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Original Research Article

Effect of chitosan-silver nanoparticle composite-treated water on selected biochemical parameters of rats

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Abstract

Purpose: *To investigate the impact of chitosan-silver nanoparticles (chitosan-AgNP) composite-treated water on some biochemical parameters in the albino rats.*

Methods: *Water samples were pretreated with chitosan-coated silver nanoparticles (chitosan-AgNPs) prior to oral administration in a rodent model. Following a 28-day experimental period, serum biochemical markers associated with hepatic and renal functions and enzymatic activities were quantitatively assessed to evaluate potential physiological and metabolic alterations.*

Results: *Biochemical analysis revealed significant alterations in liver and kidney function markers in rats exposed to contaminated water. Liver alkaline phosphatase (ALP), aspartate aminotransferase (AST), and γ-glytamyl transferase (GGT) activities were significantly decreased ($p < 0.05$), while serum ALP, AST and GGT levels were significantly elevated ($p < 0.05$) in the contaminated water group compared to control. Serum urea and creatinine levels were significantly higher in rats exposed to contaminated water (90 ± 0.08 mg/dL and 21.73 ± 4.03 mg/dL, respectively) compared to the control group (43 ± 0.13 mg/dL and 16.37 ± 1.97 mg/dL, respectively; $p < 0.05$). Conversely, administration of chitosan-AgNP-treated water significantly reduced these elevations, bringing the values closer to control levels. Bacteriological analysis showed a drastic reduction in total coliform and fecal counts from 1.44 × 10^7 CFU/mL and 7.2 × 10^6 CFU/mL, respectively, to 0 CFU/mL after 27 days of chitosan-AgNP treatment.*

Conclusion: *The findings suggest that chitosan-AgNP treatment significantly reduces bacterial load in water and positively affects selected biochemical parameters of albino rats, indicating its potential as a water treatment option.*

Keywords: *Antimicrobial, Nanoparticles, Water treatment, Chitosan*

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INTRODUCTION

Water is essential for life, food production and sustainable health, yet access to clean, uncontaminated water is a significant global challenge [1]. About 30 % of groundwater is under stress and nearly 800 million people lack safe drinking water [2]. Water availability is

further threatened by climate change, population growth, urbanization, emerging contaminants and industrialization [3]. Water-related diseases cause over 25,000 deaths daily and 3 million annually [4]. Conventional water treatment methods have limitations, such as inability to remove pesticides and certain contaminants like atrazine [5]. Microbial contamination in urban and rural water systems, especially in Nigeria, is linked to declining life expectancy due to unsafe water [6]. The global community aims to improve access to clean water for 1.5 billion people, with nanotechnology emerging as a promising solution for effective water treatment [7].

This study focuses on the Gbugudu community near Kwara State University, which depends on a shallow stream and an overburdened hand pump borehole, particularly ineffective in the dry season. Initial studies found high microbial contamination, with suspected pesticide and heavy metal contamination due to local activities. The study aims to develop an inexpensive, eco-friendly filtration and sterilization method using local resources to combat waterborne diseases. The abundance of banana trees in the area presents an opportunity to convert agricultural waste, particularly banana trunks, into a solution for clean drinking water.

EXPERIMENTALS

Banana trunks

Banana trunks were harvested from a plantation located across from the World Assembly Church, Offa-Garage Road, Ilorin, Kwara State, Nigeria. The plant sample was identified at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Kwara State, where a voucher specimen (no. UILH/001/1249) was deposited.

Collection and storage of water samples

Water samples were collected from a stream at Gbugudu village, Moro Local Government, Kwara State, Nigeria. This village lacks basic water supply system and therefore depends majorly on a shallow, slow-flowing stream for washing, cooking and drinking, as well as a single overwhelmed hand pump borehole system that does not work effectively during the dry season. Initial feasibility studies revealed a high level of microbial contamination with suspected levels of pesticide and heavy metals contamination due to the activities around the water source. The water samples collected from the stream were immediately transported on ice to the laboratory and their pH values were determined before storage at – 4 °C for further use.

