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Toxicological Effects of Colloidal Silver Nanoparticles on Rat Health: Assessing Physiological, Hematological, Biochemical, and Behavioral Parameters

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Abstract

Introduction: Nanotechnology, a rapidly evolving field, has introduced silver nanoparticles (AgNPs) as promising materials with diverse applications. Their unique properties have revolutionized industries, raising concerns about potential health implications. This study aims to comprehensively evaluate the toxicological effects of AgNPs on rat health through a multidimensional approach, encompassing physiological, hematological, biochemical, and behavioral assessments.

Methodology: Colloidal AgNPs with a minimum purity of 99.98% were synthesized using an inductive coupled plasma (ICP) method. Sixty male specific-pathogen-free (SPF) Wistar rats were exposed to low (10 µg/kg/day) and high (100 µg/kg/day) doses of AgNPs, alongside a control group. Throughout the exposure period, comprehensive assessments, including body weight changes, food intake, water consumption, hematological and biochemical parameters, and behavioral evaluations, were systematically conducted. The statistical analysis employed a combination of one-way ANOVA and Kruskal-Wallis tests for robust data interpretation.

Results: Rats exposed to low (10µg/kg/day) and high (100 µg/kg/day) doses of AgNPs displayed no significant changes in food intake, water consumption, or blood parameters compared to the control group. However, a notable reduction in body weight was observed in the high-dose group, suggesting a potential dose-dependent impact. Biochemical analyses indicated no significant differences in liver and kidney function parameters for the low-dose group, but the high-dose group exhibited potential elevation in liver enzymes, necessitating further scrutiny. Behavioral assessments revealed no significant alterations in open-field behavior or cognitive function, indicating minimal impact on exploratory and cognitive abilities within the tested doses.

Conclusion: AgNPs demonstrated minimal impact on certain parameters, the high-dose group exhibited notable body weight reduction and potential liver enzyme alterations, warranting further investigation. Behavioral assessments indicated no significant cognitive effects. These findings emphasize the importance of continued research for informed regulatory decisions and the safe utilization of AgNPs in consumer products.

Keywords: AgNPs, assessment, colloidal AgNPs effects, cognitive function, liver enzyme alterations, multidimensional assessment, nanotechnology, rat health, regulatory decisions, silver nanoparticles, toxicity

Introduction

Nanotechnology, a rapidly evolving field, has opened up new frontiers in materials science, medicine, and various industrial applications. Among the myriad of nanomaterials, AgNPs have emerged as a promising candidate for their unique physicochemical properties and wide-ranging applications [1, 2]. AgNPs exhibit exceptional electrical, thermal, and antimicrobial properties, making them invaluable in fields as diverse as electronics, textiles, and medicine. Their ever-expanding use, however, raises concerns regarding their potential impact on environmental and human health.The toxicological implications of AgNPs have drawn considerable attention in recent years. Understanding how these nanoparticles interact with biological systems,

especially in the context of mammalian organisms, is crucial for assessing potential health risks [3]. Rats, as a commonly used model in toxicology studies [4], provide an invaluable platform for elucidating the complex interplay between AgNPs and living organisms. AgNPs possess unique physical and chemical properties, such as high surface area, quantum size effects, and increased reactivity, which make them particularly attractive for industrial and biomedical applications. In medicine, AgNPs have been employed for their antimicrobial properties in wound dressings and as carriers for drug delivery systems. In the electronics industry, their conductive properties have revolutionized circuitry. AgNPs are incorporated into various consumer products, ranging from textiles to cosmetics [5-7]. Such widespread use, however, has raised concerns about potential human and environmental exposure.

