

Original Research

Biochemical and morphological alterations caused by silver nanoparticles in Wistar rats

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Abstract

Objective: This study evaluated the biochemical effect of the oral administration of silver nanoparticles on some biochemical parameters and tissue morphology.

Methods: Wistar rats of both sexes with an average weight of 160 ± 5 g were randomly assigned into four groups. Animals in Group 1 served as the control and received 0.5 mL of distilled water (drug vehicle). Those in Groups 2, 3, and 4 were administered with 10, 50, and 100 mg/kg body weight silver nanoparticles, respectively. The animals were sacrificed under slight anesthesia 24 hours after the last treatment.

Results: Silver nanoparticle exposure in rats elevated the level of rat serum total cholesterol, triacylglyceride, free glycerol, low density lipoprotein-cholesterol, and bilirubin ($p < 0.05$) when compared with the control. The level of high density lipoprotein-cholesterol was depleted by nanoparticle exposure, whereas the atherogenic index rose. The levels of albumin, urea, creatinine, as well as activities of aspartate transaminase and alkaline phosphatase were decreased by the nanoparticles, whereas the total protein and alanine transaminase were inconsistently altered relative to the control. Furthermore, the nanoparticle treatment caused morphological lesions in rat cardiac, renal, and hepatic tissues relative to the control.

Conclusion: We show evidence that silver nanoparticle potentiated biochemical changes predisposing to liver injury and cardiovascular disorder in rat.

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1. Introduction

Nanoparticles are simply defined as particles <100 nm in size.¹ Nanoparticles have unique properties that may be useful in a diverse range of applications, and consequently

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they have attracted significant interest. In the biomedical field, in particular, the use of nanovaccines and nanodrugs are being intensively researched. Nevertheless, our knowledge about the biocompatibility and risks of exposure to nanomaterials is limited.² Hence, there is an urgent need for studies that contribute to our understanding of the interaction between nanoparticles and living cells. Because of the large surface area/volume ratio and reactivity, nanoparticles can bind to and interact extensively with biomolecules within the cell, which leads to several consequences.³ This factor has

stimulated the various research efforts aimed at investigating the effects of nanoparticles or nanomaterials in living organisms.

Silver nanoparticles (AgNPs) are increasingly and widely being applied for biomedical purposes.⁴ This hinges on the fact that AgNPs have been credited with broad antimicrobial potential. AgNP, as an arch product from the field of nanotechnology, has gained interest because of its distinctive properties, such as good conductivity, chemical stability, catalytic, antibacterial activity, antifungal, antiviral, and anti-inflammatory properties.^{5–7} Silver-based medical products, ranging from topical ointments and bandages for wound healing to coated stents, have been proven to be effective in retarding and preventing bacterial infections.⁸ However, the increasing usage of AgNP for biomedical purposes far exceeds the availability of safety evaluation studies of these nanoparticles. This research gap is being gradually filled.

The present study evaluated the effect of AgNPs on some serum biochemical indices and tissue morphology in Wistar rats.

2. Materials and Methods

2.1. Nanoparticles

The AgNPs were obtained from the Nanomedicine and Biomedical Target Laboratory, Department of Biochemistry, Microbiology and Biotechnology, Rhodes University, South Africa. The preparation procedures and the characterization are as previously demonstrated in our previous report.^{4,9,10}

2.2. Experimental animals

Wistar rats of both sexes with an average weight of 160 ± 5 g were obtained from the animal unit of the Department of Biochemistry, University of Ilorin, Nigeria.

2.3. Chemicals and reagents

The assay kits for creatinine (CREA), urea, bilirubin (BIL), and albumin (ALB), total cholesterol (TC), triacylglyceride (TAG), high density lipoprotein-cholesterol (HDL-C), free glycerol (FG), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were obtained from Randox Laboratory Limited (Crumlin, United Kingdom). All other reagents used were of analytical grade and supplied by Sigma Aldrich Inc. (St. Louis, MO, USA).

2.4. Animal groupings and treatments

The animals were housed in standard plastic cages and were acclimatized for 2 weeks. They were maintained under standard conditions of 12 hours light and dark cycle and had free access to standard rat chow and clean water *ad libitum*.

