bind to thiol groups in enzymes, impairing metabolic pathways and antioxidant defenses. Mitochondrial impairment leads to decreased ATP production and apoptosis. Additionally, silver disrupts cell membrane integrity and induces inflammatory responses via cytokine release (Martínez et al., 2024). Bioaccumulation in organs such as the liver, kidneys, and lungs further contributes to organ-specific toxicity (Wang et al., 2024). In light of these concerns, the present study was designed to investigate the potential histopathological effects of silver nanoparticles when administered orally at a NOAEL dose over a period of 90 days in Wistar rats.

2. Materials and methods

A total of 35 apparently healthy Wistar rats (six weeks old) of both sexes were procured from the Laboratory Animal Resources Division, Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, India. The animals were housed in the Experimental Animal Facility of the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, and maintained under standard laboratory conditions with free access to food and water. The study protocol was reviewed and approved by the Institutional Animal Ethics Committee of the university. Following a seven-day acclimatization period, the rats were randomly divided into two groups: Group I (control; n = 20) and Group II (treatment; n = 15). Silver nitrate nanoparticles with a particle size of less than 90 nm and a molecular weight of 107.87 g/mol were obtained from Sisco Research Laboratories Pvt. Ltd., India. A fresh suspension of nanoparticles in distilled water was prepared daily. The suspension was homogenized using a sonicator prior to administration. Rats in Group II were administered silver nanoparticles orally via gavage at a NOAEL dose of 30 mg/kg body weight/day for 90 consecutive days, as recommended by Kim et al. (2010). The study design, including the number of animals sacrificed at each interval, is detailed in Table 1.

Table 1: Tabular form of experimental design

Days post treatment	Control group (GI)	Treatment group (G2) (Nano silvernitrite)
0	5 Rats	-
30th	5 Rats	5 Rats
60th	5 Rats	5 Rats
90th	5 Rats	5 Rats
Total	20 Rats	15 Rats

2.1. Pathological studies

At 0 days post-treatment (DPT), five rats from Group I (control) were randomly selected and humanely sacrificed following standard ethical guidelines. Subsequently, five rats from each group were sacrificed at 30th, 60th, and 90th DPT. All animals underwent thorough post-mortem examinations, and any gross pathological changes were carefully documented. For histopathological evaluation, tissue samples were collected from the liver, lungs, kidneys, spleen, heart, testes, and uterus, and preserved in 10% neutral buffered formalin. The preserved tissues were processed for microscopic examination following the standard histological procedures outlined by Luna (1968).

3. Results

3.1. Gross Pathological studies

No visible lesions were detected in the organs of rats from Group I. In contrast, the livers of Group II rats exhibited a diffuse pinkish discoloration on both surfaces of all lobes at 30th, 60th, and 90th days post-treatment (DPT). Emphysematous changes were observed in the lungs of Group II rats at 30th DPT, while at 60th and 90th DPT, some rats showed pronounced red discoloration across all lung lobes. The kidneys of treated rats also displayed a diffuse pinkish discoloration at 30th DPT. No notable gross pathological changes were observed in the spleen, heart, testes, and uterus of Group II rats at any time point.

3.2. Histopathological studies

No histopathological abnormalities were detected in any organs of Group I rats throughout the duration of the study.

Liver

Histopathological examination of liver in nanosilver treated rats at 30th DPT revealed congestion of large blood vessels throughout the parenchyma (Fig. 1), thrombus formation in one large vein, congestion of central veins at many places, congestion of sinusoidal spaces throughout the parenchyma (Fig. 2) and degeneration of hepatocytes throughout the parenchyma, while at other places there was shrinkage of hepatocytes leading to increase in sinusoidal spaces. At 60th DPT, liver revealed severe congestion of majority of central veins throughout the parenchyma. At places, parenchyma showed shrinkage of the hepatocytes leading to increase in sinusoidal spaces. At 90th DPT, liver revealed severe congestion of central veins throughout the parenchyma, coagulative necrosis of hepatocytes at places.