Animals

A total of twenty-five albino rats (*Rattus norvegicus*, Wistar strain), weighing 145.09 ± 6.92 g, were sourced from the Animal Holding Unit of the Department of Biochemistry, Faculty of Pure and Applied Sciences, Kwara State University, Malete. The rats were acclimatized under standard housing conditions for seven days, during which they were provided with standard rat pellets (Top Feeds) and tap water *ad libitum*. The experimental protocols adopted in the treatment of animals in this study were reviewed and approved by the Centre for Research Innovation and Training of Kwara State University, Nigeria (approval no. KWASU/CRIT/REA/2022/0002).

Preparation of aqueous banana stem extract

The outer layer of the banana trunk was stripped off, and the inner parts were chopped into smaller pieces. Exactly 300 g of these pieces were then washed and boiled in 3 liters of distilled water for 30 minutes at 90 °C. The extract obtained was filtered using filter paper (Whatman no. 1 filter paper) and the resulting filtrate was stored at 4 °C and subsequently used in the synthesis of nanoparticles.

Synthesis of silver nanoparticles using aqueous banana stem extract

Synthesis of silver nanoparticles was done as previously reported elsewhere [8]. In brief, 1 mM $AgNO_3$ solution (Sigma Aldrich, United States) was prepared and used as source of silver. The ratio of silver nitrate to banana stem extract varied. The reaction mixture was incubated in the dark at 30 °C under static conditions to prevent photoactivation of silver nitrate. Controls were set up with only banana stem extract and silver nitrate solution (1 mM). Colour changes were observed and changes in absorbance were monitored at time (T) 0, 30 min, 60 min, 90 min, 120 min, 150 min, 24, 48, 72 and 96 h.

Synthesis of chitosan–silver nanoparticles composite beads

Chitosan and chitosan-silver nanoparticle microbeads were prepared by ionic gelation of chitosan solution with anionic tripolyphosphate (TPP), following the methods described previously [9]. A 3 % TPP solution was prepared by dissolving 3 g of TPP in a small volume of phosphate buffer (pH 7.2), then adjusting the

volume to 100 mL. Chitosan solution (1.5 %) was made by dissolving 1.5 g of chitosan powder in a small amount of acetic acid solution (1 % v/v) and then bringing the total volume to 100 mL with additional acetic acid solution. One milliliter of the prepared silver nanoparticles was mixed with 9 mL of the chitosan solution and this mixture was added dropwise to 50 mL of TPP solution on an orbital shaker. Chitosan-silver nanoparticles composite bead was formed spontaneously under mild agitation (70 rpm) at room temperature. The beads were also formed by a similar procedure without adding silver nanoparticles. Thereafter, bead-containing TPP solutions were filtered through filter paper, air-dried and then stored for further studies.

Morphological characterization of chitosan-silver nanocomposites

The morphology of silver nanoparticles embedded in chitosan beads was examined using scanning electron microscopy (SEM). Bead samples were placed on a carbon-coated grid and analyzed with a Hitachi S-4500 SEM, which was equipped with Energy Dispersive X-ray Spectroscopy (EDX) for elemental analysis. The analysis was conducted at two designated spots on the nanoparticle surface.

Antimicrobial activity of synthesized chitosan-silver nanoparticles

Microbial broth dilution assays

Macrobroth dilution method using Mueller-Hinton or Luria Bertani Broth medium was carried out to evaluate microbial load before and after treatment. In these experiments, 0.4 mL of untreated water was mixed with 3.6 mL of susceptibility test broth containing two-fold serial dilutions of the AgNP-Chitosan composite in glass test tubes fitted with loose screw caps. All tubes were incubated at 37 °C and aliquots were evaluated prior to incubation (T_0) and at 6, 12, 18, 24, 36, 48, 72 and 96 h. Reduction or total eradication of microbial growth was evaluated using turbidimetric technique by measuring optical density at 540 nm using a UV spectrophotometer.

Disinfection of contaminated water

The AgNP-Chitosan composite (50 mg) was added to 10 mL of untreated water samples in a 25 mL McCartney tube. All tubes were incubated at 25, 37 and 40 °C and aliquots were sampled prior to incubation (T_0) and at 6, 12, 18, 24, 36, 48, 72 and 96 h and subsequently transferred into broth medium for macro dilution assays.