Handling of animals was humane and consistent with relevant guidelines as approved by the institutional ethics committee on scientific study. The details of animal groupings are as shown below:

Group 1 served as control and was administered 0.5 mL distilled water.

Group 2 was administered 0.5 mL of 10 mg/kg body weight of Ag nanoparticles dissolved in distilled water.

Group 3 was administered 0.5 mL of 50 mg/kg body weight of Ag nanoparticles dissolved in distilled water.

Group 4 was administered 0.5 mL of 100 mg/kg body weight of Ag nanoparticles dissolved in distilled water.

Rats were given oral and daily treatments. The treatments lasted for 30 days. The selection of dosages used is premised on our previous report.⁴

2.5. Preparation of serum and tissue homogenates

The rats were sacrificed under slight anesthesia 24 hours after the end of the last treatment. The blood was collected into a clean and sterile sample bottle. The blood was centrifuged at 1500g for 15 minutes using a Uniscope Laboratory Centrifuge (Model SM800B). The serum was aspirated and stored frozen until required for analyses. The tissues including liver, kidney, and heart were removed, blotted, and weighed. The tissues were then homogenized in ice-cold 0.25M sucrose solution (1:5 w/v). Sections from the excised tissues were fixed in buffered neutral formalin and used for the histopathological examination.

2.6. Determination of biochemical parameters

The activities of AST (E.C. 2.6.1.1), ALT (E.C. 2.6.1.2), ALP (E.C.3.1.3.1), ALB, BIL, CREA, urea, serum TC concentration, HDL-C, and TAG was assayed using the Randox assay kits (Randox Laboratory Limited). The concentration of serum low density lipoprotein (LDL-C) was estimated using the Friedewald formula,¹¹ whereas the atherogenic index (AI) was estimated by finding the ratio of TC to HDL-C concentration. The protein content of the serum and homogenates was determined using the Biuret method as previously described by Sulaiman and Adeyemi.¹²

All measurements were done using a Spectronic 21 spectrophotometer (Bausch and Lomb, Rochester, NY, USA).

2.7. Histological examination

The procedures described by Adeyemi and Akanji¹³ were used. Briefly, the heart, kidney, and liver were fixed in 10% (v/v) formaldehyde, dehydrated through ascending grades of ethanol (70%, 90%, and 95%, v/v), cleaned in xylene, and embedded in paraffin wax (melting point 56°C) and stained with hematoxylin and eosin. The photomicrographs were captured at $\times 100$ using the software Presto Image Folio package.

2.8. Statistical analysis

The data are expressed as mean $\pm$ standard error of mean. Statistical analysis was performed using analysis of variance and Duncan's multiple range test at 5% level of confidence ($p < 0.05$).

3. Results

3.1. Average weights of animals

The oral administration of Ag nanoparticles to rats for 30 days inconsistently altered the average body weights relative to the control (Figure 1). The rat organ weights as well as the organ/body weight ratios were also significantly decreased when compared with the control (Tables 1 and 2).

3.2. Lipid profile

The oral administration of Ag nanoparticles to rats caused elevation in the level of serum TC, TAG, FG, and LDL-C when compared with the control (Tables 3 and 4). By contrast, the level of serum high density lipoprotein-cholesterol was depleted by nanoparticle administration whereas the AI was significantly elevated.

3.3. Total protein

The level of rat serum and tissue total protein was inconsistently altered by the nanoparticle treatment when compared with the control (Table 5). In the rat liver and heart, total protein contents were elevated whereas reductions were recorded for protein levels in kidney and serum.

3.4. Serum metabolites

The daily administration of Ag nanoparticles to rats caused reductions in the level of serum ALB, urea, and CREA relative

Table 1

Rat organ weights following oral exposure to silver nanoparticles.

Groups	Organs weight of rats (g)		
	Heart	Liver	Kidney
Control	1.43 $\pm$ 0.00 ^a	8.97 $\pm$ 0.09 ^a	2.70 $\pm$ 0.12 ^a
Ag 10 mg/kg	1.37 $\pm$ 0.03 ^b	5.93 $\pm$ 0.35 ^c	1.60 $\pm$ 0.25 ^c
Ag 50 mg/kg	1.13 $\pm$ 0.17 ^c	6.63 $\pm$ 0.44 ^b	1.53 $\pm$ 0.32 ^d
Ag 100 mg/kg	1.06 $\pm$ 0.23 ^d	6.57 $\pm$ 0.48 ^b	1.77 $\pm$ 0.41 ^b

Values are expressed as mean $\pm$ SEM ($n = 3$). Values in each column with different superscripts are significantly different ($p < 0.05$).

