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Full Length Research

Characterization, antimicrobial and toxicological properties of silver nanoparticles synthesized from *Ocimum gratissimum* leaves

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ABSTRACT: *Ocimum gratissimum* leaves were used in the synthesis of silver nanoparticles (AgNps) that were non-toxic and also possessed antimicrobial properties against clinically isolated pathogenic strains. Some bioactive agents in the aqueous extract of *Ocimum gratissimum* leaves (AEOgL) were identified. AgNp synthesis was carried out by incubating the AEOgL and 1 mM $AgNO_3$. The brownish colour obtained upon reduction of silver by the AEOgL was observed. The phytochemicals present are tannins, glycosides, sterols, phenols, alkaloid and terpenoids. There was the presence of a surface plasmon resonance from the UV-visible scan. The synthesized silver nanoparticles were characterized by UV-visible spectrophotometry, Fourier Transform Infrared spectroscopy (FTIR), Scanning Electron Microscopy/Energy Dispersive Analysis (SEM/EDX) and X-ray diffraction analysis. The SEM/EDX analysis indicated that the morphology of the nanoparticles is of a uniform structure and the size of the AgNp was 20 nm. The AgNps showed excellent antimicrobial activity against clinically isolated multi-drug resistant human pathogens used such as *Escherichia coli* and *Staphylococcus aureus*. For most biomarkers in the blood biochemistry analysis, there was no significant difference ($p<0.05$) between the control and treated groups except for alkaline phosphatase. It can concluded from this study that AgNps synthesized from aqueous extract of *Ocimum gratissimum* had antimicrobial properties and is also non-toxic at the dosage used.

Keywords: Antimicrobial properties, characterization, *Ocimum gratissimum*, silver nanoparticles, toxicity.

INTRODUCTION

Silver nanoparticles (AgNPs) have been reported for their wide applications in several research fields (Skonieczna and Hudy, 2018). For instance, they have been shown to be promising anticancer agents, the mechanisms of which include influence on cell cycle, inhibition of cell proliferation (Dziedzic et al., 2016), induction of apoptosis (Ho et al., 2009) and induction of oxidative stress (Braydich-Stolle et al., 2010). In addition, their roles in protection against bacterial (Bello-Vieda et al., 2018), fungal (Jo et al., 2009) and viral infections (Ishwarya et al., 2017) have also been well documented. These unique qualities make AgNPs very important among other metal based nanoparticles.

There are many ways in which silver nanoparticles can be synthesized and these include physical, chemical and biological method. Unfortunately, the physical and chemical methods which are numerous in number are expensive and require the use of hazardous chemicals, low material conversions, high energy requirements, difficult and wasteful purifications and so are termed as unfavourable in nanosilver synthesis (Sarsar et al., 2014). Therefore, environmental friendly processes for nanosilver synthesis without the use of hazardous chemicals need to be developed. Green chemistry approach stresses the fact that the use of natural organisms has provided a secure, easier, non-toxic and environmental friendly way for nanoparticle synthesis (Narayanan and Sakthivel, 2010; Sathishkumar et al., 2010). The synthesis of nanoparticles by biological methods, using microorganisms, enzymes

and plant or plant extracts, is the environmental friendly alternatives to chemical and physical methods of nanoparticle synthesis (Schultz et al., 2000; Nair and Pradeep, 2002). Biogenic synthesis is useful not only because of its reduced environmental effect (Shankar et al., 2004; Anastas and Zimmerman, 2007; Dahl et al., 2007) compared with some of the physicochemical production methods, but also because it can be used to produce large quantities of nanoparticles that are free of contamination and have a well-defined size and morphology (Hutchison, 2008). Silver (Ag) and gold (Au) nanoparticles have been the particular focus of plant-based syntheses and today, these materials can be synthesized and modified by various approaches (Mohanpuria et al., 2008; Cauerhff and Castro, 2013; Aisida et al., 2019). The biogenic synthesis of nanoparticles by plants or agricultural waste like peel extracts exceeds other biological methods because it is environmentally benign and quite rapid.

The most commonly reported technique for silver nanoparticle synthesis involves the use of physical or chemical processes. These methods are expensive, toxic and involves wasteful purification procedures. Hence, this study intends to synthesize silver nanoparticle through green chemistry using aqueous extract of *Ocimum gratissimum* leaves. *O. gratissimum* (family Labiatae) commonly referred to as Clove Basil. This plant often grows in temperate climates. The medicinal benefits of this plant have been well reported in the literature. The plant is known to have importance in folklore medicine such as in the treatment of cough, skin rashes and even headache just to mention a few (Corrêa 1932, Onajobi, 1986). In Nigeria, *O. gratissimum* is also regarded as an edible vegetable and it is very ubiquitous.

There have been numerous reports on the use of several members of the Ocimum family in the synthesis of silver nanoparticles with *Ocimum sanctum* being the most used (Singhal et al., 2011; Khan et al., 2017). There have been very few reports on the use of *O. gratissimum* as the source of reducing and capping agents for the synthesis of nanoparticles. Hence in this study, a novel biogenic route for the synthesis of silver nanoparticles by Ocimum gratissimum is reported. The silver nanoparticles were characterized using FTIR, UV-visible spectroscopy, X-ray diffraction and Scanning electron microscopy-energy dispersive analysis. The antimicrobial properties of these biogenically synthesized silver nanoparticles on some pathogenic multi-drug resistant clinically isolated micro-organisms were investigated to ascertain the possible use of these nanoparticles in antimicrobial therapy. Also, since silver nanoparticles have diverse applications in numerous biotechnological and medical fields, there are concerns regarding how safe it is upon exposure to human beings, hence, the possible toxicity to Wistar rats were also established by repeated administration of the silver nanoparticles. This will provide necessary information regarding their safe use.

MATERIALS AND METHODS

Materials

Silver nitrate ($AgNO_3$), was obtained from Sigma Chemical Company, St. Louis, USA. Reagent kits for the biochemical assay were obtained from Randox, USA. All other reagents and chemicals were of analytical grade and used without further purifications. The *Ocimum gratissimum* was collected from the National Horticultural Research Institute (NIHORT) and it was further identified at the Ife Herbarium, Obafemi Awolowo University, Ile-Ife. Representative microorganisms of Gram-negative long rod (*Klebsiella pneumonia* (NCIB 418 240816)), Gram-positive cocci (*Staphylococcus aureus* (NCIB 8588 260816)), Gram-negative short rod (*Escherichia coli* (NCIB 86 240816)), Gram positive bacilli (*Bacillus cereus* (NCIB 6349 250816)) bacterial strains as well as the yeast, *Candida albicans* (240816) were provided by Mr. T.O. Oni, Department of Microbiology, Obafemi Awolowo University, Ile-Ife. Bacterial strains were maintained on nutrient agar slants and the yeast was maintained on potato-dextrose agar slants at room temperature.

Methods

Preparation of aqueous extract of Ocimum gratissimum

Freshly collected *O. gratissimum* leaves were washed under running water as well as deionized water and dried in an oven for 30 minutes at 80℃. The leaves were then boiled using distilled water for 1 hour. The extract was collected after filtration using a cheese cloth. The extract was stored at 4℃ for subsequent use. The extract served as the source of reducing and capping agent for silver nitrate.

Phytochemical screening of the aqueous extract of the O. gratissimum

Phytochemicals such as anthraquinones, flavonoids, reducing sugars, saponin, steroids, tannins and terpenoids were determined according to the method described by Harborne (1998) and Evans (2002).

Phytosynthesis of silver nanoparticles using the aqueous extract of O. gratissimum

The reaction mixtures contained 90 ml silver nitrate solution (1 mM) in 10 ml of aqueous extract of *Ocimum* sp. The reaction mixtures were incubated in the dark at 30°C to avoid the photo activation of silver nitrate under static conditions. The brownish colour of the solution was

observed, indicating the formation of AgNPs. The effect of reaction time was evaluated by incubating the reaction mixtures for 2 hours. Colour change was routinely observed every 10 min.

