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Antibiofilm and Toxicity potential of Biogenically Synthesized Silver Nanoparticles

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ABSTRACT

In this study, biogenically synthesized Ag silver nanoparticles (NPs) using four bacterial strains *viz*., *Escherichia coli* (MT448673), *Pseudomonas aeruginosa* (MN900691), *Bacillus subtilis* (MN900684) and *Bacillus licheniformis* (MN900686) were tested for their antibiofilm potential and toxicity evaluation using mice model. The biofilm time kinetics of four bacterial isolates was analysed using crystal violet staining assay followed by antibiofilm activity of biogenically synthesized AgNPs (10 - 100 μgmL^{-1}). For toxicity evaluation, albino male mice (BALB/c) were administered with 50 $mgkg^{-1}$, 100 $mgkg^{-1}$ and 150 $mgkg^{-1}$ of AgNPs, respectively except for control. Log-probit regression analysis was used to measure the dosage response to determine the median lethal dose (LD_{50}). Histopathology of male albino mice liver and kidney was studied after 30 days experimental period. Results revealed that all four strains had ability to form strong biofilms between 48 to 72 h of incubation. Two strains (*B. subtilis* and *B. licheniformis*) formed significant ($p < 0.05$) biofilm after 3 days while other two strains (*E. coli* and *P. aeruginosa*) showed strong biofilm formation after 2 days. Antibiofilm study revealed that 60-90% biofilm inhibition was observed at 60 μgmL^{-1} of AgNPs, while maximum inhibition (*i.e.,*100%) was found at highest concentration (90 μgmL^{-1}). Exposure to AgNPs caused significant changes in the levels of serum AST ($P < 0.05$) at the 100$mgkg^{-1}$ and 150$mgkg^{-1}$ of AgNPs exposure, while ALT and serum creatinine ($P > 0.05$) levels remained normal. Mice exposed to heavy dose (150 $mgkg^{-1}$) of biogenic AgNPs showed slight cell distortion and detachment of hepatocytes in the liver. Regarding kidney, at higher concentration (150 $mgkg^{-1}$) kidneys showed smooth surface and dark red colour with proliferation of podocytes. It can be concluded from present study that biologically synthesized AgNPs caused significant ($p < 0.05$) biofilm decrease at higher concentration. Toxicity evaluation revealed that biologically synthesized AgNPs are small to be eliminated easily by kidney and therefore the liver and kidney did not show toxicity at low concentrations. However, this is a short-term study and slight toxic effects at high concentrations necessitates further investigation of interactions between AgNPs and full biological systems in a time-dose dependent manner.

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IL designed research and supervised the study. IL and ST performed research. HA, AAL and AB contributed analytic tools. SN, SA and AK analyzed data. IL wrote the manuscript. All authors approved the final version of manuscript.

Key words
Biofilm formation, Antibiofilm potential, Nanotechnology, Pathogenic bacteria, Biogenic silver Nanoparticles, Toxicity assessment, Histopathology

INTRODUCTION

Nanomaterials have been considered one of the most forefront materials as biological, therapeutic, medical and antimicrobial agents in recent decades. Compared to conventional materials, nanoparticles exhibit unique electrical, light-emitting and catalytic properties. They have been reported to be the material of the 21st century because of their unique designs (Tufail *et al*., 2022). Silver nanoparticles (AgNPs) have recently gained popularity and are widely used in medicine, medicinal devices,

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pharmacology, food industries, cosmetics, electronics, paints and also in environmental remediation (Recordati *et al*., 2015). The small size (1–100 nm) of AgNPs enhances their physical, chemical, magnetic, and optical properties. Furthermore, AgNPs have become one of the most common nanomaterials used as antimicrobial agents and disinfectants in medicine and industry due to their highly effective antibacterial activity both in solution and in components. The investigation of bactericidal activity of AgNPs is intensifying because of the emergence of new drug-resistant bacterial strains (Banerjee *et al*., 2014).

The bacterial infection and multidrug-resistant phenotype arise from the ability of the bacteria to form biofilms which make them resistant to antibiotics. The bacteria such as *Staphylococcus aureus*, *Acinetobacter baumannii, Escherichia coli, Pseudomonas aeruginosa*, are known to cause infections due to biofilm formation. Bacterial biofilms are complex surface attached communities of bacteria held together by self-produced polymer matrix primarily composed of polysaccharides, proteins and extracellular DNAs (Liaqat *et al*., 2009). The bacterial biofilms are associated with major infectious diseases and their resistance against antibiotics. In this case, the AgNPs has the strong potential to penetrate in to the mature biofilm due to their small size and higher volume ratio (Liaqat *et al*., 2014). Previously, the AgNPs were analyzed to inhibit the biofilm production of *E. coli* and *P. aeruginosa* at very low concentration (0.2 μgmL^{-1}) (Barapatre *et al*., 2016). Shahwany *et al*. (2016) also reported that AgNPs inhibited the biofilm formation by *S. aureus* and *Klebsiella pneumoniae* at the concentration of 0.0683 μgmL^{-1} and 0.0596 μgmL^{-1}, respectively.