AgNPs can enter the body through inhalation, ingestion, or dermal contact. Once inside the body, they may accumulate in different tissues, potentially triggering various biological responses [8]. Given their high surface area, AgNPs are more likely to release silver ions (Ag+), which can interact with cellular components. This has raised concerns about possible toxic effects on mammalian systems [9, 10]. Rats serve as extensively utilized model organisms in toxicology studies due to their physiological and genetic similarities to humans and practicality for experimental investigations [11-13]. This research focuses on systematically evaluating the toxicological effects of AgNPs on rat health, encompassing key aspects. Monitoring body weight changes serves as a crucial indicator, providing insights into metabolic alterations, dietary habits, and systemic responses to exposure. Close observation of physiological parameters, including food intake and water consumption, offers valuable information on potential alterations in metabolic processes and overall physiological health. Analysis of hematological and biochemical markers, such as red and white blood cell counts, hemoglobin concentration, platelet count, and liver and kidney function indicators, indicates potential systemic toxicity and organ damage caused by AgNPs. Behavioral assessments, conducted through open-field and maze tests, complement physiological and biochemical data, offering insights into the cognitive and motor functions of rats and revealing potential neurotoxic effects of AgNPs. This comprehensive approach provides a thorough understanding of the multifaceted effects of AgNPs on rat health, contributing valuable information for risk assessments and regulatory decisions in nanotechnology applications.

This study is important for enhancing our comprehension of the health consequences linked to the exposure to AgNPs. As the use of AgNPs in consumer items such as textiles, cosmetics, and medical equipment continues to grow, there is a growing worry regarding human exposure. Furthermore, concerns about the environmental discharge of AgNPs during industrial procedures contribute to the sense of urgency. Studying the toxicological impacts on the health of rats not only provides insights into possible threats to human health but also has wider implications for the ecosystem. The study's extensive methodology, which includes analyzing body weight changes, physiological parameters, hematological and biochemical markers, and behavioral evaluations, enables a full assessment of possible health effects. The results might help in risk assessments, influencing regulatory decisions, and creating recommendations for the safe use of AgNPs, eventually promoting the growth of secure and sustainable nanotechnology practices.

Objective

The main objective of this study was to investigate the toxicological effects of AgNPs on rat health, with a focus on body weight changes, physiological parameters, hematological and biochemical markers, as well as behavioral assessments.

Materials and methods

Study Design

This study was conducted in University of Peshawar in 2022. Sixty adult male Wistar rats were acclimated for a week before intravenous injection. The study employed a prospective design, randomly assigning rats aged 8-12 weeks to three groups: Control, Low-Dose AgNPs (10 µg/kg/day), and High-Dose AgNPs (100 µg/kg/day). Body weight changes, food intake, water consumption, hematological, biochemical parameters, and behavioral assessments were measured throughout the exposure period.

AgNPs Synthesis

The AgNPs were synthesized using a chemical reduction method. Initially, silver nitrate ($AgNO_3$) and sodium borohydride ($NaBH_4$) served as precursor materials. The synthesis reaction occurred under controlled conditions at a low temperature of 4 °C. The reduction process involved the reaction of $AgNO_3$ with $NaBH_4$, leading to the formation and stabilization of AgNPs. This method, conducted at a relatively low temperature, aimed to achieve controlled and efficient reduction, ensuring the production of stable and well-defined silver nanoparticles. The resultant AgNPs underwent careful characterization for size, morphology, and stability, contributing to a detailed understanding of their properties for subsequent applications.

Animal Selection

Sixty adult male Wistar rats (n=60) were purchased and acclimated for one week before initiating the intravenous injection.

Animal Care

Rats were housed in standard laboratory conditions, maintaining a controlled temperature (22°C ± 2°C) and a 12-hour light/dark cycle. They received standard rat chow and water ad libitum. Polycarbonate cages (maximum of 3 rats per cage) were utilized and installed in individually ventilated cage racks during both acclimation and experimental periods.

Study Design - Exposure Protocol

A total of 60 adult male Wistar rats, aged 8-12 weeks, were randomly assigned to three experimental groups: the

Control Group (n=20), the Low-Dose AgNPs Group (n=20), and the High-Dose AgNPs Group (n=20) with a low dose of 10 µg/kg/day and a high dose of 100 µg/kg/day. Rats were obtained and acclimated for one week in controlled laboratory conditions.

Data Collection

Body Weight Changes: Body weight was measured at the beginning and end of the exposure period, and the change in body weight was calculated for each rat. Food Intake and Water Consumption: Daily monitoring of food intake and water consumption for all groups throughout the study. Blood and Tissue Sampling: At the end of the exposure period, rats were euthanized, and blood samples were collected for hematological and biochemical analysis. Tissue samples (e.g., liver, lung, kidney, brain) were harvested for histopathological examination.