Characterization of the synthesized silver nanoparticles

UV-visible scan

The nanoparticles were primarily characterized by UV–Visible spectroscopy, which has proved to be a very useful technique for the analysis of nanoparticles. Ultraviolet-Visible spectra were obtained using a Shimadzu UV-1650 pc Spectrophotometer.

Fourier transform infra-red spectroscopy

The changes in the surface chemical bonding and surface composition were characterized by using Fourier Transform Infrared (FT-IR) spectroscopy (Nicolet Avatar series 330) ranging from 4000 to 400 cm^{-1} with samples powder dispersed in the pressed potassium bromide (KBr) discs.

Scanning electron microscopy energy-disperse X-ray spectroscopy

The morphology and chemical composition of the synthesized nanoparticles were examined by scanning electron microscopy (SEM, JEOL JSM-6490A) equipped with an energy-dispersive X-ray spectrometer (EDX) (6490 LA). EDX was carried out at an acceleration voltage of 20.0 kV. Sample was prepared by sprinkling the dispersed nanoparticles onto double-sided adhesive carbon conductive tape which was mounted on a microscopic stub of silver nanoparticles. Then the sample was sputter-coated with gold using an ion sputtering device (JFC 1500) (Lyman et al., 2012)

X-ray diffraction (XRD)

XRD patterns were recorded on a Rigaku Ultima IV X-ray diffractometer equipped with a graphite-monochromatic Cu Kα radiation source (40 kV, 30 mA) at iThemba Laboratory, Department of Material Research, Cape Town South Africa. A diffractogram was collected in the 2θ range between 3º and 90º with a step size of 0.01 º, and a scan speed of 1º/min. The XRD pattern was processed using JCPDS card numbers. Scherrer equation was used to determine the crystallite size from XRD diffraction pattern measured for nanoparticles (Baron, 2015).

$$d = \frac{k\lambda}{xCos2\theta}$$

Where k is the Scherrer constant (shape factor, its value is 0.9), λ is the X-ray wavelength (λ = 0.154 nm), x is the line broadening at half the maximum intensity (FWHM) in radians, θ is the Bragg angle (the position of the diffraction peak maximum) and d is the averaged dimension of crystallites in nanometers

Antimicrobial potentials of the photosynthesized silver nanoparticles

The antibacterial potential of biosynthesized silver nanoparticles was tested against gram-positive cocci (*Staphylococcus aureus*), gram-negative short rod (*Escherichia coli*), gram-positive bacilli (*Bacillus cereus*) and gram-negative long rod (*Klebsiella pneumonia*) bacteria, as well as the yeast *Candida albicans*, by disc diffusion method (Cruickshank, 1968). Petri plates were prepared by pouring 20 ml of potato dextrose agar for bacteria and malt extract agar for the yeast. The plates were then allowed to solidify and used in susceptibility test. The standard inoculum using bacterial suspensions were swabbed on the top of the solidified respective media and allowed to dry for 10 minutes. Sterile discs were impregnated with prepared silver nanoparticles of 200 mg/disc. The discs with compounds were placed on the surface of the medium with sterile forceps and gently pressed to ensure contact with inoculated agar surface. Finally, the inoculated plates were incubated at 37°C for 24 to 72 hours for all the bacterial strains. The zone of inhibition was observed after every 24 hours and measured in millimeters.

Investigations of the possible toxicity of the silver nanoparticles

Animals

Male mice of 6 weeks were allowed to adapt to the animal room conditions for 1 week prior to the initiation of the study. The environmental conditions were set at a temperature of 23±1°C and a 12 hours light/dark cycle. All the animals used in this study were cared for in accordance with the principles outlined in the “Guide for the Care and Use of Laboratory Animals” issued by the Animal Care and Committee of NVRQS (National Veterinary Research and Quarantine Service). The animals were divided into three of 6 animals per group. The control group was treated with de-ionized water without AgNPs, which was prepared by the same process used to prepare AgNPs suspension. Groups 1 and 2 were treated with AgNPs doses of 0.5 mg/kg and 1 mg/kg respectively by oral administration for 28 days according to the procedure described by Kim et al. (2010). Twenty-four (24) hours after administration, blood was collected from the retro-orbital venous plexus of the rats using heparinized capillary tubes. The animals were sacrificed by cervical dislocation, and the liver, kidney and intestine tissues were collected, rinsed in 1.15% KCl and used for the assays.

Table 1. Phytochemical screening of aqueous extract of *Ocimum gratissimum*.

Phytochemicals	Confirmation
Tannins	++
Glycosides	++
Sterols	+
Anthraquinones	++
Phenols	+
Alkaloids	+
Terpenoids	+
Resins	-
Phlobatannins	-
Carbohydrates	-

(+) indicates presence while (-) indicates absence.

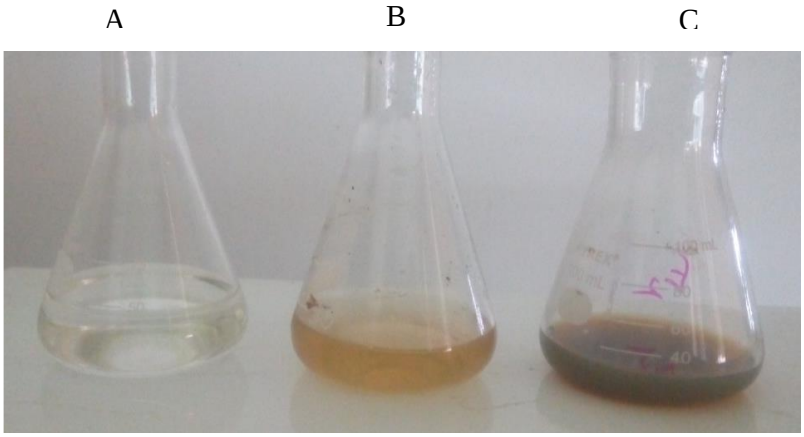


Figure 1. Silver nanoparticle synthesis (A.) Silver nitrate solution only (B.) Aqueous extract and (C.) Silver nanoparticles.

Blood biochemistry

Whole blood was centrifuged at 3000 rpm for 10 min to make serum. The sera were stored in the freezer prior to blood biochemical analysis; total protein, albumin, AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase) and creatinine were measured using Randox Kits.

RESULT

Phytochemical screening of the aqueous extract of *Ocimum gratissimum*

The presence of tannins, glycosides, sterols, phenols, alkaloid and terpenoids were confirmed. There were not renins, phlobatannins and carbohydrates in the aqueous extract of the *Ocimum* sp. The result of the phytochemical screening is shown in Table 1.

Synthesis and characterization of the silver nanoparticles

The synthesis of the nanoparticles was observed from the colour change that occurs within the first 5 minutes after the interaction of the silver nitrate with the aqueous extract as seen in Figure 1.

UV-visible spectroscopy

The UV-visible scan is shown in Figure 2. There was the presence of a surface plasmon resonance that is characteristic of silver nanoparticles at the 400 to 450 nm region.

Fourier transform infrared spectroscopy

The FTIR spectra of the aqueous extract of *Ocimum* sp.