Ag = silver; SEM = standard error of the mean.

Table 2

Organ/body weight ratios of Wistar rats orally administered with silver nanoparticles.

Groups	Percentage organ/body weight ratios		
	Heart	Liver	Kidney
Control	0.91 $\pm$ 0.04 ^a	4.92 $\pm$ 0.26 ^a	1.84 $\pm$ 0.12 ^a
Ag 10 mg/kg	0.82 $\pm$ 0.07 ^a	3.55 $\pm$ 0.41 ^d	0.96 $\pm$ 0.17 ^c
Ag 50 mg/kg	0.64 $\pm$ 0.09 ^c	3.78 $\pm$ 0.25 ^c	0.88 $\pm$ 0.19 ^d
Ag 100 mg/kg	0.67 $\pm$ 0.17 ^c	4.05 $\pm$ 0.52 ^b	1.11 $\pm$ 0.29 ^b

Values are expressed as mean $\pm$ SEM ($n = 3$). Values in each column with different superscripts are significantly different ($p < 0.05$).

Ag = silver; SEM = standard error of the mean.

Table 3

Effect of silver nanoparticles on the level of rat serum total cholesterol (TC), triacylglyceride (TAG), and free glycerol (FG).

Groups	TC (mmol/L)	TAG (mmol/L)	FG (mmol/L)
Control	0.30 $\pm$ 0.05 ^a	0.46 $\pm$ 0.30 ^a	0.35 $\pm$ 0.30 ^a
Ag 10 mg/kg	1.54 $\pm$ 0.74 ^c	0.86 $\pm$ 1.00 ^b	0.75 $\pm$ 0.10 ^b
Ag 50 mg/kg	1.78 $\pm$ 0.24 ^c	0.79 $\pm$ 0.01 ^c	0.68 $\pm$ 0.01 ^c
Ag 100 mg/kg	4.58 $\pm$ 0.68 ^b	0.61 $\pm$ 0.34 ^d	0.51 $\pm$ 0.26 ^d

Values are expressed as mean $\pm$ SEM ($n = 3$). Values in each column with different superscripts are significantly different ($p < 0.05$).

Ag = silver; SEM = standard error of the mean.

Table 4

Effect of silver nanoparticles on the level of rat serum high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), and atherogenic index.

Group	HDL-C (mmol/L)	LDL-C (mmol/L)	Atherogenic index
Control	0.49 $\pm$ 0.17 ^a	0.06 $\pm$ 0.03 ^a	1.80 $\pm$ 0.22 ^a
Ag 10 mg/kg	0.46 $\pm$ 0.08 ^a	0.17 $\pm$ 0.01 ^b	10.37 $\pm$ 0.32 ^c
Ag 50 mg/kg	0.62 $\pm$ 20.06 ^b	0.16 $\pm$ 0.00 ^b	7.18 $\pm$ 0.35 ^b
Ag 100 mg/kg	0.66 $\pm$ 0.06 ^b	0.12 $\pm$ 0.03 ^c	17.64 $\pm$ 0.19 ^d

Values are expressed as mean $\pm$ SEM ($n = 3$). Values in each column with different superscripts are significantly different ($p < 0.05$).

Ag = silver; SEM = standard error of the mean.

to the control (Table 6). By contrast, the level of rat serum BIL was elevated by the nanoparticle treatment.

3.5. Other biochemical indices

In order to evaluate whether the administration of Ag nanoparticle could cause cellular toxicity, the levels of AST, ALT, and ALP were determined in rat serum and tissues