Administration of treated water

Twenty male albino rats were randomly assigned to five groups (A – E), with four rats in each group. The treatment for each group was as follows: Group A (Positive Control) received distilled water, Group B (Negative Control) was exposed to contaminated water, while Group C was given chitosan bead-treated water. Also, Groups D and E were administered silver nanoparticle-treated water and chitosan-silver nanoparticle composite bead-treated water, respectively. All water samples were supplied *ad libitum* and administered orally for 28 days.

Preparation of serum and tissue homogenate

The method outlined by Yakubu *et al* [10] was used for preparing serum and tissue homogenates. After the 28-day treatment period, experimental animals were anesthetized with diethyl ether and sacrificed, and venous blood samples were collected in plain sample bottles by making a small incision in the jugular vein with a sterile blade after slight displacement from the neck region. Sera were obtained by spinning the tube containing collected blood sample at 5000 rpm for 20 minutes. Kidneys and liver of each experimental rat were also excised and transferred to 0.25 M sucrose solution. Thereafter, 1 g of excised kidney and liver was homogenized in 5 mL of cold sucrose solution (0.25 M) and the homogenates were then centrifuged at 5000 rpm for 15 minutes, after which the supernatants obtained were stored at 4 ˚C. The sera and the homogenates obtained were used for biochemical assays.

Determination of biochemical parameters

The quantitative analysis of serum biomarkers, including albumin, total bilirubin, urea, creatinine, aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT), was performed using standardized colorimetric or enzymatic methodologies. All experimental protocols adhered strictly to the manufacturer's specifications provided in the commercially available assay kits (Randox Laboratories Ltd, Crumlin, UK). Assays were conducted under controlled laboratory conditions to ensure precision, with absorbance or kinetic changes monitored using a microplate spectrophotometer. Calibration curves, internal quality controls and blank samples were integrated into each assay batch to validate reproducibility and minimize inter-experimental variability.

Statistical analysis

Biochemical analysis data are presented as means of four replicates ± Standard Error of the Mean (SEM). One-way ANOVA, followed by Dunnett's post hoc test, was used for multiple comparisons among treatment groups using GraphPad Prism version 5.02. Statistical significance was determined at a 95 % confidence level.

RESULTS

Optimization of silver nanoparticle synthesis

The optimum ratio of extract to 1 mM $AgNO_3$ was found to be 1:9 after 72 h reaction time with a peak absorbance of 1.31 at a wavelength of 433 nm (Figure 1).

Characteristics of AgNPs and chitosan-AgNPS

The EDX spectrum, used to characterize all elements or chemicals present in the synthesized chitosan, AgNPs and chitosan-AgNPs, is shown in Figures 2 A - C, respectively. Figure 2 A revealed the presence of C, O, Na, P and Si, while Figure 2 B confirmed the presence of Cl, Ar, Al, Ag, Cd and K in addition to the elements found in Figure 2 A. Chitosan-AgNPs EDX spectra peaks revealed numerous additional elements, including K and Fe (Figure 2 C).