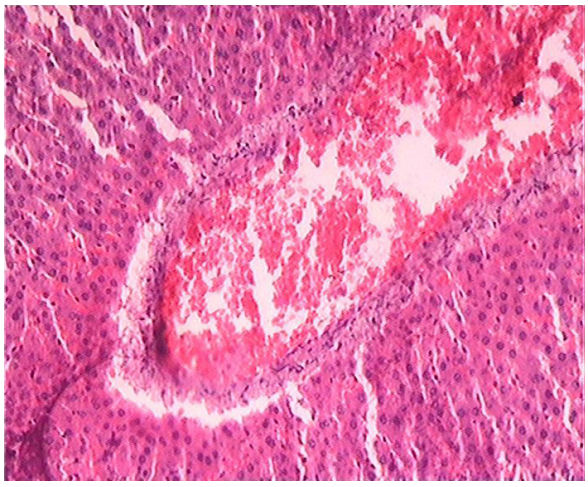

Fig. 1: Photomicrograph of liver showing congestion of large vein. Group II, 30th DPT, HE x 200

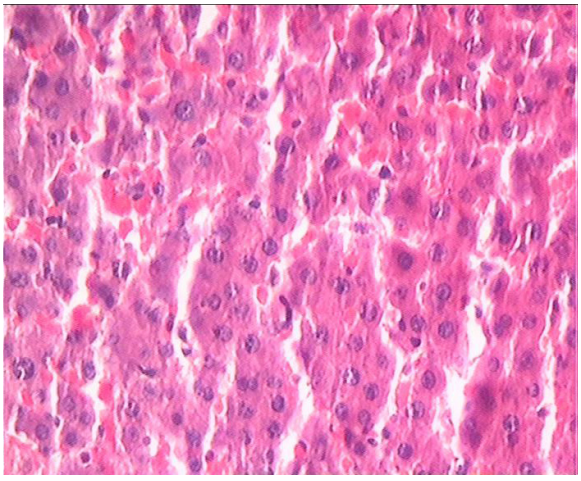

Fig. 2: Photomicrograph of liver showing congestion of sinusoids. Group II, 30th DPT, HE x 200

Lungs

Histopathological examination of lungs in nanosilver treated rats at 30th DPT revealed thickening of the interalobular wall throughout the parenchyma due to congestion of interalveolar wall capillaries, presence of few mononuclear cells, congestion of large blood vessels and emphysema at many places. Many bronchioles of the lungs contained exudate, desquamated epithelium, and few mononuclear cells (Fig. 3). In many bronchioles there was desquamation of epithelium. At 60th DPT, lung revealed thickening of interalveolar septa due to infiltration of mononuclear cells (Fig. 4) and congestion of the interalveolar wall capillaries. At 90th DPT, lung revealed severe thickening of interalveolar septal capillaries due to severe congestion. At places there was severe emphysema and formation of giant alveoli.

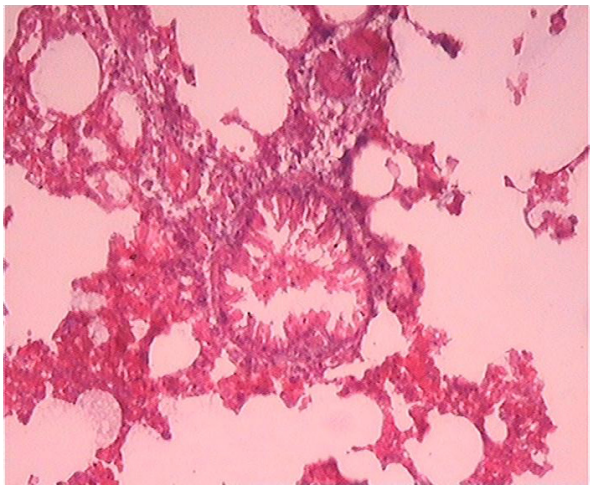

Fig. 3: Photomicrograph of lung showing thickening of the interalveolar wall due to congestion of capillaries throughout the parenchyma, and congestion of large blood vessels, bronchioles showing exudates, mononuclear cells and desquamation of epithelium. Group II, 30th DPT, HE x 100