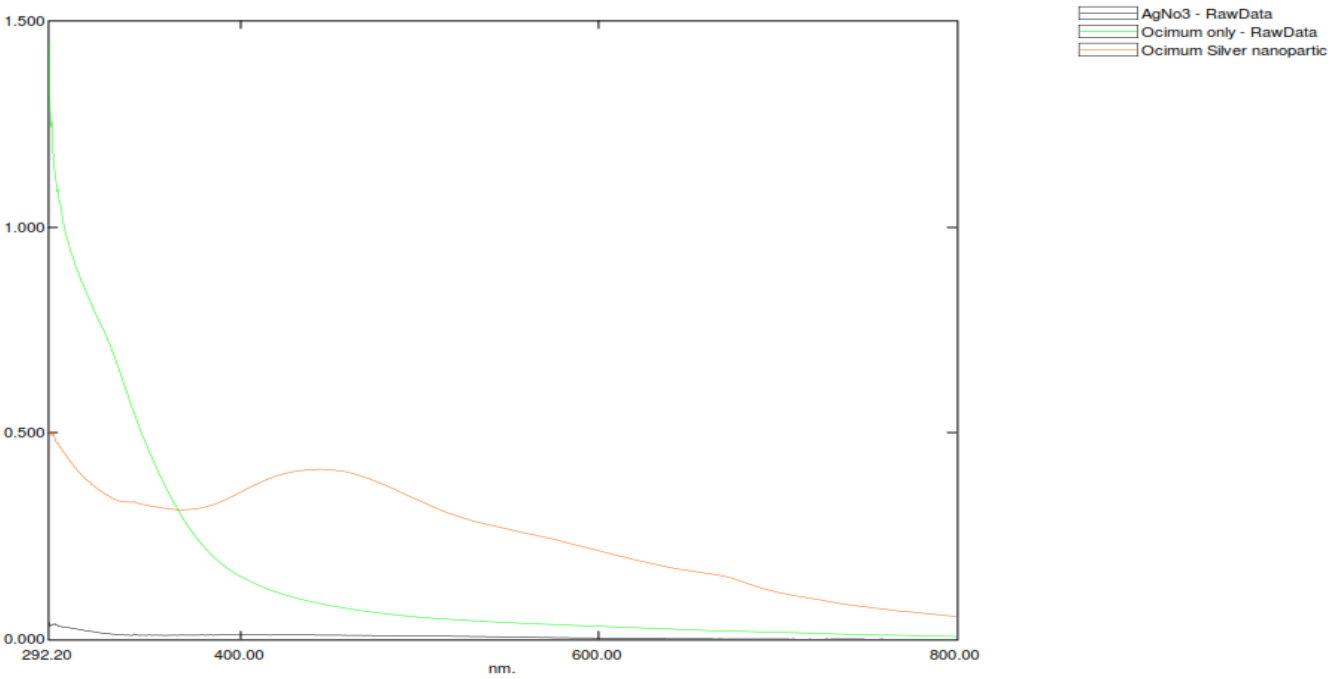


Figure 2. Comparative UV-Visible spectroscopy of the silver nanoparticles.

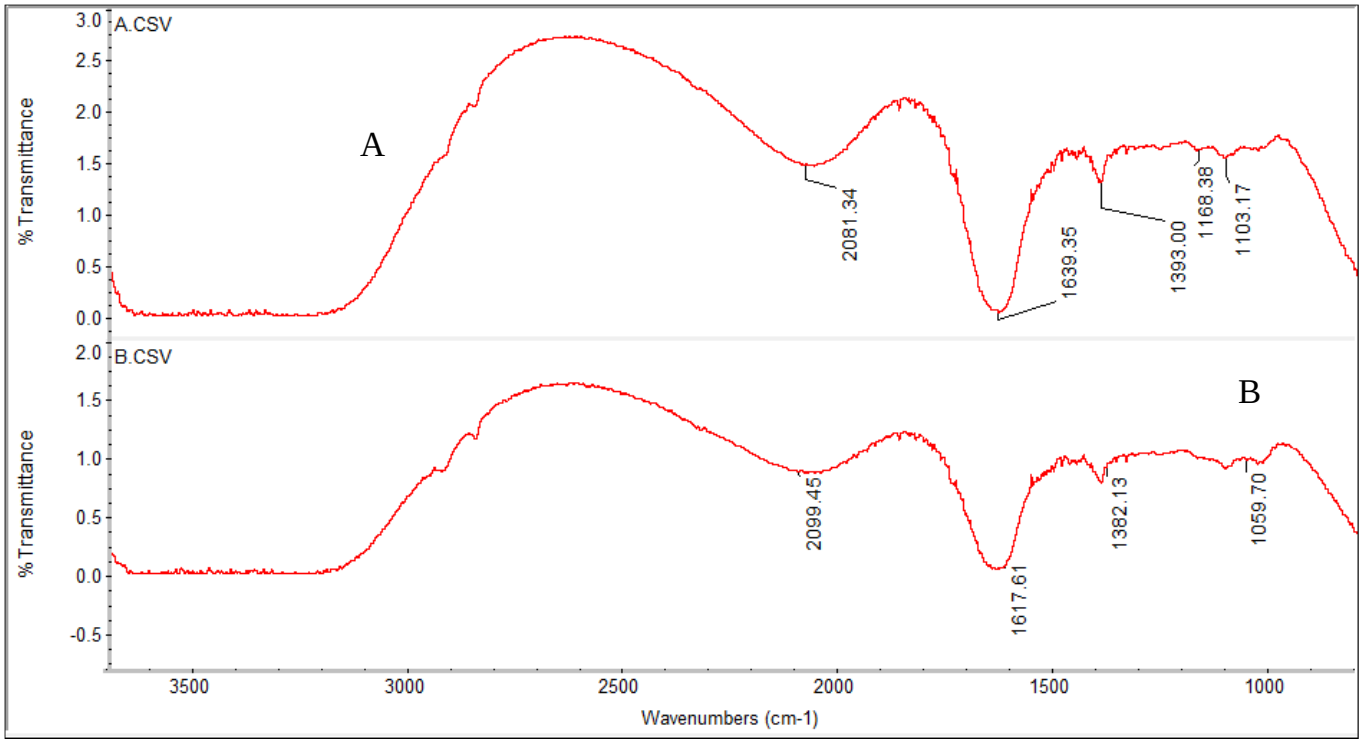


Figure 3. FTIR-spectroscopy of the (A) silver nanoparticles and (B) *Ocimum grattisimum* extract.

and the silver nanoparticles are shown in Figure 3. It showed an absorption band at 2081.34 and 2099.45 cm^{-1} respectively, which is the characteristic peak of –C-N triple bond stretching vibration of nitriles. The bands at 1639.35 cm^{-1} and 1617.61 cm^{-1} are assigned as amide bond of proteins (N-C=O). The bands (sharp) at 1393 cm^{-1} and

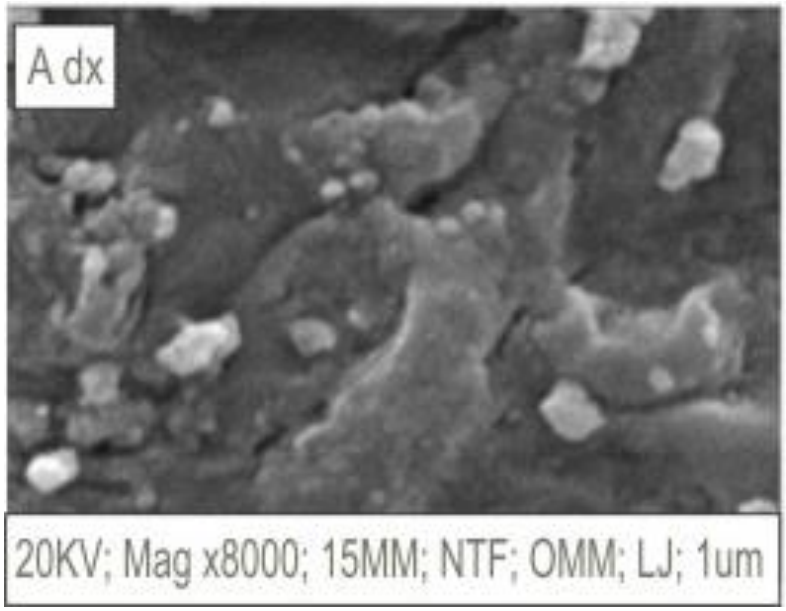


Figure 4. Scanning electron microscopy of the synthesized silver nanoparticles.