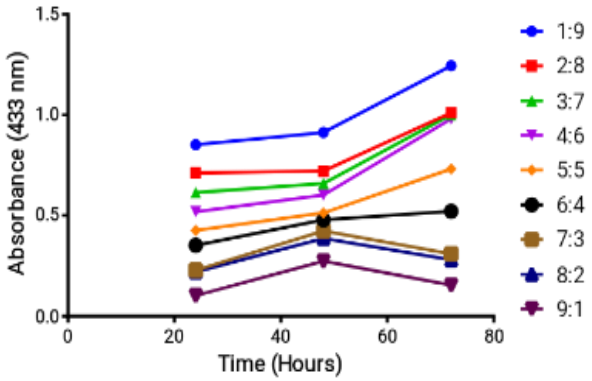
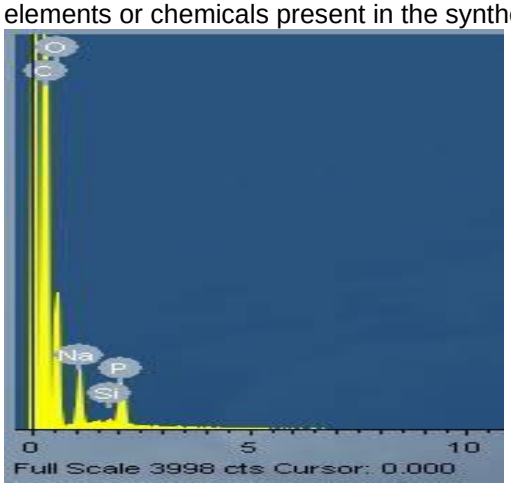
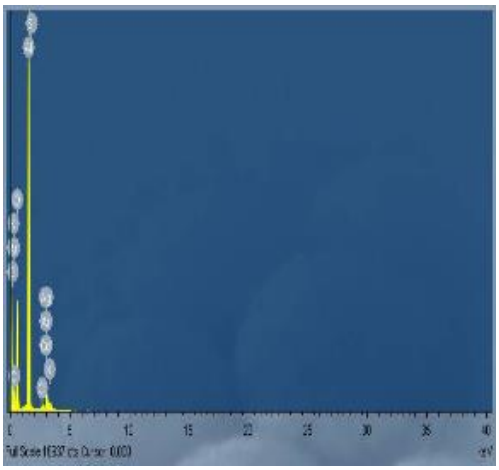


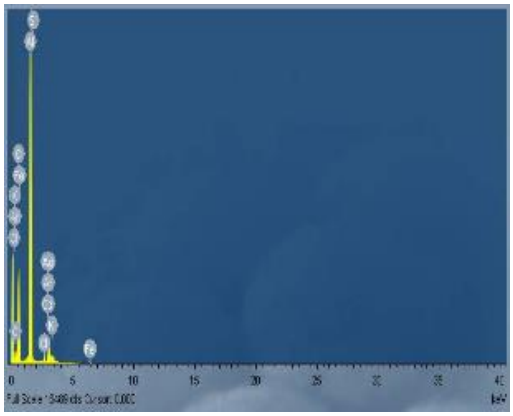
Figure 1: Absorbance of synthesized silver nanoparticles at different incubation times



A



B



C

Figure 2: (a) Energy dispersive X-ray **(**EDX) of chitosan. (**b)** Energy dispersive X-ray of AgNP, and (**c)** Energy dispersive X-ray of chitosan-AgNPs

Effect of chitosan-AgNP-treated water on some biochemical parameters of rats

Figure 3 shows the effect of chitosan-AgNP-treated water on the liver, kidney and serum ALP activity of rats. Alkaline phosphatase activity of rats placed on contaminated water was found to be significantly lowered in the liver and kidney ($p < 0.05$), compared to the control and the rat administered with chitosan-AgNPs-treated water (Figure 3). However, there was a significant increase in serum ALP ($p < 0.05$) of rats that received contaminated and chitosan-treated water relative to control, AgNP and chitosan-AgNP-treated rats (Figure 3). The AST activity in liver, kidney and serum of rats administered chitosan-AgNP-treated water is presented in Figure 4. There was a significant reduction in AST activity in the liver and kidneys of the rats administered contaminated water in comparison with the group placed on distilled water ($p < 0.05$) and the treated rats. However, serum AST activity of rats placed on contaminated water increased significantly relative to the control ($p < 0.05$) and other treated rats (Figure 4). Similarly, a marked loss of liver GGT activity of rats administered contaminated water was observed compared to control and chitosan-AgNP administered rats (Figure 5). This was in sharp contrast to the significantly ($p < 0.05$) increased serum activity of GGT of rats placed on contaminated water relative to control and chitosan-AgNP-treated groups (Figure 5).

Furthermore, serum urea and creatinine levels in rats exposed to contaminated water for 28 days are presented in Table 1. Overall, rats administered contaminated water and chitosan-treated water showed significantly higher ($p < 0.05$) serum urea and creatinine levels compared to the control group and those receiving chitosan-AgNP-treated water. Conversely, serum bilirubin levels were significantly elevated ($p < 0.05$) in rats exposed to contaminated water compared to the control and chitosan-AgNP-treated groups, while serum albumin levels were significantly lower ($p < 0.05$) in the contaminated water group than in the control and chitosan-AgNP-treated groups.