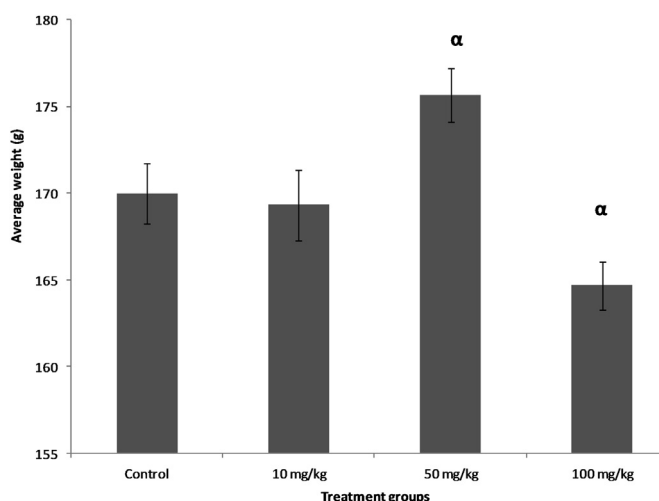


Figure 1. Average weight of rat following oral exposure to silver nanoparticles at different dosages. Data are expressed as mean $\pm$ standard error of the mean ($n = 3$). α is significant at $p < 0.05$ relative to the control.

Table 5
Effect of silver nanoparticles on the level of rat serum and tissue total protein.

Group	Total protein concentration (g/dL)			
	Serum	Heart	Kidney	Liver
Control	2.73 ± 0.51 ^a	3.68 ± 1.47 ^a	6.72 ± 1.94 ^a	7.54 ± 2.34 ^a
Ag 10 mg/kg	2.34 ± 0.52 ^a	6.72 ± 2.31 ^c	10.84 ± 1.94 ^a	11.04 ± 1.12 ^c
Ag 50 mg/kg	1.34 ± 0.60 ^a	3.16 ± 0.87 ^a	7.00 ± 2.80 ^a	7.00 ± 2.80 ^a
Ag 100 mg/kg	1.17 ± 0.42 ^a	14.29 ± 6.01 ^b	3.33 ± 0.90 ^b	19.76 ± 4.58 ^b

Values are expressed as mean ± SEM ($n = 3$). Values in each column with different superscripts are significantly different ($p < 0.05$).

Ag = silver; SEM = standard error of the mean.

Table 6
Effect of silver nanoparticle on the level of rat serum metabolites.

Group	ALB (g/dL)	BIL (mmol/L)	Urea (mmol/L)	CREA (μmol/L)
Control	2.15 ± 0.25 ^a	2.78 ± 0.32 ^a	39.21 ± 0.00 ^a	16.43 ± 1.71 ^a
Ag 10 mg/kg	2.21 ± 0.20 ^a	8.94 ± 0.16 ^c	20.29 ± 0.72 ^c	15.33 ± 0.31 ^d
Ag 50 mg/kg	0.40 ± 0.06 ^b	9.43 ± 0.11 ^c	25.33 ± 1.33 ^b	16.37 ± 0.21 ^b
Ag 100 mg/kg	0.08 ± 0.01 ^c	11.03 ± 0.65 ^b	41.81 ± 2.57 ^a	15.58 ± 0.60 ^c

Values are expressed as mean ± SEM ($n = 3$). Values in each column with different superscripts are significantly different ($p < 0.05$).

Ag = silver; ALB = albumin; BIL = bilirubin; CREA = creatinine; SEM = standard error of the mean.

Table 7
Effect of silver nanoparticles on the activity of aspartate transaminase (AST) in rat serum and tissues.

Groups	AST (U/L)			
	Serum	Liver	Kidney	Heart
Control	48.98 ± 5.78 ^a	56.87 ± 4.44 ^a	51.99 ± 3.24 ^a	51.01 ± 9.49 ^a
Ag 10 mg/kg	21.17 ± 6.44 ^b	14.50 ± 1.04 ^d	25.17 ± 1.67 ^b	34.00 ± 9.28 ^b
Ag 50 mg/kg	11.17 ± 0.88 ^c	23.00 ± 3.75 ^c	21.17 ± 1.83 ^c	24.00 ± 2.25 ^c
Ag 100 mg/kg	21.50 ± 3.75 ^b	45.67 ± 5.67 ^b	26.67 ± 2.35 ^b	34.50 ± 5.53 ^b

Values are expressed as mean ± SEM ($n = 3$). Values in each column with different superscripts are significantly different ($p < 0.05$).

Ag = silver; SEM = standard error of the mean.

Table 8
Effect of silver nanoparticles on the activity of alanine transaminase (ALT) in rat serum and tissues.