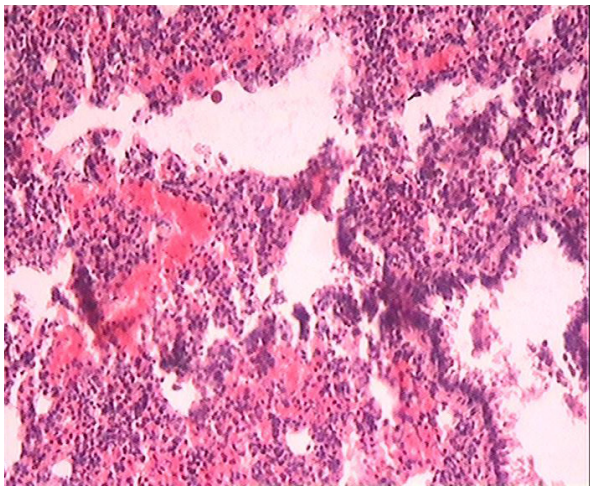

Fig. 4: Photomicrograph of lung showing thickening of interalveolar septa due to infiltration of mononuclear cells and congestion of the intralobular capillaries. Group II, 60th DPT, HE x 100

Kidneys

Histopathology of kidney in nanosilver treated rats at 30th DPT revealed congestion of glomerular capillaries and interstitial haemorrhages throughout the parenchyma and coagulative necrosis in kidney tubular epithelial cells in many of tubules throughout the parenchyma (Fig. 5). At 60th DPT, kidneys revealed congestion of glomerular capillaries, presence of necrosis in most tubules throughout the parenchyma and shrinkage of glomeruli at places. At many places, there were interstitial haemorrhages in the kidney (Fig. 6). At 90th DPT, kidney revealed shrinkage of glomerulus at majority of places throughout the parenchyma leading to increase in Bowman's space, degeneration, and necrosis of many of the kidney tubular epithelial cells in majority the tubule throughout parenchyma and interstitial haemorrhages at many places.

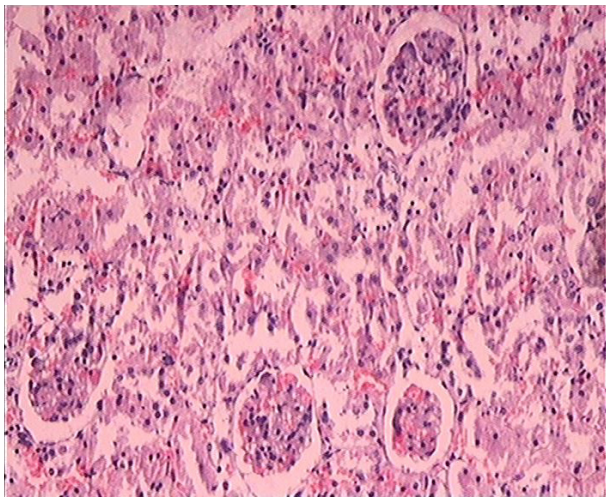

Fig. 5: Photomicrograph of kidney showing congestion of glomerular capillaries, presence of necrosis of epithelial cells in most of tubules throughout the parenchyma and interstitial hemorrhages. Group II, 30th DPT, HE x 100

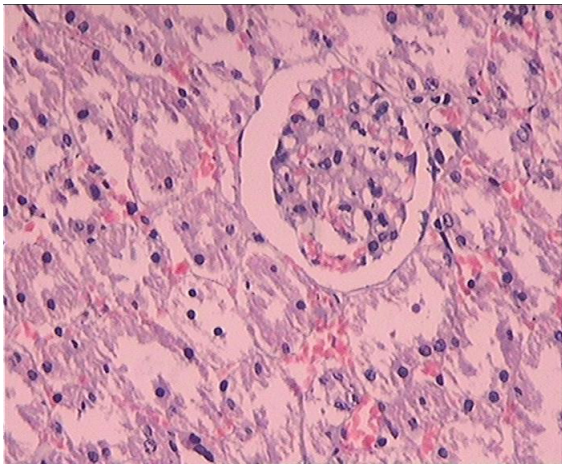

Fig. 6: Photomicrograph of kidney showing congestion of glomerular capillaries, interstitial hemorrhage, and coagulative necrosis in kidney tubular epithelial cells in many of tubules. Group II, 60th DPT, HE x 200.