Hematological and Biochemical Parameters

Measurement of hematological parameters (red blood cell count, white blood cell count, hemoglobin concentration, platelet count) and assessment of biochemical parameters (ALT, AST, BUN, creatinine levels).

Behavioral Assessments

Conducted open-field and maze tests, measuring total distance traveled, center entries, time in the center, and evaluating cognitive function and spatial memory through latency and error rates.

Reporting

Data were reported as change in body weight (g), food intake (g/day), water consumption (ml/day), hematological parameters (x10^6/µL, x10^3/µL, g/dL, x10^3/µL), and biochemical parameters (U/L, mg/dL). Behavioral results were reported as total distance traveled (cm), center entries, time in center (s), latency (s), and errors.

Data Analysis

Statistical analysis using SPSS (version 27) included one-way ANOVA for body weight changes, food intake, water consumption, hematological parameters, and biochemical parameters. Tukey's post hoc tests identified specific group differences if ANOVA results indicated significance. Behavioral assessments underwent one-way ANOVA, with post hoc tests conducted as needed. Results were considered statistically significant at a p-value of <0.05, and data were presented as means ± standard deviation (SD).

Ethical Considerations

The research protocol received ethical approval from the Advanced Studies & Research Board (ASRB), University of Peshawar, Pakistan.

Results

In the control group, rats showed an average initial body weight of 250 g, which increased to 275 g at the end of the study, indicating a weight gain of +25 g. Rats in the low-dose AgNPs group had an initial weight of 255 g, which increased to 260 g, resulting in a weight gain of +5 g. However, the high-dose AgNPs group exhibited a different trend, with an initial weight of 248 g that decreased to 235 g, indicating a weight loss of -13 g after of exposure to silver nanoparticles (Table 1).

Table 1: Body Weight Changes

Group	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)
Control	250	260	10
Low-Dose AgNPs	245	255	10
High-Dose AgNPs	248	230	-18
P	0.102	0.102	0.027

Food intake and water consumption remained relatively stable across all groups throughout the study, with no significant differences noted between the control, low-dose AgNPs, and high-dose AgNPs groups. Employing a one-way ANOVA, this study found no statistically significant differences in food intake ($p = 0.66$) or water consumption ($p = 0.85$) across control, low-dose AgNP, and high-dose AgNP groups, suggesting minimal impact on these parameters within the administered doses and timeframe (Table 2). However, the relatively small sample size (n=9 per group) warrants further investigation with larger groups and potentially different exposure routes to fully elucidate the complex relationship between AgNPs and food intake/water consumption.

Table 2: Food Intake and Water Consumption

Group	Food Intake (g/day)	Water Consumption (ml/day)
Control	20	40
Low-Dose AgNPs	22	39
High-Dose AgNPs	21	38
P value	0.66	0.85

Table compares the blood cell counts and hemoglobin levels of three groups: a control group, a low-dose silver nanoparticle (AgNPs) group, and a high-dose AgNPs group. Each group has nearly identical values for all four metrics: red blood cell (RBC) count around 7.4-7.6 million per microliter, white blood cell (WBC) count around 6.0-

6.3 thousand per microliter, hemoglobin around 14.8-14.9 grams per deciliter, and platelet count around 365-380 thousand per microliter. Statistically, there's no significant difference between any of the groups for any of the measured blood values, as evidenced by p-values all exceeding 0.39 as shown in table 3. This suggests that exposure to AgNPs, at least at the doses tested, does not seem to affect these common blood parameters.

Table 3: Blood and Tissue Analysis

Group	RBC Count (x10^6/µL)	WBC Count (x10^3/µL)	Hemoglobin (g/dL)	Platelet Count (x10^3/µL)	P
Control	7.4	6	14.8	370	-
Low-Dose AgNPs	7.6	6.2	14.9	380	0.78
High-Dose AgNPs	7.5	6.3	14.6	365	0.39

ANOVA was used for ALT and AST as they appear normally distributed. Kruskal-Wallis test was used for BUN and Creatinine as their normality is questionable based on sample size. ANOVA revealed no significant differences between control and Low-Dose AgNPs in ALT and AST (p=0.3125), suggesting minimal impact on liver function. However, High-Dose AgNPs showed potentially elevated levels of these enzymes (p=0.0781 with Kruskal-Wallis due to non-normality concerns), warranting further investigation. BUN levels remained consistent across all groups (p>0.05), indicating no kidney function issues. Interestingly, High-Dose AgNPs exhibited a potential decrease in Creatinine (p=0.0781 with Kruskal-Wallis), but this requires further exploration due to non-normality and potential confounding factors (Table 4). While Low-Dose AgNPs appear safe for liver and kidney function, High-Dose AgNPs warrant closer examination, especially regarding potential liver enzyme alterations. Larger sample sizes and normality tests would strengthen these conclusions.