Group	ALT (U/L)			
	Serum	Liver	Kidney	Heart
Control	52.37 ± 2.52 ^a	27.89 ± 1.20 ^a	85.42 ± 3.84 ^a	16.98 ± 1.45 ^a
Ag 10 mg/kg	62.00 ± 1.00 ^b	37.00 ± 8.02 ^b	25.00 ± 0.58 ^d	32.00 ± 2.00 ^d
Ag 50 mg/kg	36.70 ± 1.67 ^c	17.30 ± 1.33 ^c	84.30 ± 8.67 ^b	35.30 ± 2.67 ^b
Ag 100 mg/kg	34.00 ± 8.89 ^c	32.70 ± 10.50 ^a	34.00 ± 7.21 ^c	35.70 ± 1.67 ^b

Values are expressed as mean ± SEM ($n = 3$). Values in each column with different superscripts are significantly different ($p < 0.05$).

Ag = silver; SEM = standard error of the mean.

(Tables 7–9). The administration of Ag nanoparticles decreased the activities of rat serum and tissue AST and ALP relative to the control. However, the activity of rat serum and tissue ALT was inconsistently altered. In rat liver and heart, the highest dosage of nanoparticle produced an increment in ALT level.

3.6. Histopathological examination

Rat tissue sections were prepared and observed under a light microscope for morphological changes following exposure to Ag nanoparticles. The histological examinations revealed cellular lesions in the rat cardiac, renal, and hepatic tissue caused by nanoparticle administration (Figures 2–4). The morphological lesions included inflammation, hyperchromic anemia, polymorph infiltration of the glomeruli, and cellular degeneration. These inimical cellular alterations were nonincident in the control group.

4. Discussion

There is increasing usage of nanoparticles for biomedical purposes.¹⁴ However, our knowledge and understanding of the effect of these nanoparticles on living cells or biochemical indices remain insufficient.¹⁵ It has therefore become imperative that studies aiming to determine the biochemical evaluations of nanoparticles be encouraged. A previous study reported the LD₅₀ of AgNPs to be > 5000 mg/kg¹⁶; however, the present study evaluated that rate at much lower concentrations between 10 and 100 mg/kg rat weight.

4.1. Average body weights

The nanoparticle administration produced significant alterations to body and organ weights in rats relative to the control. This may form part of the symptoms of toxic events consequent upon Ag nanoparticle exposure. Studies have linked changes in body or organ weight to toxicity of administered chemical or toxicant.^{17,18}

4.2. Lipid profile

The nanoparticles showed a capability to alter the lipid profile by elevating the TC, TAG, LDL-C, and FG content. The HDL-C level was depleted, thereby raising the AI. HDL-C is termed “good” cholesterol and has been reported to reduce the risk for atherosclerosis development.^{19,20} By contrast, LDL-C is usually referred to as “bad” cholesterol and predisposes to atherosclerosis. At present, no reason could be advanced for the effect of Ag nanoparticles on lipid profile. However, it should be noted that Ag nanoparticles raised the AI, thus displaying capability to predispose to cardiovascular disorder.

4.3. Total protein

The AgNP caused alteration to the level of rat serum and tissue total protein. The elevation of liver total protein content may be an adaptive mechanism used by the animals to cope with stress imposed by the nanoparticle administration. It has been previously demonstrated that administration of drugs or chemical agents could promote *de novo* synthesis of protein as part of adaptive mechanisms.²¹

Table 9

Effect of silver nanoparticles on the activity of alkaline phosphatase (ALP) in rat serum and tissues.

Group	ALP (U/L)			
	Serum	Liver	Kidney	Heart
Control	454.45 ± 74.21 ^a	424.99 ± 69.74 ^a	488.98 ± 26.61 ^a	179.02 ± 38.95 ^a
Ag 10 mg/kg	388.19 ± 27.65 ^b	304.52 ± 123.74 ^b	381.85 ± 40.87 ^b	96.19 ± 1.83 ^b
Ag 50 mg/kg	76.91 ± 6.45 ^c	65.04 ± 2.40 ^c	74.45 ± 9.28 ^d	42.87 ± 11.35 ^d
Ag 100 mg/kg	61.87 ± 11.66 ^d	65.32 ± 14.09 ^c	85.93 ± 24.95 ^c	50.97 ± 5.13 ^c

Values are expressed as mean ± SEM ($n = 3$). Values in each column with different superscripts are significantly different ($p < 0.05$).