No histopathological lesion was present in the spleen and testes of group II rats at any interval of experimentation. Uterus in group II rats, at 30th DPT, revealed mild to moderate congestion of large blood vessels in myometrium. Heart, in group II, at 60th DPT, manifested slight edema in between the muscle fibre bundles. While at 90th DPT, heart of group II rats showed mild congestion in blood vessels in myocardium.

4. Discussion

Examination of various organs was done at every 30-day interval of experiment till 90th day. Grossly, major pathological lesions were seen in liver and lungs. Liver showed presence of few red patches at 30th, 60th and 90th DPT. Presence of red patches indicate liver injury. This finding is in agreement that of Almansour et al. (2015). Grossly, lungs showed emphysematous lesion at 30th DPT. At 60th and 90th DPT, lungs of treated rats revealed severe red discoloration of all lobes. The silver nanoparticle administration caused severe tissue damage in liver and kidneys of affected organs in comparison to that of control group. The observed inflammation and cellular degeneration align with evidence suggesting that nanoparticles can induce biochemical alterations resulting in cellular injury. Additionally, research indicates that nanoparticles can accumulate in tissues, contributing further to cellular damage (Yang et al., 2008).

Histopathologically, most of the lesions were seen in liver, lungs, and kidneys. Uterus also showed mild microscopic lesions. Liver revealed moderate congestion of large blood vessels, thrombus formation, congestion of central veins, and congestion of sinusoidal spaces and degeneration of hepatocytes. The results of the present study are accordance with that of previous studies with respect to the liver. Liver is the main target organ and the tissue deposition of silver originating from nanoparticles (Kim et al., 2010; Sulaiman et al., 2015). Silver ions have a strong affinity for the thiol groups present in enzymes within the liver and other organs, which can lead to tissue toxicity through accumulation (Baldi et al., 1988). Additionally, Kim et al. (2009) reported that silver nanoparticles can induce liver cell toxicity through mechanisms involving oxidative stress. Lungs showed thickening of the interalobular wall due to congestion of interalveolar wall capillaries, and presence of few mononuclear cells, congestion of large blood vessels, emphysema at many places with formation of giant alveoli. All lesions indicate lung injury. Recent studies have also shown that the application of AgNPs to the rat lung led to transient lung inflammation and congestion (Wiemann

et al., 2017). Kidneys showed congestion of glomerular capillaries, interstitial haemorrhage, and coagulative necrosis in kidney tubular epithelial cells. Kidney is also an important site for nanoparticle deposition. Similar results have been reported by Sulaiman et al., (2015) and Kim et al., (2008). Uterus also showed mild to moderate congestion of large blood vessels in myometrium which might be present because of the various phases of estrous cycle.

5. Conclusion

The present study clearly demonstrates that oral administration of silver nanoparticles at a NOAEL dose of 30 mg/kg body weight/day for 90 days induces significant histopathological changes in vital organs of Wistar rats, particularly the liver, lungs, and kidneys. Microscopic lesions observed in these organs. In liver found vascular congestion, hepatocellular degeneration and necrosis, in lungs alveolar damage, interstitial haemorrhages, and in kidneys tubular necrosis indicate the potential of silver nanoparticles to cause toxicity even at doses considered non-toxic based on conventional assessments. The findings emphasize the need for caution in the long-term use and exposure to nanosilver, especially given its widespread applications in medical, industrial, and consumer products. Further investigations on the biodistribution, accumulation, and mechanistic pathways of silver nanoparticle toxicity are warranted to ensure safe usage and regulatory oversight.

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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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