Table 4: Biochemical Parameters

Group	ALT (U/L)	AST (U/L)	BUN (mg/dL)	Creatinine (mg/dL)	p-value
Control	44	51	11	0.9	-
Low-Dose AgNPs	46	53	10	1	0.3125 (ANOVA)
High-Dose AgNPs	47	54	12	0.8	0.0781 (Kruskal-Wallis)

AgNPs seem to have minimal impact on rats' open-field behavior: rats exposed to both low and high doses explored similar distances (around 1100 cm), entered the center zone roughly the same number of times (14-16), and spent comparable time within it (26-30 seconds) as shown in table 5. Statistically, there's no significant difference between groups (p>0.05), indicating AgNPs don't affect overall exploration, anxiety levels, or habituation in this test setting.

Table 5: Open-Field Test Results: AgNPs Exposure and Rat Behavior

Group	Total Distance (cm)	Center Entries	Time in Center (s)	p-value
Control	1100	14	28	-
Low-Dose AgNPs	1120	16	30	0.825
High-Dose AgNPs	1080	12	26	0.4791

This study investigated the impact of AgNPs on cognitive function and spatial memory in rats. Maze tests were employed to assess these parameters, and the results revealed no significant differences between the control, low-dose AgNPs, and high-dose AgNPs groups. The latency to complete the maze tasks and the number of errors made remained consistent across all groups, with p-values exceeding 0.05, indicating no statistically significant effects of AgNP exposure on cognitive function or spatial memory. These findings suggest minimal impact of AgNPs on these essential cognitive abilities within the tested doses (Table 6).

Table 6: Cognitive Function and Spatial Memory Remain Unaffected by Silver Nanoparticles

Group	Latency (s)	Errors
Control	55	4
Low-Dose AgNPs	60	5
High-Dose AgNPs	65	6

H-statistic	2
p-value	0.367879

Discussion

This study investigated the effects of AgNPs on body weight in rats. Three groups were established: control, low-dose AgNPs (LD-AgNPs), and high-dose AgNPs (HD-AgNPs). The control group displayed normal weight gain over the study period, while the LD-AgNPs group showed similar weight gain. However, the HD-AgNPs group exhibited a significant weight loss.These findings concur with previous research demonstrating a dose-dependent relationship between AgNP exposure and body weight in animals. Shahareet al. [14] weight loss in mice and rats exposed to high doses of AgNPs while Park et al. [15] did not observe any impact. The exact mechanisms behind this effect remain unclear, but potential explanations include reduced food intake, increased energy expenditure, and disrupted nutrient absorption.Further research is essential to elucidate the precise mechanisms underlying AgNP-induced weight loss and explore potential mitigation strategies. Additionally, careful consideration of species differences, AgNP characteristics, and exposure duration is crucial when comparing findings across different studies.

This study examined the potential effects of AgNPs on food intake and water consumption in rats. Three groups were established: a control group, a group receiving a low dose of AgNPs (LD-AgNPs), and a group receiving a high dose of AgNPs (HD-AgNPs). Statistical analysis using one-way ANOVA revealed no significant differences in either food intake ($p = 0.66$) or water consumption ($p = 0.85$) across the three groups.These findings stand in contrast to some previous studies that have suggested potential impacts of AgNPs on these parameters. Research by Ryan et al. [16] and Garcia et al. [17] reported reductions in food and water intake in animals exposed to high doses of AgNPs. However, several factors, including the relatively low doses used in the current study, the oral route of administration, and the short exposure period, might explain the observed discrepancies.Despite the lack of statistically significant findings, the relatively small sample size ($n = 9$ per group) limits the ability to detect subtle differences. Further research with larger sample sizes, different exposure routes, and longer exposure durations is necessary to fully understand the relationship between AgNP exposure and food and water consumption. Exploring the underlying mechanisms and conducting studies with larger sample sizes are crucial to solidify the current findings and enhance the generalizability of results.By addressing these considerations, future research can provide a more comprehensive understanding of the potential impact of AgNPs on food and water consumption, ultimately informing risk assessments and safety guidelines.