Bacteriological analyses of Gbugudu water sample

The results of bacteriological analyses of Gbugudu water sample are presented in Table 2. After 27 days of chitosan-AgNP treatment, it was observed that the total coliform and faecal counts of the bacteria dropped from 1.44 x 10^7 and 7.2 × 10^6 CFU/mL to 0 CFU/mL, respectively. There was also a reduction in the total heterotrophic count from 1.77 x 10^7 to 9.0 x 10^1 after 27 days of treatment.

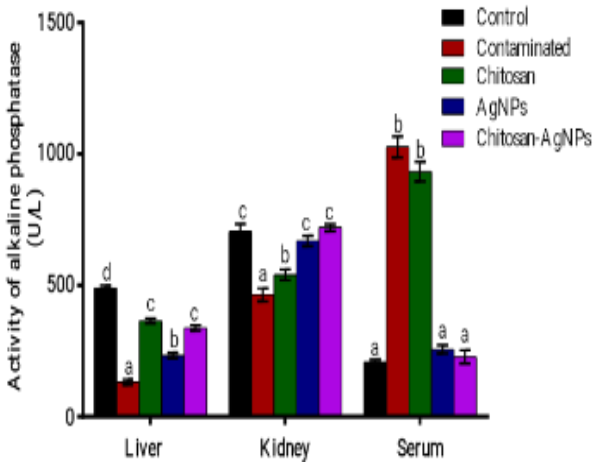


Figure 3: Effect of Chitosan-AgNPs treated water on the liver, kidney and serum ALP activity of rats

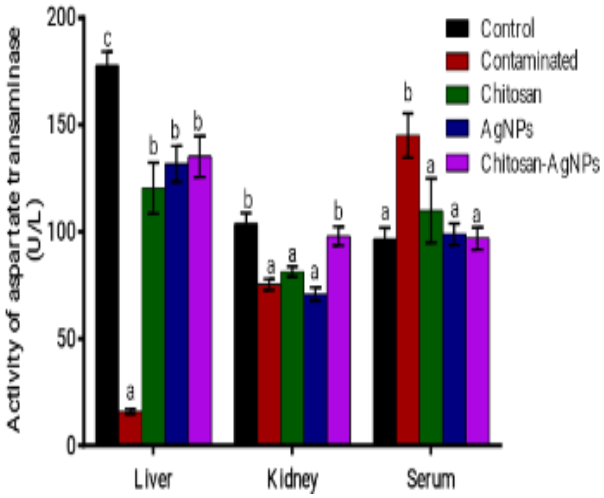


Figure 4: Effect of Chitosan-AgNPs treated water on the liver, kidney and serum AST activity of rats

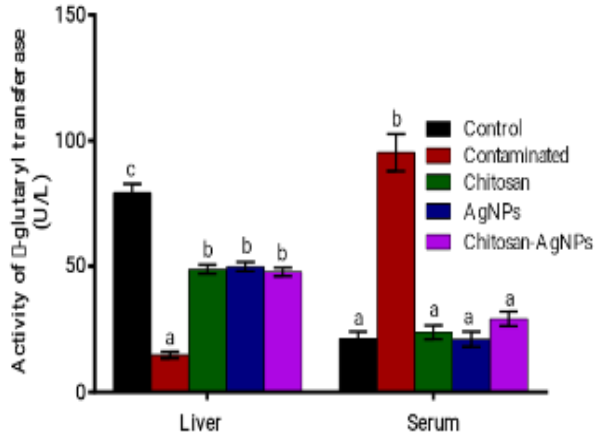


Figure 5: Effect of chitosan-AgNP-treated water on the liver and serum GGT activity of rats

Table 1: Effect of chitosan-AgNPs treated water on other kidney and liver function indices of rats