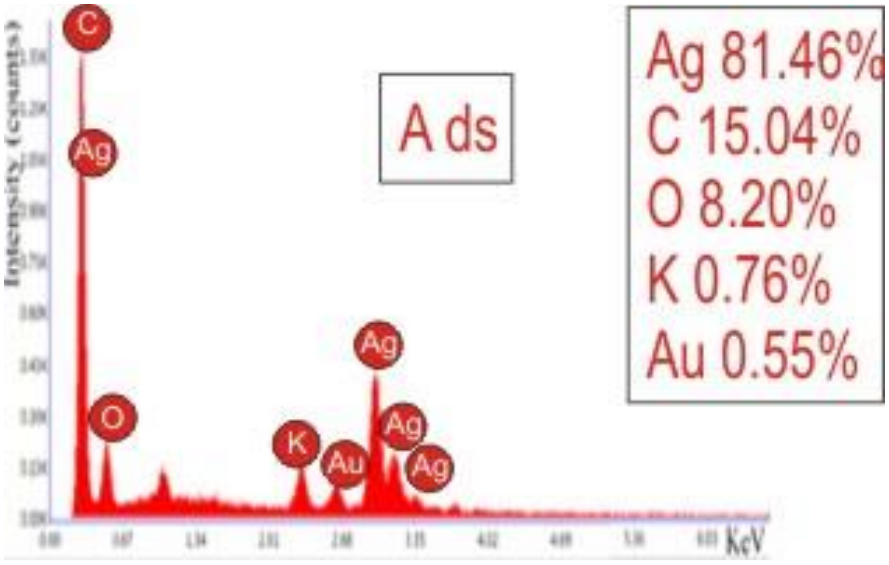


Figure 5. Electron Diffraction X-ray of the synthesized silver nanoparticles.

1382.13 cm^{-1} for silver nanoparticles and *Ocimum grattisimum,* respectively indicates the presence of a nitrate ion. The bands appearing at 1168.38, 1103.17 and 1059.70 cm^{-1} is the –C–O-C- stretching vibration of esters or ethers.

Scanning electron microscopy- energy dispersive X-ray

The SEM spectroscopy of the silver nanoparticles is shown in Figure 4 while the energy dispersive x-ray is shown in Figure 5. The morphology of the nanoparticles indicates a less uniform structure. The elemental composition of the nanoparticles showed the presence of silver, carbon, oxygen, potassium and gold.

X-ray diffraction analysis

The XRD pattern of dried silver nanoparticles is shown in Figure 6. The four diffraction peaks at 34°, 38°, 65° and

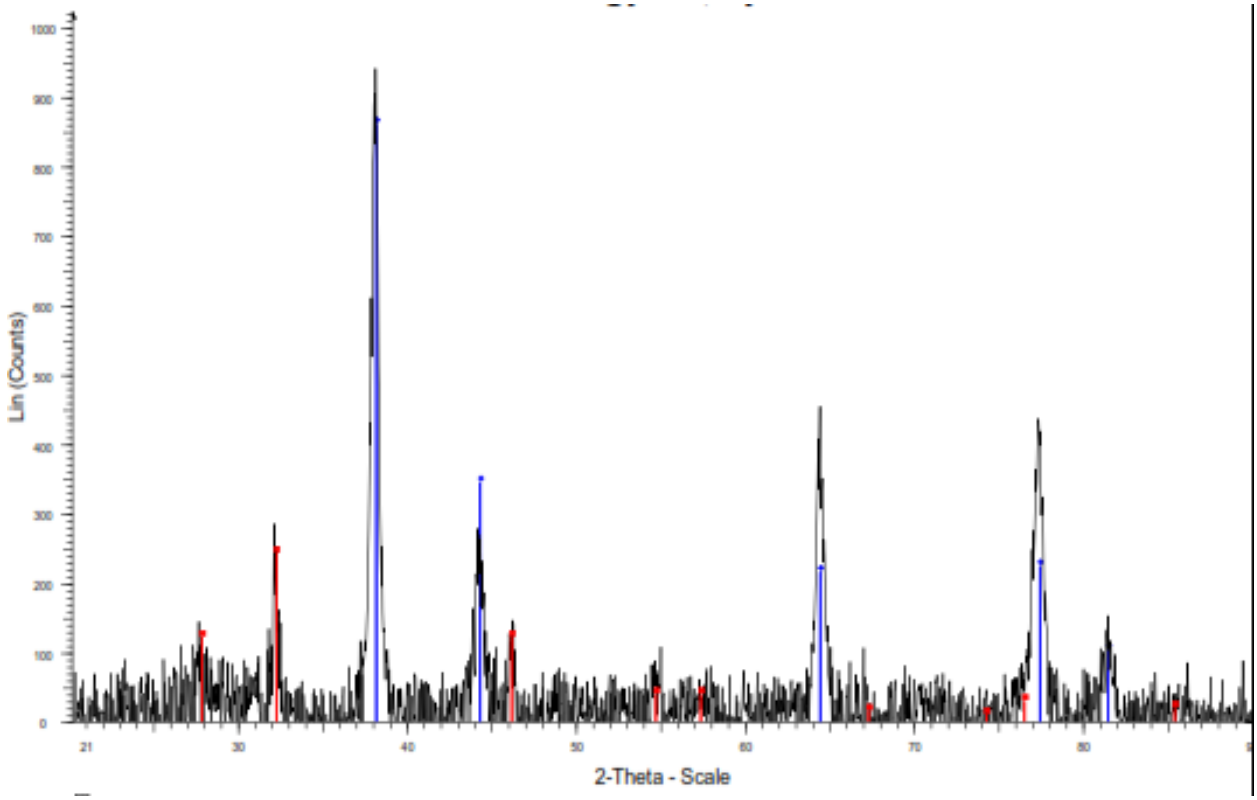


Figure 6. X-Ray Diffraction of the synthesized nanoparticles.

78° are indexed as (111), (200), (220) and (311) planes of face centered cubic silver. The data obtained was matched with the database of Joint Committee on Powder Diffraction Standards (JCPDS) file No. 04-0783. The (200), (220) and (311) Bragg reflections are weak and broadened relative to the intense (111) reflection. This feature indicates that the nanocrystals are (111)-oriented. The crystal size of the nanoparticles was estimated to be 25 nm using INSTANANO online software.

Antimicrobial properties of the silver nanoparticles

The synthesized silver nanoparticles showed excellent antimicrobial activity against clinically isolated multi drug resistant human pathogens such as Gram-positive bacteria *Bacillus cereus*, *Staphylococcus aureus* and Gram-negative bacteria *Klebsiella pneumoniae*, *Escherichia coli* (Figure 7 (a-c)). The mean inhibitory zone diameter was measured after 24 hours and re-measured after 48 hours. Both measurements were found to be the same further indicating that the microorganisms were indeed susceptible.

Toxicity studies

Blood biochemistry

Treatment of mice with AgNPs of *Ocimum gratissimum* at doses of 0.5 and 1 mg/kg did not produce any significant difference in the concentrations of albumin, bilirubin, total protein, urea and creatinine and activity of aspartate aminotransferase at $p<0.05$ when compared with the control (Figures 8 (a-f)). The activities of alanine aminotransferase and alkaline phosphatase reduced significantly when compared with the control group at $p< 0.05$ (Figures 8 g and h).

Histological examination

Histological examination of the liver, small intestine and kidney of the rats treated with silver nanoparticles (0.5 and 1 mg/kg) showed similar architecture with the control group and no visible lesion (Figures 9, 10 and 11).

DISCUSSION

The roles of green synthesis in nanotechnology and nano-science fields are very significant in the synthesis of diverse nanomaterials. In this study, a report of the synthesis of silver nanoparticles (Ag NPs) was carried out using biological methods. The colloidal AgNPs were synthesized in aqueous solutions using aqueous extract of *Ocimum gratissimum* leaves which served as the stabilizing and reducing agents. The biosynthesis of

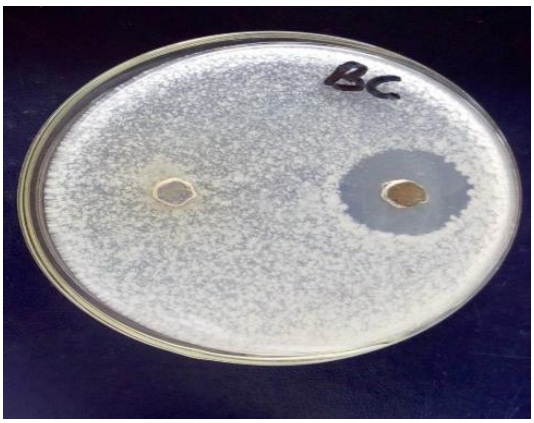


Figure 7a. Zone of inhibition of biogenically synthesized silver nanoparticles against *Bacillus cereus* (BC).