Nanotoxicology has emerged as a new discipline to investigate the adverse effects of NPs. Different size and shapes of AgNPs have different toxicities (Elkhawass *et al*., 2015). Also, the uses of AgNPs in human activities have been gradually increasing (Aziz *et al*., 2014). Hence, the study on biological effect of AgNPs and particularly their effects on animal and human organisms is also increasing (Zhang *et al*., 2016). Accordingly, among studies performed on various metal NPs, AgNPs have shown more toxicity compared to other metals such as nickel, iron, aluminium and manganese (Iravani *et al*., 2014).

Organization for Economic Cooperation and Development, guidelines recommend oral toxicity test, eye irritation, corrosion and dermal toxicity, and lethal $Dose_{50}$ (LD_{50}) in order to determine the acute *in vivo* toxicity of nanomaterials (Deepak *et al*., 2011). Many parameters such as dose, route of exposure, excretion and immune response, metabolism are important to perform *in vivo* toxicity studies. Since the last decade, there is a substantial increase in the number of ongoing nanotoxicology studies to investigate the biological pathways taken by nanoparticles and induced toxic effects (Aziz *et al*., 2014). Free radical formation leading to oxidative stress is the most important mechanism of *in vivo* nanotoxicity. In case of AgNPs, *in vivo* generated hydrogen peroxide will react with AgNPs and release Ag^{+} ions. The excess free radical generations will damage cellular macromolecules via oxidation of proteins, lipids and DNA. Injury of cell membranes will thus result in leakage of cytoplasmic contents and necrosis (Liaqat, 2009; Khan *et al*., 2019).

The current study was aimed to assess the antibiofilm potential and toxicity of biogenically synthesized AgNPs in male albino mice. To achieve this, biofilm forming ability of four pathogenic isolates was determined along with histopathological studies of liver and kidney.

MATERIALS AND METHODS

Synthesis of biogenic AgNPs

Biogenic synthesis of AgNP was made using four human pathogenic bacteria (both Gram negative and Gram positive), such as *E. coli* (MT448673), *P. aeruginosa* (MN900691), *B. subtilis* (MN900684) and *B. licheniformis* (MN900686) following method of Tufail *et al*. (2022). Briefly, sterilized nutrient broth was prepared and inoculated with fresh culture of isolates followed by incubation at 37 °C. 10mM silver nitrate ($AgNO_3$) solution was added and incubated in dark. The AgNPs were collected by centrifugation and stored for further study.

Antibiotic susceptibility testing

Antibiogram study of four test pathogens against antibiotics was checked using the Kerby-Bauer disc diffusion method (Liaqat *et al*., 2009). Briefly, Muller Hinton agar (MHA) was prepared. Bacterial cultures, OD_{600} adjusted to 0.5 McFarland turbidity standard, were spread on the MHA plates with the help of sterile cotton buds. Four antibiotic discs were used to check the antibiotic sensitivity of *E. coli*, *P. aeruginosa*, *B. subtilis*, and *B. licheniformis*. The antibiotics used were ampicillin (Am-50 μgml^{-1}), rifampicin (Rif-50 μgml^{-1}), erythromycin (Ery-20 μgml^{-1}) and lincomycin (Linc-50 μgml^{-1}). The prepared antibiotic discs were placed on the media plates and labeled properly. The plates were incubated at 37 °C for 24 h. The appearance of the zones around the disc showed sensitivity against the antibiotics and vice versa. The zones of inhibition were measured in mm.

Determination of time kinetics of biofilm formation

In order to determine time kinetics of biofilm formation, test strains were quantified using the method of

Abdalla *et al*. (2020). Overnight pre-inoculum of bacterial pathogens were prepared. Glass tubes containing 5 mL nutrient broth were prepared and autoclaved. 50 µL of pre-inocula of four strains were added to test tubes except for control and incubated at 37 °C with shaking for 2, 3 and 5 days. The first set of glass tubes was taken out after two days, cultures were discarded and tubes were dried at 37 °C for 10 min. After drying, 5 mL of 0.1% crystal violet was added for a few min, discarded, tubes were rinsed with 0.85% NaCl. Later, 5 mL of 33% glacial acetic acid was added and OD_{523} was noted. Similar procedure was repeated with tubes incubated for 3 and 5 days. The experiment was carried out in triplicates.

Determination of antibiofilm activity of biogenic AgNPs

Antibiofilm activity of AgNPs was measured using the test tube method (Guideline, 2001; Al-Aboody, 2019). 03 mL of prepared nutrient broth was added to the sterile test tubes and 100 µL of pre-inoculum was added. Following that, varying concentrations (10–100 μgmL^{-1}) of biogenic AgNPs were added to test tubes and incubated at 37 °C for 24 h except for controls. Two controls, one with cell free supernatant of each respective strains and other with $AgNO_3$ were run in parallel. After incubation, the content of each test tube was removed and washed with saline solution 3X to remove free-floating planktonic bacteria. Biofilm formed by the adherent sessile organism on the test tube's wall was stained with crystal violet dye and tubes were allowed to dry. After drying, the test tubes were washed with 33% glacial acetic acid. The absorbance at 523 nm was measured, which showed the bacterial adhering to the tube's wall for developing biofilm. The experiment was repeated thrice.