Parameter/ Group	Control	Contaminated water	Chitosan-treated water	AgNP-treated water	Chitosan-AgNPs treated water
Creatinine (mg/dL)	43±0.13^{a}	90±0.08^{d}	69±0.09^{c}	60±0.04^{b}	47±0.03^{a}
Urea (mg/dL)	16.37±1.97^{a}	21.73±4.03^{b}	20.12±3.79^{b}	17.13±4.05^{a,b}	16.82±2.14^{a}
Albumin (mg/dL)	10.15±2.84^{d}	5.10 ±1.58^{a}	8.89±1.73^{b,c}	7.06±2.37^{b}	9.8±1.23^{c,d}
Total Bil (µmol/L)	6.23±0.89^{a,b}	9.15±0.75^{c}	7.89±0.58^{b}	7.21±0.41^{a,b}	6.37±0.43^{a}
Dir Bil (µmol/L)	3.70±0.56^{a}	6.06±1.71^{c,d}	6.04±0.32^{c}	4.81±0.54^{b}	3.90±0.57^{a}

Values with different superscripts are significantly different ($p < 0.05$) from each other

Table 2: Bacteriological analyses of Gbugudu water sample

Day	Total heterotrophic count (CFU/mL)	Total Coliform count (CFU/100 mL)	Total faecal count (CFU/100 mL)
1	1.77 x10^{7}	1.44x10^{7}	7.20x10^{6}
3	1.55 x10^{7}	1.08x10^{7}	6.50x10^{6}
5	1.40x10^{6}	9.70x10^{5}	5.00x10^{5}
7	1.32x10^{6}	8.90x10^{5}	4.30x10^{5}
9	1.19x10^{6}	6.90x10^{5}	3.70x10^{5}
11	9.50x10^{5}	6.30x10^{4}	2.80x10^{4}
13	8.30x10^{5}	3.90x10^{4}	1.90x10^{4}
15	7.00x10^{4}	2.10x10^{3}	1.10x10^{3}
17	5.50x10^{4}	1.20x10^{2}	5.00x10^{2}
19	4.30x10^{3}	5.00x10^{2}	1.00 x10^{2}
21	3.50x10^{2}	0.00	0.00
23	2.50x10^{2}	0.00	0.00
25	1.50x10^{2}	0.00	0.00
27	9.0x10^{1}	0.00	0.00

DISCUSSION

Noble metals such as Ag and Au, as well as ZrN, are well-known for their unique optical properties resulting from Surface Plasmon Resonance (SPR). In this study, the synthesis of AgNP was monitored using visual observation and ultraviolet-visible light (UV-Vis) spectroscopy. The color change and progressive decolorization of the NP solution within the first 10 minutes, followed by a reddish-brown color after 1 hour, confirmed the formation of AgNP from Ag. This color change indicated the reduction of silver metal ions (Ag^{+}) into AgNP (Ag0) through the active molecules present in the banana stem extract.

The characterization of nanoparticle formation in water treatment applications is crucial in understanding both the physicochemical behavior and efficacy of the synthesized materials. In the context of chitosan-silver nanoparticle composites, the formation and stability of nanoparticles are influenced by precursor concentration, surface chemistry, and the interaction with stabilizing agents like biopolymers. These dynamics are essential when developing point-of-use filtration systems, particularly for rural or household water treatment purposes. The optical and elemental characterization techniques such as UV–Vis spectroscopy and energy dispersive X-ray (EDX) provide robust evidence of successful nanoparticle synthesis, which directly relates to the efficiency of pollutant capture and microbial inhibition. The integration of silver nanoparticles with biopolymers like chitosan has demonstrated promising results in various studies, highlighting its potential in domestic water purification applications by effectively removing organic and microbial contaminants from drinking water sources. This aligns with previous findings that emphasized the practicality of such composites in resource-limited settings, ensuring access to safer water through innovative material engineering [8,9,11].

Bacterial contamination levels in water, including total coliform, fecal coliform and heterotrophic counts, are key indicators of water quality [11]. The WHO and National Agencies set permissible bacterial limits for safe drinking water, with acceptable levels ranging from 100 to 500 CFU/mL [12]. In this study, untreated raw water exceeded WHO standards, with bacterial counts of 1.77 x 10^{7} CFU/100 mL for total heterotrophic, total coliform and fecal coliform counts [13]. After treatment with chitosan-AgNP, bacterial levels significantly decreased, indicating improved water quality for reuse. Heterotrophic bacteria, which naturally occur in humans and animals, are used to assess water quality but do not specifically indicate fecal contamination [14].