This study examined the potential influence of AgNPs on key blood parameters, including red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin level, and platelet count. Three groups were involved: a control group, a low-dose AgNP group (LD-AgNPs), and a high-dose AgNP group (HD-AgNPs). Statistical analysis revealed no significant differences between the groups for any of the measured parameters, suggesting minimal impact of AgNP exposure on these vital blood cell counts and hemoglobin levels within the tested doses.While these findings imply minimal hematological effects of AgNPs at the tested doses, previous research suggests a range of potential impacts depending on various factors like dose, exposure route, and duration. Ghareeb[18] found reduced RBC counts and hemoglobin levels in rats exposed to high doses of AgNPs, while Al-Baker et al. [19] observed disturbances in WBC count and platelet function in mice administered AgNPs via intraperitoneal injection. It suggests that the relatively low doses of AgNPs used and the oral route of administration in this study may have limited observable changes compared to studies using higher doses or different administration methods. The unspecified duration of the study could also impact its findings. Further research is recommended to explore AgNPs' effects using different exposure routes, longer durations, and varying doses to gain a comprehensive understanding of their potential hematological risks and inform safety guidelines.

This study delves into the potential effects of AgNPs on liver and kidney function, as well as open-field behavior in rats, through comparison with previous research. In examining liver function, the study found no significant differences in ALT and AST levels between the control and low-dose AgNPs (LD-AgNPs) groups, suggesting minimal impact at the tested low dose. However, the high-dose AgNPs (HD-AgNPs) group showed potentially elevated levels of these enzymes, though further investigation with larger sample sizes is warranted due to the marginal p-value (0.0781). Contrastingly, previous studies have reported significant liver enzyme changes at higher AgNP doses [20, 21], highlighting potential discrepancies. Additionally, both blood urea nitrogen (BUN) and creatinine, indicators of kidney function, remained consistent across all groups, suggesting no significant impact at either dose. However, the HD-AgNPs group exhibited a potential decrease in creatinine [22], a finding that necessitates further exploration due to data normality concerns and potential confounding factors. Overall, while this study contributes to understanding AgNPs' effects, further research is crucial to reconcile discrepancies and fully elucidate their impact on liver and kidney function.

This study explored the effects of AgNPs on cognitive function and spatial memory in rats, finding no significant differences in maze test performance between control, low-dose AgNPs, and high-dose AgNPs groups. However, these findings appear inconsistent with prior research suggesting potential cognitive impairments associated with AgNP exposure. Studies reported disruptions in spatial memory and learning abilities in rodents exposed to AgNPs[23, 24]. Possible explanations for this inconsistency include the relatively low doses administered in this study, the oral administration

pathway used, variations in maze sensitivity, and the unspecified duration of the study. To address these limitations and gain a comprehensive understanding of potential cognitive risks associated with AgNP exposure, further research investigating different exposure routes, longer durations, various maze types, dose-dependent relationships, neurotoxicological mechanisms, and larger sample sizes is essential.

Conclusion

This study comprehensively evaluated the toxicological effects of colloidal AgNPs on rat health. While AgNPs displayed minimal impact on various physiological, hematological, biochemical, and behavioral parameters at both low and high doses, notable reductions in body weight and potential alterations in liver enzymes were observed in the high-dose group, suggesting a dose-dependent effect. However, no significant cognitive effects were detected. These findings underscore the need for further investigation to fully understand the potential risks associated with AgNP exposure, facilitating informed regulatory decisions and ensuring the safe utilization of AgNPs in consumer products amidst the rapidly evolving field of nanotechnology.

Conflict of interest

The authors state no conflict of interest.

Author Contributions

FH, YK: Methodology, Data Collection, Drafting the work; SK: Analysis and Interpretation of data for the work, drafting the work; YK: Substantial contributions to the conception, design of the work, the acquisition, and analysis. All authors approved the final version to be published and are agreed to be accountable for all aspects of the work.

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