The biofilm inhibition percentage was calculated by using the formula:

$$\% \text{ Biofilm inhibition} = \frac{1 - OD_{523}\text{nm of cells treated with biogenic nanoparticles}}{OD_{523}\text{of non} - \text{treated}} \times 100$$

Experimental animals

Albino male mice (BALB/c) (10–14 weeks old with 26–40 g body weight) were obtained from animal house of the Government College University, Lahore, Pakistan. They were kept in cages and supplied with commercially available standard pellet diet and water *ad libitum*. They were housed in an air-conditioned room with a constant temperature range (22 ± 1 °C) and constant light/dark cycle (12:12 h). Acclimatization of the animals to the laboratory condition was achieved by keeping the animals in the cages for at least 15 days prior to dosing.

Toxicity assessment

The subacute toxicity assessment of AgNPs was performed using albino male mice. Mice were acclimatized for 15 days before the delivery of AgNPs.

LD_{50} determination

Lethal dose (LD_{50}) for AgNPs was determined using commercially available procedures (Hussain *et al*., 2005). The mice were administered with 200, 400, 600, 800 and 1000 $mgkg^{-1}$ (per body weight) biogenic AgNPs via oral route. Signs of toxicity and death of animal were monitored for 24 h. Log-probit regression analysis was used to measure the dosage response to determine the median lethal dose (LD_{50}).

Sub-lethal dose determination and experimental groups

Afterwards, another set of mice was divided into various groups as detailed in Supplementary Fig. S1 and sub lethal doses of AgNPs were administered. In brief, 10 albino mice were taken in each group. The mice were supplied with 50 $mgkg^{-1}$, 100 $mgkg^{-1}$ and 150 $mgkg^{-1}$ of AgNPs, respectively except for control. The treatment was run for 30 days. Group 1mice with no AgNPs served as control. Group 2 mice were fed with 50 $mgkg^{-1}$ of AgNPs, group 3 with 100 $mgkg^{-1}$ of AgNPs and group 4 mice were fed with 150 $mgkg^{-1}$ of AgNPs.

Study of liver and kidney function biomarkers

Considering the fact that AgNPs are accumulated mostly into the liver and to some extent in lung and kidney, the current study analysed the toxic effects of biogenic AgNPs in liver and kidney by measuring concentration of liver and renal enzymes. Levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine of the experimental male albino mice were measured and compared with control mice.

Histopathological study

After 30 days of treatment, 2 mice from each group (treated and control) were sacrificed to obtain their liver and kidneys for further histopathological analysis. The collected organs were fixed in formaldehyde solution and embedded in paraffin. Following suitable processing, organ sections were stained with hematoxylin and eosin. The sections of kidney and liver from respective mice were observed under light microscope at 40 and 100X magnification. These sections were compared for any pathological changes implicated upon treatment with biogenic AgNPs.

Statistical analysis

Data collected from all the biochemical analysis were expressed as Mean ± SEM. Significant differences

between the groups were determined by one-way ANOVA. All the statistical calculations were performed using Graph Pad Prism. The significance level was set at $P \leq 0$ .05.

RESULTS

Biogenic AgNPs

The biogenic AgNPs used in this study were reported to be stable for almost 8-12 weeks (Tufail *et al*., 2022). The specific biomolecules present in the supernatant of bacteria play important role as capping and reducing agent and provide great stability to the AgNPs (Khan *et al*., 2019). The results of characterization of biogenic AgNPs has already been described in our previous study (Tufail *et al*., 2022).

Antibiotic susceptibility testing

Disc diffusion method was used to isolate the antibiotic resistant strains. Four antibiotic discs (ampicillin 50 µgmL^{-1}, rifampicin 50 µgmL^{-1}, erythromycin 20 µgmL^{-1} and lincomycin 50 µgmL^{-1}) were used to check the antibiotic sensitivity of *P. aeruginosa, E. coli, B. subtilis* and *B. licheniformis*. The presence of inhibitory zones (ZOI) around the discs showed sensitivity of bacterial strains against the tested antibiotics and vice versa. The antibiotic ampicillin (Am-50 µgmL^{-1}) showed 3-6 mm ZOI, rifampicin (Rif-50 µgmL^{-1}) 2-6 mm, erythromycin (Ery-20 µgmL^{-1}) 3-7 mm and lincomycin (Linc-50 µgmL^{-1}) 4-7 mm ZOI against test strains. All tested strains showed ZOI smaller than 10 mm so, they were considered as resistant against antibiotics (Table I).

Time kinetics of biofilm formation assay

Quantification of biofilm was done using test tube assay following crystal violet staining over a period of different time intervals *i.e.,* 2, 3, 5 and 7 days in test tubes. All the four strains have an ability to form strong biofilm. Two strain (*B. subtilis* and *B. licheniformis*) formed significant ($p < 0.05$) biofilm after 3 days of incubation period and the other two strains (*E. coli* and *P. aeruginosa*) showed strong biofilm formation after 2 days of incubation (Fig. 1).