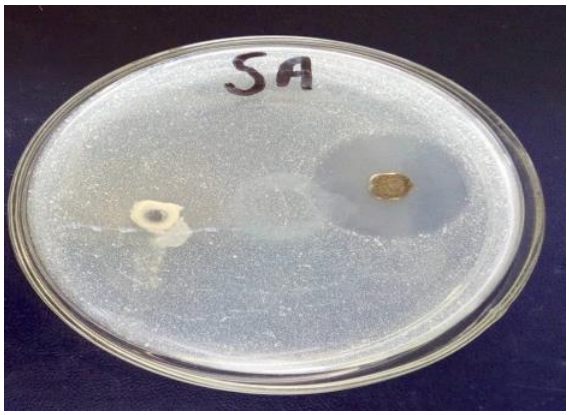


Figure 7b. Zone of inhibition of biogenically synthesized silver nanoparticles against *Staphylococcus aureus (*SA).

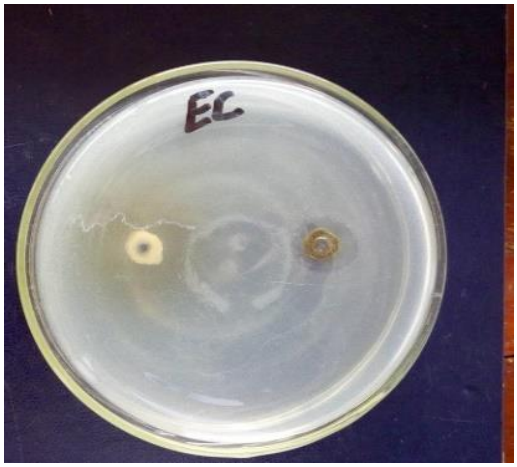
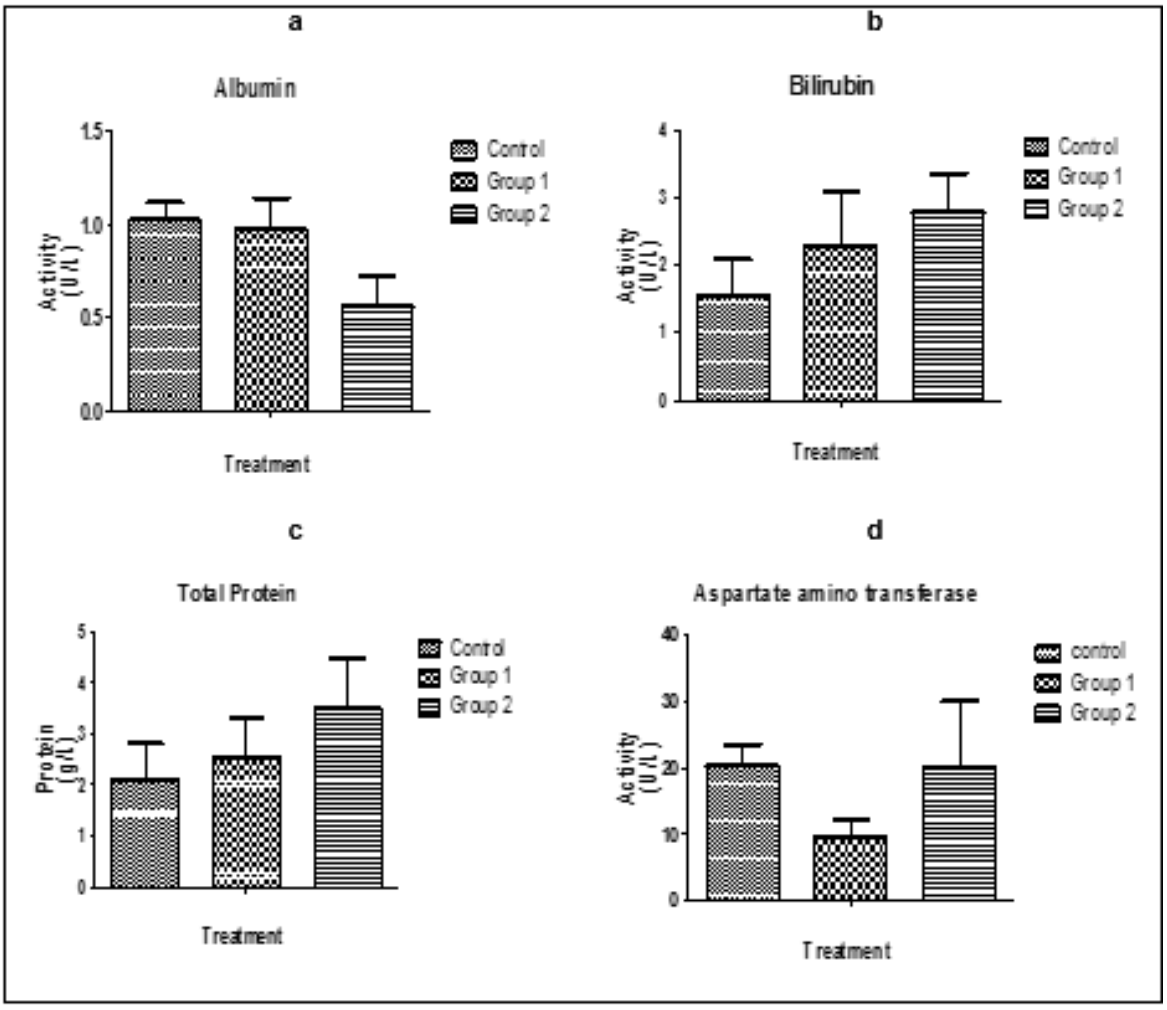


Figure 7c. Zone of inhibition of biogenically synthesized silver nanoparticles against *Escherichia coli* (EC).