Ag = silver; SEM = standard error of the mean.

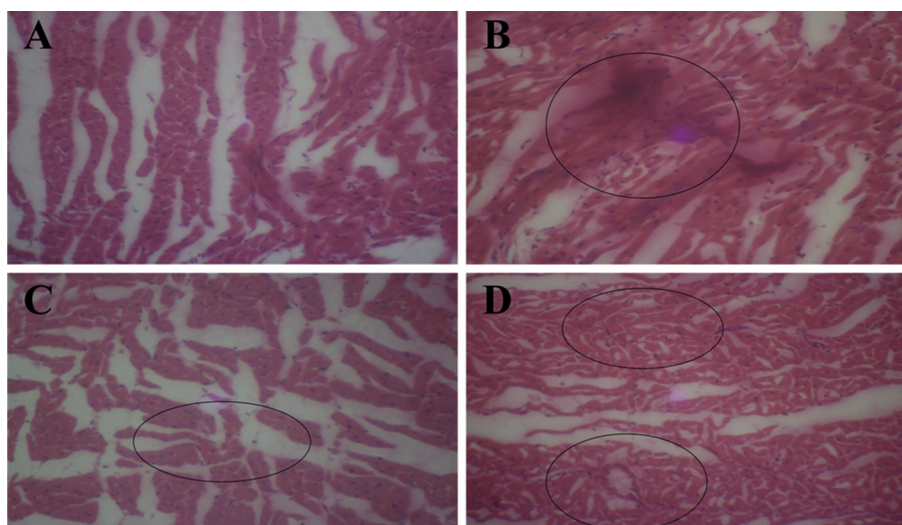


Figure 2. Photomicrographs of rat heart following oral exposure to silver nanoparticles at different dosages. (A) Control group given distilled water showing intact cellular morphology; (B) group given silver nanoparticles at 10 mg/kg body weight showing mild inflammation and cellular degeneration; (C) group given silver nanoparticles at 50 mg/kg body weight showing inflammation and cellular degeneration; and (D) group given silver nanoparticles at 100 mg/kg body weight showing inflammation and cellular degeneration. Hematoxylin and eosin staining ($\times 400$).

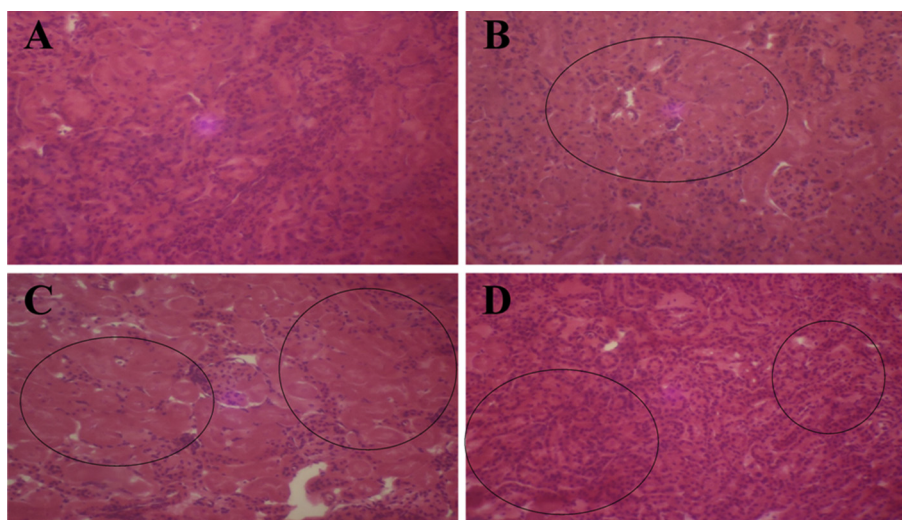


Figure 3. Photomicrographs of rat kidney following oral exposure to silver nanoparticles at different dosages. (A) Control group given distilled water showing intact cellular morphology; (B) group given silver nanoparticles at 10 mg/kg body weight showing mild inflammation and hyperchromic anemia; (C) group given silver nanoparticles at 50 mg/kg body weight showing inflammation and cellular degeneration; and (D) group given silver nanoparticles at 100 mg/kg body weight showing inflammation and cellular degeneration. Hematoxylin and eosin staining ($\times 400$).