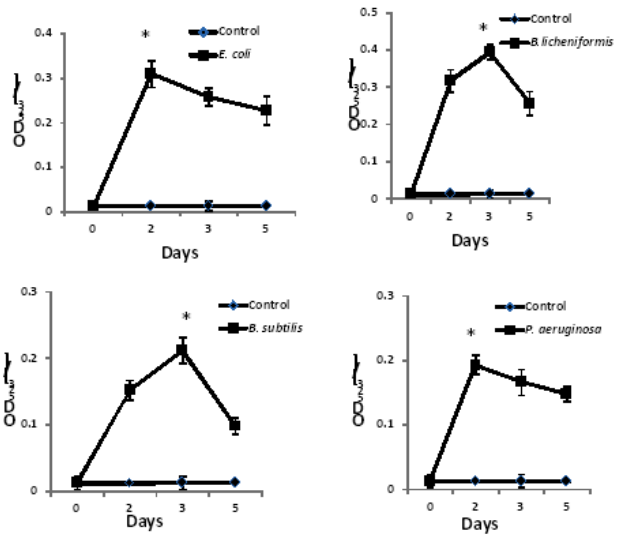

Fig. 1. Biofilm time kinteics by four human pathogenic isolates. *B. subtilis* and *B. licheniformis* formed significant ($p < 0.05$) biofilm at 3 days of incubation period compared to *E. coli* and *P. aeruginosa*, which showed strong biofilm formation at 2 days of incubation.

Antibiofilm activity of biogenically synthesized AgNPs

AgNPs synthesized extracellularly using *P. aeruginosa, B. subtilis, E. coli* and *B. licheniformis* were assessed for antibiofilm activity against biofilm-forming bacterial strains. The inhibition of microbial biofilm by AgNPs was evaluated by treating with AgNPs (90 µgmL^{-1} and 100 µgmL^{-1}) for 24 h. It was observed that concentration dependent decrease in biofilm formation of all strains was observed following treatment with AgNPs. Highest percent biofilm inhibition (100.0 ± 0.0; $p < 0.05$) was measured at 90 µgmL^{-1} and 100 µgmL^{-1}concentration of AgNPs while least percent biofilm inhibition (10.3 ± 0.3; $p < 0.05$) was measured at 10 µgmL^{-1} of AgNPs against test pathogens.

Table I. Antibiotic susceptibility test of test strains.

	Control (C)	Zones of inhibition (ZOI) in mm			
		Ampicillin 50 µg.mL^{-1}	Rifampicin 50 µg.mL^{-1}	Erythromycin 20 µg.mL^{-1}	Lincomycin 50 µg.mL^{-1}
E. coli	R	4.0 ±0.02	4.1±0.02	5.0±0.01	6.0±0.02
P. aeruginosa	R	3.0±0.03	2.2±0.01	3.0±0.02	4.0±0.01
B. subtilis	R	5.2±0.03	6.0±0.01	4.0±0.01	7.0±0.02
B. licheniformis	R	6.1±0.02	5.0±0.02	7.0±0.02	5.0±0.01