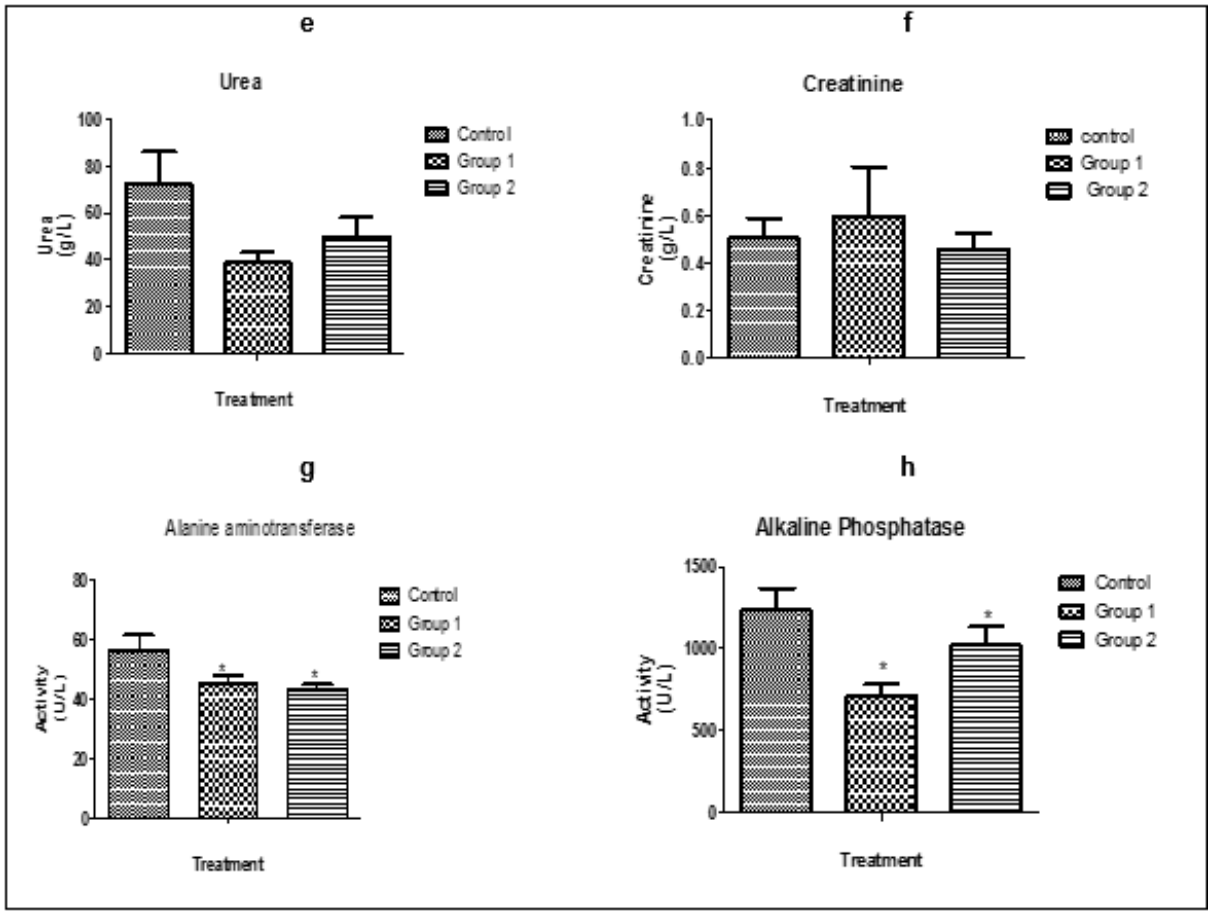
AgNPs initially involved observation of the change in the colour of the solution. The intensity of the change in colour observed increased with an increase in time which could be due to the excitation of surface plasmon vibrations. The surface plasmon resonance (SPR) is the collective oscillations of the conductive electrons of AgNPs. Excitation of the surface plasmon causes strong light scattering by an electric field of the wavelength where resonance occurs (Jebakumar Immanuel Edison and Sethuraman, 2013). Surface plasmon resonance bands are influenced by the size, shape, morphology, composition and dielectric environment of the prepared AgNPs (Kelly et al., 2003). Previous studies have shown that spherical AgNPs contribute to the absorption bands at around 400 nm in the UV-Vis spectra (Stamplecoskie and Scaiano, 2010). The SPR bands characteristics of Ag NPs synthesized from aqueous extract of *Ocimum gratissimum* (AEOG) were detected around 400 to 450 nm, which strongly suggests that the Ag NPs are spherical. FTIR analysis was carried out to identify the major functional groups on the biogenically synthesized AgNPs-AEOgL interface/surface and their possible involvement in the synthesis and stabilization of silver nanoparticles. The control spectrum of AEOgL showed several peaks indicating the nature of the biological material. The bands appearing at 2081.34, 1639.35, 1393, 1168.38 and 1103.17 cm^{-1} were assigned to the stretching vibration of –C-N triple bond of nitriles, N-C=O amide bond of proteins, nitrate ion, –C–O-C- of esters or ethers, respectively (Socrates, 1980). After reaction with 1 mM silver nitrate solution, there was a shift in the following peaks: 2081.34 to 2099.45 cm^{-1}, 1639.35 to 1617.61 cm^{-1}, 1393 to 1382.12 cm^{-1}, 1103.17 to 1059.70 cm^{-1}, and the disappearance of the peak at 1168.38 cm^{-1} indicating that nitrile, amide, nitrate and carboxyl groups on the surface of the AEOG may be participating in the synthesis of nanoparticles. Phytochemically, the AEOG contains tannins, glycosides, anthraquinones, sterols, alkaloids, phenols and terpenoids may be involved in reducing the Ag^{+} to Ag^{0}. Thus, these biological components are known to interact with the metal salts through these functional groups in order to carry out their reduction to nanoparticles.

The antimicrobial effects of silver (Ag) ion or salts are well known, but the effects of silver nanoparticles (AgNPs) on microorganisms and antimicrobial mechanism have not been revealed clearly. It is believed that DNA loses its replication ability and cellular proteins become inactivated on Ag^{+} treatment (Feng et al., 2008). In addition, it was also shown that Ag^{+} binds to functional groups of proteins, resulting in protein denaturation (Spadaro et al., 1974). Stable AgNPs prepared using the *Ocimum gratissimum* leaves were subjected and its antimicrobial activity was investigated against yeast (*Candida albicans*), gram negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*), and gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*). As results, *Candida albicans* was completely resistant by Ag NPs due to the fact that it can create a biofilm consisting of cellulose, polynucleotides,



Figures 8 (a,b,c and d). Effect of AgNPs of *Ocimum gratissimum* on bilirubin, albumin, total protein, AST respectively. Data are presented as the mean ± SD (n = 6). * values are significantly ($p<0.05$) different from control, Group1= control, Group 2= 0.5mg/kg AgNPs of *Ocimum gratissimum*, 1mg/kg AgNPs of *Ocimum gratissimum*,

polypeptides and fibrinogen as it becomes multicellular, thus, preventing the penetration of silver nanoparticles into its cell. *Candida albicans* can take on either a unicellular or multicellular form but can be maintained by a transcription repressor. *Escherichia coli* and *Klebsiella pneumonia* were inhibited by AgNPs to a smaller extent compared to *Staphylococcus aureus* and *Bacillus cereus.* Gram positive and gram-negative bacteria have differences in their membrane structure, the most distinctive of which is the thickness of the peptidoglycan layer. It is expected that there should be lower efficacy of the silver nanoparticles against gram positive bacteria due to the difference at a point of membrane structure as reported by Kim et al. (2010). However, results show that the inhibitory effect of AgNPs was mild in gram negative bacteria as compared to gram positive bacteria; these results suggest that the antimicrobial effects of AgNPs is not just keyed to the characteristics of the certain bacterial species but to other factors such as the type of extraction media. Therefore, if the antibacterial effect of AgNPs is associated with the peptidoglycan layer, it will be easier and more specific to use AgNPs as an antibacterial agent. The mechanism of the inhibitory effects of Ag ions on microorganisms is partially known. Some studies have reported that the positive charge on the Ag ion is crucial for its antimicrobial activity through the electrostatic attraction between negative charged cell membrane of microorganism and positive charged nanoparticles (Dibrov et al., 2002), while others reported that the antimicrobial activity of silver nanoparticles on gram negative bacteria was dependent on the concentration of AgNPs (Sondi and Salopek-Sondi, 2009), and was closely associated with the formation of ‘pits’ in the cell wall of bacteria, so that, AgNPs accumulated in the bacterial membrane causing the permeability, resulting in cell death. However, because those studies included both positively charged Ag ions and



Figures 8 (e, f, g and h) contd. Effect of AgNPs of *Ocimum gratissimum* on Urea, creatinine, ALT and ALP respectively. Data are presented as the mean ± SD (n = 6). * values are significantly ($p<0.05$) different from control, Group1= control, Group 2= 0.5mg/kg AgNPs of *Ocimum gratissimum*, 1mg/kg AgNPs of *Ocimum gratissimum*,

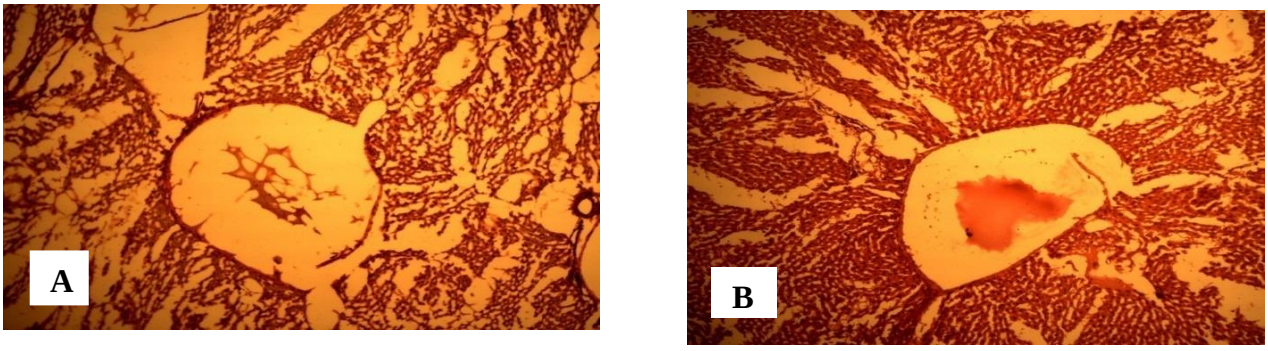


Figure 9. Representative photomicrographs of liver section viewed under light microscope at magnification 100x; A is the control group showing no visible lesion. B is AgNPs of *Ocimum gratissimum* (1mg/kg) showing no visible lesion.

negatively charged AgNPs, it is insufficient to explain the antimicrobial mechanism of positively charged AgNPs in this study.