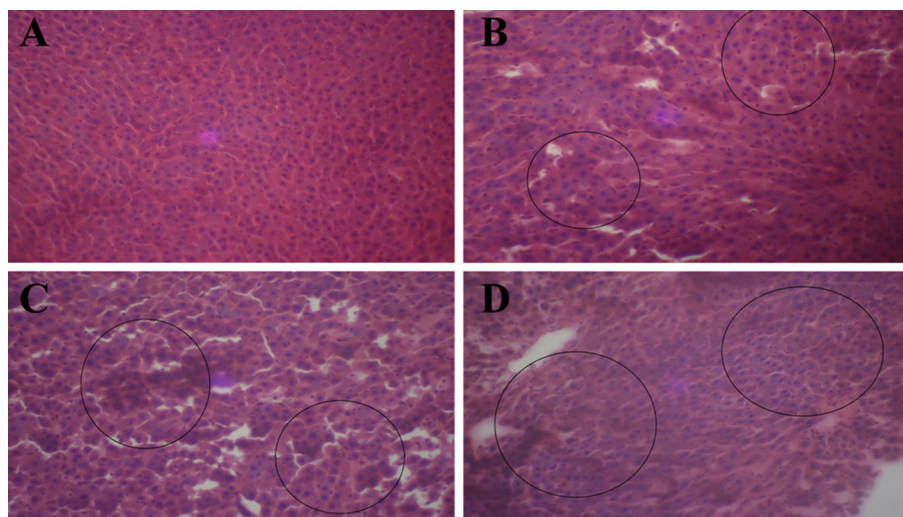


Figure 4. Photomicrographs of rat liver following oral exposure to silver nanoparticles at different dosages. (A) Control group given distilled water showing intact cellular morphology; (B) group given silver nanoparticles at 10 mg/kg body weight showing mild inflammation and intercellular cracking; (C) group given silver nanoparticles at 50 mg/kg body weight showing inflammation and cellular degeneration; and (D) group given silver nanoparticles at 100 mg/kg body weight showing inflammation and cellular degeneration. Hematoxylin and eosin staining ($\times 400$).

4.4. Serum metabolites

The nanoparticle administration to rats decreased the level of serum ALB, CREA, and urea. By contrast, the level of rat serum BIL rose. The alteration in serum ALB and BIL levels may denote stress on the liver imposed by the nanoparticles. The ALB is synthesized in the liver and has been useful in the measurement of the synthetic functions of the liver.^{21,22} BIL is a product of hemoglobin degradation following red cell hemolysis. Thus, a rise in the level of BIL may be associated with increased hemolysis or hepatic complications either in metabolism or secretion of conjugated products.¹³

4.5. Other biochemical parameters

The decreases in AST and ALP activities caused by the nanoparticle may be due to inactivation arising from the affinity of the Ag nanoparticles for thiol ($-SH$) groups, thereby causing change in the functional state of proteins.⁹ In a separate study by Abbas et al.,²³ it has been shown that Ag nanoparticles inactivated amino transaminases. Inactivation of enzymes or proteins may affect reactions in key metabolism with dire consequences for cellular integrity. The elevation of the activities of serum AST and ALT may be linked to cellular toxicity particularly in the liver.^{24,25} There was elevation in the activity of rat liver ALT relative to the control. However, there was no corresponding increment on serum ALT level relative to the control. This may point in the direction of an adaptive mechanism by animals trying to cope with stress imposed by the nanoparticle exposure. Recent studies have shown that Ag nanoparticles caused cellular stress both *in vitro*^{26,27} and in rats after repeated exposure for 21 days.²⁸

4.6. Histopathological examination

The Ag nanoparticle administration caused several alterations to the cellular architecture of rat cardiac, renal, and hepatic tissue relative to the control. The inflammation and cellular degeneration caused by the nanoparticle are lines of evidence supporting the potential of the nanoparticle to cause biochemical alterations leading to cellular damage. Recent studies have shown the potential of nanoparticles to sequester in tissues and thereby cause cellular damage.^{15,29}

5. Conclusion

The study presented evidence showing the potential of AgNPs to elevate rat AI as well as cause a series of biochemical and morphological alterations at the doses investigated.

Conflicts of interest

None declared.

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