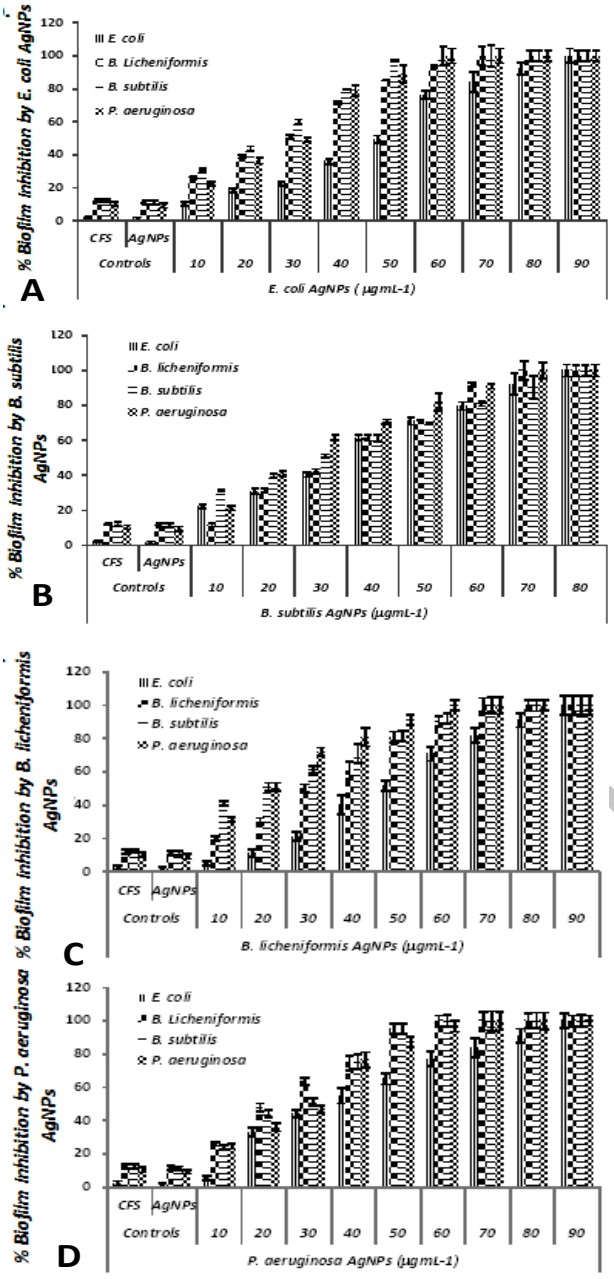

Fig. 2. The antibiofilm activity of biogenic AgNPs against *E. coli, B. licheniformis, B. subtilis* and *P. aeruginosa* at different concentrations (10-90 µgmL^{-1}). (A) *E. coli* AgNPs antibiofilm potential against four human pathogenic isolates. (B) *B. subtilis* AgNPs antibiofilm potential against four human pathogenic isolates. (C) *B. licheniformis* AgNPs antibiofilm potential against four human pathogenic isolates. (D) *P. aeruginosa* AgNPs antibiofilm potential against four human pathogenic isolates. The results were expressed as the Mean±S.E of three replicates and compared with control. Treated groups showed statistically significant differences from the control group by the student's *t* test p (< 0.05).

E. coli AgNPs exhibited highest antibiofilm activity at 90 µgmL^{-1} against all tested strains while the least biofilm inhibition (10.3 ± 0.3; $p < 0.05$) was observed against *E. coli* at 10 µgmL^{-1} (Fig. 2A). *B. subtilis* AgNPs showed greater antibiofilm activity at 80 µgmL^{-1} against all tested strains and least biofilm inhibition (11.6± 0.8; $p < 0.05$) was observed at 10 µgmL^{-1} against *B. licheniformis* (Fig. 2B). *B. licheniformis* and *P. aeruginosa* AgNPs inhibited biofilm formation at all concentrations but most successfully reduced at 60 to 90 µgmL^{-1}concentration. Least biofilm inhibition (5.0 ± 0.5; $p < 0.05$ was observed at 10µgmL^{-1} against *E. coli* and highest biofilm inhibition was observed against *P. aeruginosa* (Fig. 2C, D).

Biochemical analysis

The serum AST level of untreated control groups varied from 155 to 159 U/L. There were no significant differences ($P > 0.05$) in serum AST levels between the control and the treatment groups at 50 mgkg^{-1} concentration after 30 days treatment period. However, there was significant increase ($P < 0.05$) in AST levels at 100 mgkg^{-1} and 150 mgkg^{-1} concentrations. There was no significant difference ($P > 0.05$) in serum ALT and creatinine levels of untreated groups and treated concentration at 50, 100 and 150 mgkg^{-1} after 30 days of treatment (Fig. 3).

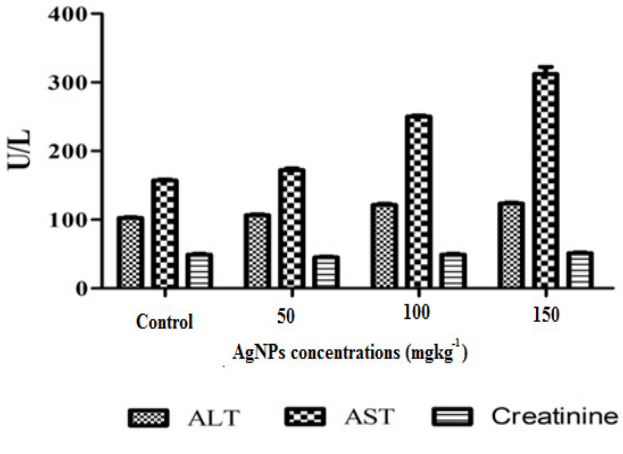

Fig. 3. Effect of AgNPs on alanine aminotransferase (ALT), aspartate transaminase (AST) and creatinine levels in male albino mice.

Lethal dose value (LD_{50})

The LD_{50} value was determined to be 600 mgkg^{-1} for *P. aeruginosa* based AgNPs. It was observed that higher value of LD_{50} make the NPs less toxic to the environment as well as the living tissues. The size of the NPs also play significant role in toxicity assessment because smaller particles have more ability to penetrate inside the cell as compared to larger size nanoparticles (Rosas-Hernández

et al., 2009). The concentration (dose) is also important to evaluate the LD_{50} concentration. However, more concentration (dose) lowers the value of LD_{50}, make the particles more toxic (Fig. 4).

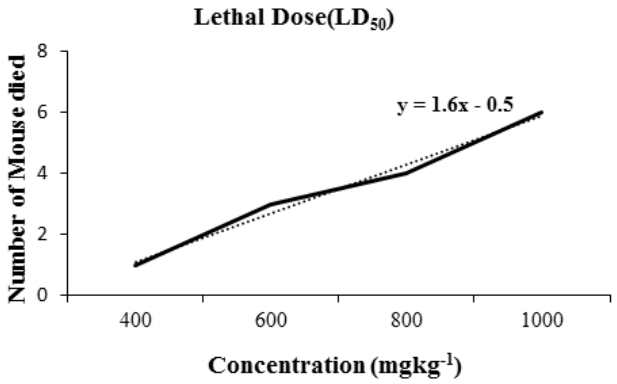

Fig. 4. Evaluation of LD_{50} of AgNPs in male albino mice through probit method.