The current study revealed that the green synthesis approach for the synthesis of AgNPs using *Ocimum gratissimum* appeared to be a cost effective, non-toxic, easily available, ecofriendly alternative to the conventional microbiological, physical and chemical methods. However, gram negative bacteria were found to be more resistant to the antimicrobial agent than gram positive bacteria which was not expected and so further studies is required on this. In this study, repeated oral administration of Ag NPs had

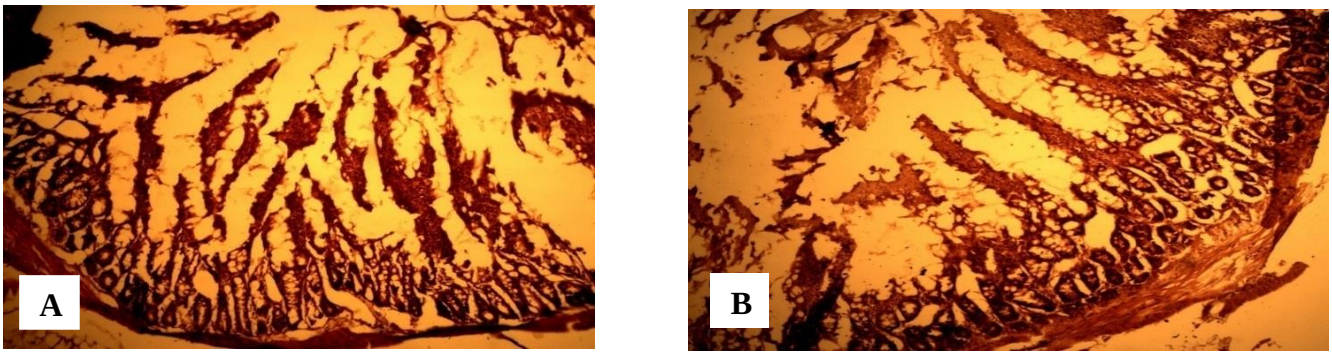


Figure 10. Representative photomicrographs of small intestine section viewed under light microscope at magnification 100x; A is the control group showing no visible lesion. B is AgNPs of *Ocimum gratissimum* (1mg/kg) showing no visible lesion.

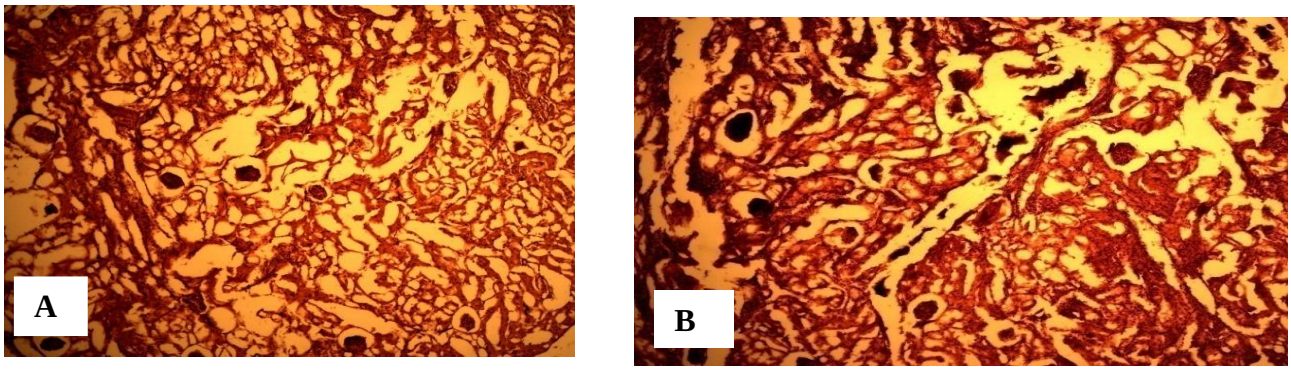


Figure 11. Representative photomicrographs of kidney section viewed under light microscope at magnification 100x; A is the control group showing no visible lesion. B is AgNPs of *Ocimum gratissimum* (1mg/kg) showing no visible lesion.

no adverse effects on the rats. When mice were treated with AgNPs, changes in body weight were observed; however, alkaline phosphatase (ALP) was significantly increased in the blood of the groups treated with more than the control group. The levels of aspartate transaminase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were not significantly increased in only the group treated with 1 mg/kg Ag NPs, which is the highest dosage in this study. Histopathological evidence of toxicity was not observed in liver, kidney or small intestines. Although AgNPs have been widely applied in consumer products, enough toxicological data have not been generated. Furthermore, toxicity studies of AgNPs have been focused on mechanism of action using *in vitro* models, rather than on toxicological and pathological changes using *in vivo* model through possible exposure scenario. Exposure to AgNPs can occur through inhalation, dermal contact and ingestion.

Other exposure route of AgNPs, such as oral route may be important in many consumer products such as toothpaste, reusable bottles, nursing nipples, kitchen utensils, and toys (Chen and Schluesener, 2008; Edwards-Jones, 2009). AgNPs are known to be translocated to the blood during circulation and distributed throughout the main organs, especially in the kidney, liver, spleen, brain and lung in the form of particles (Tang et al., 2009). It has been reported that no significant toxicological change occurred during the 28 days inhalation of AgNPs (Hyun et al., 2008). This finding was also established in this study as no noticeable effect of Ag NPs toxicity was observed either in the blood biochemistry parameters or histopathological examinations of the liver, kidney and small intestine. Lung and liver were the major target tissues for prolonged AgNPs exposure and no adverse effect was observed. These findings were similar to that observed by Sung et al. (2009) where no observable adverse effect level (NOAEL) of AgNPs was determined at 100 µg/m^3.

Conclusion

In conclusion, a non-toxic silver nanoparticle that possessed antimicrobial properties was synthesized using an aqueous extract of *Ocimum gratissimum* leaves in this study. This could offer a great biogenic route for the synthesis of silver nanoparticles for immense industrial application. However, more studies are warranted with

experimental models especially at molecular level in order to obtain beneficial and medicinal values of AgNPs synthesized with *Ocimum gratissimum*.

CONFLICT OF INTEREST

The authors declare no conflict of interest

REFERENCES

Aisida, S. O., Ugwu, K., Akpa, P. A., Nwanya, A. C., Ejikeme, P. M., Botha, S., Ahmad, I., Maaza, M., & Ezema, F. I. (2019). Biogenic synthesis and antibacterial activity of controlled silver nanoparticles using an extract of Gongronema Latifolium. *Materials Chemistry and Physics*, *237*, 121859.

Anastas, P. T., & Zimmerman, J. B. (2007). Green nanotechnology. Why we need a green nano award and how to make it happen. Center for Green Chemistry and Green Engineering, Yale University, U.S. state. 29p.

Baron, A. Q. (2015). Introduction to high-resolution inelastic x-ray scattering. *arXiv preprint arXiv:1504.01098*.

Bello-Vieda, N., Pastrana, H., Garavito, M., Ávila, A., Celis, A., Muñoz-Castro, A., Restrepo, S., & Hurtado, J. (2018). Antibacterial activities of azole complexes combined with silver nanoparticles. *Molecules*, *23*(2), 361.

Braydich-Stolle, L. K., Lucas, B., Schrand, A., Murdock, R. C., Lee, T., Schlager, J. J., & Hofmann, M. C. (2010). Silver nanoparticles disrupt GDNF/Fyn kinase signaling in spermatogonial stem cells. *Toxicological Sciences*, 116(2), 577-589.

Cauerhff, A., & Castro, G.R. (2013). Bionanoparticles: a green chemistry approach. *Electronic Journal of Biotechnology,* 16(3), 234-241.

Chen, X., & Schluesener, H. J. (2008). Nanosilver: a nanoproduct in medical application. *Toxicology letters*, 176(1), 1-12.