Alkaline phosphatase (ALP) serves as a marker enzyme for the plasma membrane integrity [15].

Abnormal levels of ALP indicate membrane damage. The reduction in ALP activity observed in the liver and kidneys of rats administered contaminated water, compared to the control and other groups, may be attributed to the presence of pathogens and other contaminants that attacked the membrane, causing enzyme leakage from the cell to the extracellular matrix. This is supported by the significantly higher serum ALP levels in rats maintained on contaminated water compared to the control and rats administered with chitosan-AgNPs-treated water. Also, AST activity provides evidence of tissue dysfunction resulting from consuming contaminated water. Reduction in liver and kidney AST activities, along with an increase in serum AST activity in rats exposed to contaminated water, suggests that constituents of contaminated water may inhibit the enzymes or cause their leakage into the serum. Elevated serum levels of AST and ALT imply impaired liver function [16]. Similarly, exposure to heavy metal ions-polluted water resulted in reduced activities of AST and ALT in the small intestine of freshwater bivalves [17]. Increased serum AST activity has been reported in animals exposed to polluted water [18], further supporting the hypothesis of enzyme leakage into the serum.

Elevated concentrations of urea and creatinine in rats exposed to contaminated water indicate kidney injury. The increased bilirubin levels in rats exposed to contaminated water may be due to oxidative damage induced by the contaminants, potentially impacting the liver, red blood cells, or heart. Also, reduced albumin levels in these rats further indicate liver impairment.

CONCLUSION

The findings from this study reveal that chitosan, AgNP and chitosan-AgNP elicit antimicrobial effects. However, chitosan-AgNP is more effective as it is more potent than chitosan, and AgNP and presents no marked signs of toxicity.

DECLARATIONS

Acknowledgement/Funding

We would like to thank the Technologists of the Department of Biochemistry for their technical support.

Ethical approval

The study was approved by the Institutional Review Board (or Ethics Committee) of Research Ethics Committee of Kwara State University Research Council (protocol code KWASU/CRIT/REA/2022/002 and 01/05/2022).

Use of Artificial intelligence/Large language models

We declare also that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Conceptualization, Raliat Abimbola Aladodo, Abdulhakeem Olarewaju Sulyman and Saheed Sabiu; Methodology, Raliat Abimbola Aladodo, Abdulhakeem Olarewaju Sulyman and Mutiu Adewunmi Alabi; Software, Abdulhakeem Olarewaju Sulyman, Mutiu Adewunmi Alabi, and Saheed Sabiu; Validation, Abdulhakeem Olarewaju Sulyman, Rasheed Bolaji Ibrahim, and Saheed Sabiu; Formal analysis, Abdulhakeem Olarewaju Sulyman, Rasheed Bolaji Ibrahim, and Saheed Sabiu; Resources, Fausat Abimbola Jimoh, Yusuf Ayodeji Iyanda, Juwon Samuel Afolayan and Ibrahim Opeyemi Ibrahim; Data curation, Abdulhakeem Olarewaju Sulyman, Rasheed Bolaji Ibrahim; Investigation, Juwon Samuel Afolayan and Ibrahim Opeyemi Ibrahim; Writing—original draft preparation, Abdulhakeem Olarewaju Sulyman, Juwon Samuel Afolayan and Ibrahim Opeyemi Ibrahim; Writing— review and editing, Abdulhakeem Olarewaju Sulyman, Raliat Abimbola Aladodo, Mutiu Adewunmi Alabi, Juwon Samuel Afolayan, Ibrahim Opeyemi Ibrahim, C.C.K; visualization, Abdulhakeem Olarewaju Sulyman and Saheed Sabiu; Supervision, Abdulhakeem Olarewaju Sulyman, Fausat Abimbola Jimoh and Yusuf Ayodeji Iyanda; Project administration, Abdulhakeem Olarewaju Sulyman, Saheed Sabiu and Chidolue Chinenye Kingsley. All authors read and agreed on the published version of the manuscript.

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