Histopathological study of liver and kidney

The histopathological studies of liver and kidney after exposure to biogenically synthesized AgNPs showed slight toxicity at higher concentration (dose), i.e., 150 $mgkg^{-1}$ compared to the groups administered with 50 $mgkg^{-1}$ and 100 $mgkg^{-1}$ concentrations. Male albino mice administered with 50 $mgkg^{-1}$ and 100 $mgkg^{-1}$ AgNPs showed normal hepatocellular architecture. *P. aeruginosa* AgNPs showed no histopathological changes in liver and kidney except for slight inflammatory infiltration of the mice kidney cells (indicated by yellow arrows) on treatment with high dosage 150 mgkg-1 of AgNPs. *B. licheniformis* AgNPs showed slight interstitial fibrosis with high dosage treatment (Fig. 5A, B). *B. subtilis* AgNPs shows slight histopathological changes in liver and kidney on treatment with high dosage 100 and 150 $mgkg^{-1}$ of AgNPs. *E. coli* AgNPs showed slight proliferation of podocytes with high dosage treatment of AgNPs (Fig. 5C, D).

DISCUSSION

Bio-synthesized AgNPs have been proved as therapeutic agent and valuable compounds. They also have excellent antiviral and antimicrobial activity (Tufail *et al*., 2022; Rosas-Hernández *et al*., 2009; Al-Taee, 2020; Ong *et al*., 2016). There are a lot of methods which can be utilized for AgNPs synthesis. However, biogenic synthesis of AgNPs is more eco-friendly approach (Ong *et al*., 2016; Murray *et al*., 2015). In the present study, bacterial strain *P. aeruginosa* based AgNPs were used for antibiofilm potential and toxicity evaluation *in vivo*.

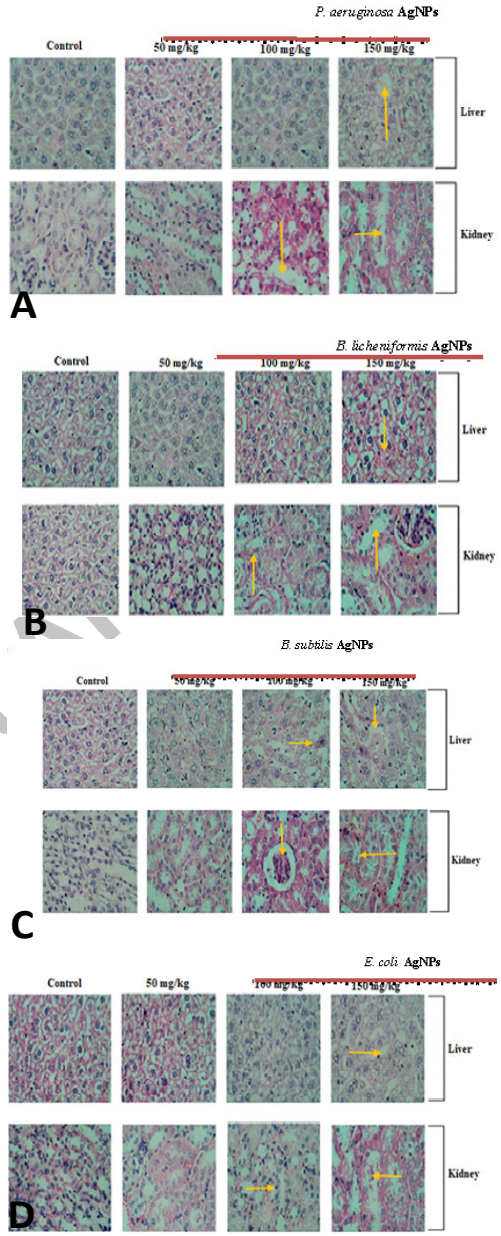

Fig. 5. Effect of oral administration of biogenic AgNPs on liver and renal histology in male albino mice following 30 days experimental period. (A) *P. aeruginosa* AgNPs showed no histopathological changes in liver and kidney except for slight inflammatory infiltration of the mice kidney cells (indicated by yellow arrows) on treatment with high dosage 150 mgkg-1 of AgNPs. (B) *B. licheniformis* AgNPs showed slight interstitial fibrosis with high dosage treatment. (C) *B. subtilis* AgNPs shows slight histopathological changes in liver and kidney on treatment with high dosage 100 and 150 $mgkg^{-1}$ of AgNPs. (D) *E. coli* AgNPs showed slight proliferation of podocytes with high dosage treatment of AgNPs.

As first step, antibiotic susceptibility test was performed to analyse the antibiotic resistance pattern of selected bacterial strains. It was observed that *P. aeruginosa* was highly resistant against all tested antibiotics (ampicillin, rifampicin, erythromycin and lincomycin). Previously, Gelband *et al*. (2015) also observed that *P. aeruginosa* is known as superbug and is highly resistant towards all the antibiotics, which might be due to its production of high extracellular polymeric substance and strong biofilm forming ability. Among tested four isolates, rifampicin was found to be highly effective against *B. subtilis* and *B. licheniformis* (Gram positive) and was used as positive control in subsequent study. The increased bacterial resistance observed in this study might be due to over and miss-use of the antibiotics (Haney *et al*., 2018).