Correa, M. P. (1932) *Dicictionary of Plant Utilized in Brazil.* Ministry of Agriculture, Rio de Janeiro, p. 63.

Cruickshank, R. (1968). *Medical microbiology: A guide to diagnosis and control of infection, 11th (ed)*, Edinburgh and London: E&S. Livingston Ltd. p. 888.

Dahl, J. A., Maddux, B. L., & Hutchison, J. E. (2007). Toward greener nanosynthesis. *Chemical Reviews*, 107(6), 2228-2269.

Dibrov, P., Dzioba, J., Gosink, K. K., & Häse, C. C. (2002). Chemiosmotic mechanism of antimicrobial activity of Ag^+ in Vibrio cholerae. *Antimicrobial Agents and Chemotherapy*, 46(8), 2668-2670.

Dziedzic, A., Kubina, R., Bułdak, R., Skonieczna, M., & Cholewa, K. (2016). Silver nanoparticles exhibit the dose-dependent anti-proliferative effect against human squamous carcinoma cells attenuated in the presence of berberine. *Molecules*, 21(3), 365.

Edwards-Jones, V. (2009). The benefits of silver in hygiene, personal care and healthcare. *Letters in Applied Microbiology*, 49(2), 147-152.

Evans, W. C. (2002). *Trease and Evans Pharmacognosy, 9th Edition*. Published by Saunders Elsevier. Pp. 553-557.

Feng, Q. L., Wu, J., Chen, G. Q., Cui, F.Z., Kim, T. N., & Kim, J. O. (2000). A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *Journal of Biomedical Materials Research*, *52*(4), 662-668.

Harborne, A. J. (1998). *Phytochemical methods a guide to modern techniques of plant analysis*. Springer Science and Business Media.

Ho, Y. T., Lu, C. C., Yang, J. S., Chiang, J. H., Li, T. C., Ip, S. W., & Chung, J. G. (2009). Berberine induced apoptosis via promoting the expression of caspase-8,-9 and-3, apoptosis-inducing factor and endonuclease G in SCC-4 human tongue squamous carcinoma cancer cells. *Anticancer Research*, 29(10), 4063-4070.

Hutchison, J. E. (2008). Greener Nanoscience: a proactive approach to advancing applications and reducing implications of nanotechnology. *ACS Nano*, 2(3), 395-402.

Hyun, J. S., Lee, B. S., Ryu, H. Y., Sung, J. H., Chung, K. H., & Yu, I. J. (2008). Effects of repeated silver nanoparticles exposure on the histological structure and mucins of nasal respiratory mucosa in rats. *Toxicology Letters*, 182(1-3), 24-28.

Ishwarya, R., Vaseeharan, B., Anuradha, R., Rekha, R., Govindarajan, M., Alharbi, N. S., & Benelli, G. (2017). Eco-friendly fabrication of Ag nanostructures using the seed extract of Pedalium murex, an ancient Indian medicinal plant: Histopathological effects on the Zika virus vector Aedes aegypti and inhibition of biofilm-forming pathogenic bacteria. *Journal of Photochemistry and Photobiology B: Biology*, 174, 133-143.

Jebakumar Immanuel Edison, T. N., & Sethuraman, M. G. (2013). Electrocatalytic reduction of benzyl chloride by green synthesized silver nanoparticles using pod extract of Acacia nilotica. *ACS Sustainable Chemistry and Engineering*, 1(10), 1326-1332.

Jo, Y. K., Kim, B. H., & Jung, G. (2009). Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi. *Plant Disease*, 93(10), 1037-1043.

Kelly, K. L., Coronado, E., Zhao, L. L., and Schatz, G.C. (2003). The optical properties of metal nanoparticles: the influence of size, shape, and dielectric environment. *The Journal of Physical Chemistry B*, 107(3), 668-677.

Khan, M. Z. H., Tarek, F. K., Nuzat, M., Momin, M. A., & Hasan, M. R. (2017). Rapid biological synthesis of silver nanoparticles from Ocimum sanctum and their characterization. *Journal of Nanoscience,* vol. 2017, Article ID 1693416, 6 pages.

Kim, Y. S., Song, M. Y., Park, J. D., Song, K. S., Ryu, H. R., Chung, Y. H., & Hwang, I. K. (2010). Subchronic oral toxicity of silver nanoparticles. *Particle and Fibre Toxicology*, *7*(1), 20.

Lyman, C. E., Newbury, D. E., Goldstein, J., Williams, D. B., Romig Jr, A. D., Armstrong, J., Echlin, P., Fiori, C., Joy, D. C., Lifshin, E., & Peters, K. R. (2012). *Scanning electron microscopy, X-ray microanalysis, and analytical electron microscopy: a laboratory workbook*. Springer Science & Business Media.

Mohanpuria, P., Rana, N. K., & Yadav, S. K. (2008). Biosynthesis of nanoparticles: technological concepts and future applications. *Journal of Nanoparticle Research*, 10(3), 507-517.

Nair, B., & Pradeep, T. (2002). Coalescence of nanoclusters and formation of submicron crystallites assisted by Lactobacillus strains. *Crystal Growth and Design*, 2(4), 293-298.

Narayanan, K. B., & Sakthivel, N. (2010). Biological synthesis of metal nanoparticles by microbes. *Advances in Colloid and Interface Science*, 156(1-2), 1-13.

Onajobi, F. D. (1986). Smooth muscle contracting lipid-soluble principles in chromatographic fractions of *Ocimum gratissimum*. *Journal of Ethnopharmacology*, 18(1), 3-11.

Sarsar, V., Selwal, K.K., and Selwal, M.K. (2014). Nanosilver: potent antimicrobial agent and its biosynthesis. *African Journal of Biotechnology*, 13(4), 546-554.

Sathishkumar, M., Sneha, K., & Yun, Y. S. (2010). Immobilization of silver nanoparticles synthesized using Curcuma longa tuber powder and extract on cotton cloth for bactericidal activity. *Bioresource Technology*, 101(20), 7958-7965.

Schultz, S., Smith, D.R., Mock, J.J., and Schultz, D.A. (2000). Single-target molecule detection with nonbleaching multicolor optical immunolabels. *Proceedings of the National Academy of Sciences*, *97*(3), 996-1001.

Shankar, S. S., Rai, A., Ahmad, A., & Sastry, M. (2004). Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (Azadirachta indica) leaf broth. *Journal of Colloid and Interface Science*, 275(2), 496-502.

Singhal, G., Bhavesh, R., Kasariya, K., Sharma, A. R., & Singh, R. P. (2011). Biosynthesis of silver nanoparticles using Ocimum sanctum (Tulsi) leaf extract and screening its antimicrobial activity. *Journal of Nanoparticle Research*, 13(7), 2981-2988.

Skonieczna, M., & Hudy, D. (2018). Biological activity of Silver Nanoparticles and their applications in Anticancer Therapy. *Silver Nanoparticles: Fabrication, Characterization and Applications*, 131.

Socrates, G. (1980). Infrared characteristic group frequencies. Infrared spectrometry.

Sondi, I., & Salopek-Sondi, B. (2004). Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. *Journal of colloid and interface science*, 275(1), 177-182.

Spadaro, J. A., Berger, T. J., Barranco, S. D., Chapin, S. E., & Becker, R. O. (1974). Antibacterial effects of silver electrodes with weak direct current. *Antimicrobial agents and chemotherapy*, 6(5), 637-642.

Stamplecoskie, K. G., & Scaiano, J. C. (2010). Light emitting diode irradiation can control the morphology and optical properties of silver nanoparticles. *Journal of the American Chemical Society*, 132(6), 1825-1827.

Sung, J. H., Ji, J. H., Park, J. D., Yoon, J. U., Kim, D. S., Jeon, K. S. and Chung, Y. H. (2008). Subchronic inhalation toxicity of silver nanoparticles. *Toxicological sciences*, 108(2), 452-461.

Tang, J., Xiong, L., Wang, S., Wang, J., Liu, L., Li, J., & Xi, T. (2009). Distribution, translocation and accumulation of silver nanoparticles in rats. *Journal of Nanoscience and Nanotechnology*, 9(8), 4924-4932.