The biofilm formation time kinetics of four bacterial isolates (*P. aeruginosa*, *E. coli*, *B. licheniformis* and *B. subtilis*) were analysed following crystal violet staining method. Previously, various researchers followed crystal violet method to measure biofilms developed by different bacteria (Pericolini *et al*., 2018; Liaqat *et al*., 2021). All the four strains showed ability to form strong biofilms between 48 to 72 h of incubation. Two strain (*B. subtilis* and *B. licheniformis*) formed significant ($p < 0.05$) biofilm after 3 days of incubation period. Similarly, Uhlich *et al*. (2014) determined biofilm time kinetics of 11 multispecies biofilm groups and found that all 11 multispecies biofilm groups showed highly significant biofilm development at 72 h incubation, after which biofilm decreased with time. The other two strains (*E. coli* and *P. aeruginosa*) showed strong biofilm formation after 2 days of incubation. Our results sustained that *E. coli* formed strong biofilm at 48 h incubation (Chan *et al*., 2007), while the findings by Liaqat *et al*. (2019) showed biofilm development after 3 days (72 h) incubation with an increased number of *E. coli* cells. The results of this study corroborate with the findings of (Liaqat *et al*., 2021), who reported that *P. aeruginosa* formed significant ($p < 0.05$) biofilm after 2 days (48 h). The above result showed contradiction with (Gallo and Schillaci, 2021), who reported that *P. aeruginosa* produced strong biofilm formation after 3 days (72 h).

Next, the antibiofilm activity of biogenically synthesized AgNPs (10-100 μgmL^{-1}) was analysed against *P. aeruginosa, B. licheniformis, E. coli* and *B. subtilis*. Almost 60-90% biofilm inhibition was observed at 60 μgmL^{-1} of AgNPs. While maximum inhibition (*i.e.,* 100%) was found at highest concentration (90 μgmL^{-1}). From this work, it was evident that by increasing the concentration of AgNPs, decrease in biofilm formation was observed. Highest antibiofilm activity at 90 μgmL^{-1} by *E. coli* AgNPs is in accordance with (Ansari *et al*., 2015), who reported *E. coli* assisted AgNPs possessed antibacterial and antibiofilm potential against *P. aeruginosa*, *E. coli*, *S. aureus* and *K. pneumoniae* due to low level of polysaccharides, proteins, nucleic acids and lipids in AgNPs treated biofilm. Similarly, Ansari *et al*. (2014) reported the complete antibiofilm potential of AgNPs against pathogenic bacteria at 50 μgmL^{-1} concentrations. In another study, Shanthi *et al*. (2016) observed that at reduced concentration (10 μgmL^{-1}), the organisms continued to grow but it was analysed that 20 μgmL^{-1} concentrations of AgNPs significantly arrested biofilm formation without affecting viability, whereas 50 μgmL^{-1} completely blocked the biofilm formation. Similarly, *B. licheniformis* AgNPs inhibited biofilm formation at all concentrations of 60-90 μgmL^{-1}. It was reported previously, that *B. licheniformis* based AgNPs treatment for 24 h at different concentrations of 20, 50 and 100 μgmL^{-1} developed poor biofilm growth and showed high reduction (85%) of biofilm architecture of *V. parahaemolyticus* Dav1 at 100 μgmL^{-1}concentration (Ramachandran and Sangeetha, 2017). Our findings are consistent with the results of Liaqat *et al*. (2017), who observed that biofilm formation decreased with increasing AgNPs concentration.

The study revealed the toxic effects of AgNPs in male albino mice. The AgNPs treatments did not produce any significant change in body weight of the mice and even no mortality was observed after oral administration of dosage up to150 $mgkg^{-1}$. The results support previous report (Ong *et al*., 2016) which showed no death and no effect on the percentage weight between control and treatments groups of mice at similar concentration.

The biochemical parameters such as ALT, AST and creatinine are among the biomarkers for the liver and kidneys functions. The ALT levels observed from 105-125 U/L in this experiment were comparable with the results of Ong *et al*. (2016) who published that ALT levels of albino mice ranged from 55.0-208.2 U/L. There was significant increase of the AST at the dosage of 100 $mgkg^{-1}$ and 150 $mgkg^{-1}$ treatment group after 30 days of AgNPs administration. Moreover, a separate study by Srivastava and Mukhopadhyay (2015) demonstrated that AgNPs affect the activity of transaminase enzymes but did not produce adverse effects on the health of male albino mice. The antibacterial activity of AgNPs has already been explained in our previous studies (Liaqat *et al*., 2008, 2019). The biochemical tests revealed that biogenic AgNPs caused insignificant effects on ALT and creatinine levels in current study.

Histopathology of liver and kidney revealed that mice exposed to heavy dose (150 $mgkg^{-1}$) of AgNPs showed necrosis, cell distortion and detachment of hepatocytes in the liver, while at lower dose (50 $mgkg^{-1}$) kidney showed normal renal structure with normal glomeruli. However, at

higher concentration (150 $mgkg^{-1}$) kidneys showed normal surface and dark red colour with proliferated podocytes. The findings of this study are contradictory to Hussain *et al*. (2005) who reported that AgNPs at 5–50 μgmL^{-1} are highly toxic to liver cell of rat. The liver hepatocytes were gradually distorted and some degree of necrosis and apoptosis was observed at higher dosing rates of bacterial synthesized AgNPs. At lower concentration (4 μgmL^{-1}), normal renal structure with normal glomeruli was observed. The above results authenticate the previous findings of Yaqub *et al*. (2019) who reported that higher concentration (100 $mgkg^{-1}$) of AgNPs cause liver complications in albino mice. The above-mentioned results are similar to Al-Taee (2020) who observed that high AgNPs concentration caused liver complications in mice. Similarly, Rosas *et al*. (2009), who reported that high concentrations of AgNPs (50–100 gmL^{-1}) induced a significant proliferative effect, indicating the association of proliferation with endothelial nitrogen oxide production.

CONCLUSION

In conclusion, the current study reported significant antibiofilm activity of easy, non-toxic and low cost prepared AgNPs. The biochemical analysis showed that AgNPs caused insignificant effects on the body of male albino mice. Meanwhile, the present results agreed with those who did not support any toxic effect of biogenically synthesized AgNPs on liver and kidney functions at low concentration. Though at high concentration, significant effect on liver morphology and biomarkers were noted. It can be concluded from the current study that biologically synthesized AgNPs are potent antimicrobial agents and small to be eliminated easily by kidney and therefore kidney did not show any toxicity effect. However, this is a short-term study and slight toxic effects at high concentrations necessitates further investigation of interactions between AgNPs and full biological systems in a time-dose dependent manner.

DECLARATIONS

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IRB approval

The study was approved by the Bioethics Committee of the Government College University, Lahore, Pakistan.

Ethical statement

Animal care and handling was performed following the official guidelines of OECD. The study was approved by the Bioethics Committee of the Government College University, Lahore, Pakistan. The experimental protocol was approved by the Bioethics Committee and conducted in accordance with the National Institute of Health guide for the care and use of laboratory animals while making efforts to minimize animal suffering.

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20240105143243

Statement of conflict of interest

The authors have declared no conflict of interest.

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Supplementary Material

Antibiofilm and Toxicity potential of Biogenically Synthesized Silver Nanoparticles

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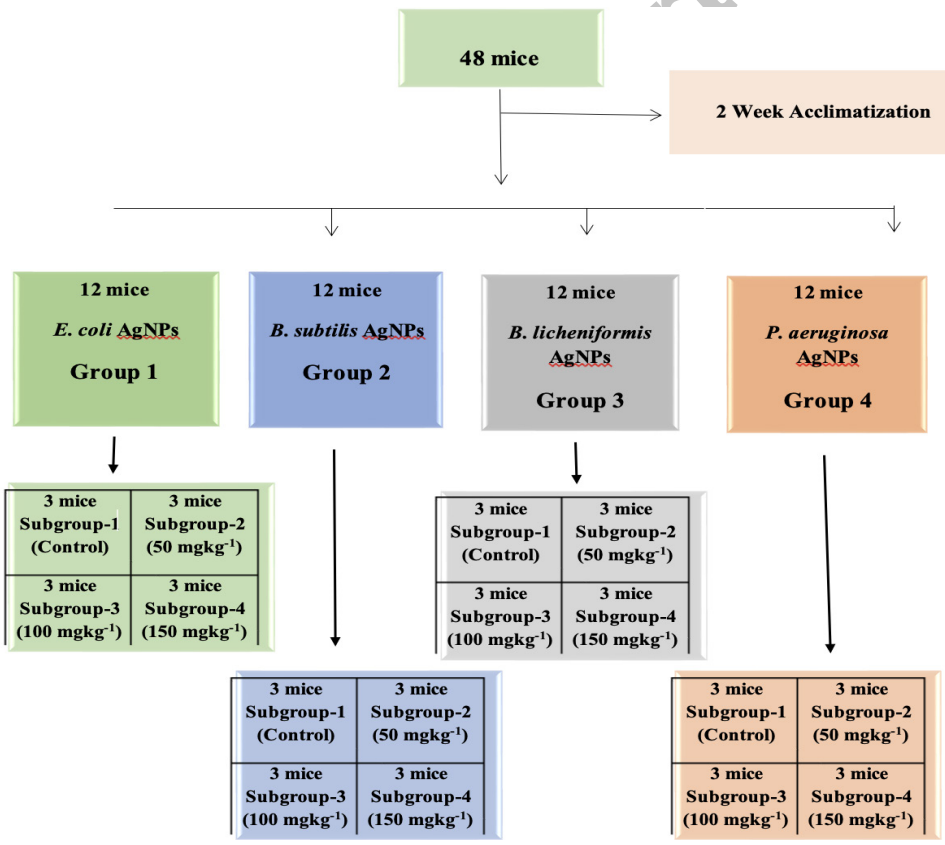

Supplementary Fig. S1. Details of various groups of mice in the study.

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