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ORIGINAL ARTICLE

Biogenic Synthesis of Silver Nanoparticles with Bitter Leaf (*Vernonia amygdalina*) Aqueous Extract and Its Effects on Testosterone-Induced Benign Prostatic Hyperplasia (BPH) in Wistar Rat

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Abstract

Purpose This study aims to perform biogenic synthesis of silver nanoparticles (AgNPs) with the aqueous extract of *Vernonia amygdalina* (*V. amygdalina*) (bitter leaf) and determine their therapeutic effects on testosterone-induced benign prostatic hyperplasia (BPH) in a rat model.

Methods In a reaction involving *V. amygdalina* plant extract and a silver nitrate ($AgNO_3$) solution, the resultant biogenic AgNPs were characterised by Fourier transformed infrared spectrophotometric, SEM, TEM and X-ray diffraction analysis. Animal experiments involved thirty (30) adult male Wistar rats randomly divided into six groups (A to F; $n=5$). Group A received only subcutaneous injection of olive oil daily while the other groups got 3 mg/kg/daily of testosterone propionate (TP) subcutaneously plus 50 mg/kg/daily of AgNPs intraperitoneally (B), TP plus 25 mg/kg/daily of AgNPs (C), TP only (D), 25 mg/kg/daily of AgNPs only (E) and TP plus 10 mg/kg/daily of Finasteride orally (F). The animals were sacrificed after 14 days, serum collected and assayed for four reproductive hormones, AST, ALT, urea and creatinine while the prostate was collected for histological analysis.

Results Biogenic AgNPs with an average diameter of 34 nm were synthesized. Biogenic AgNPs ameliorated hormone-induced prostate enlargement with significant increase in prostate weight ($p<0.0001$) compared to placebo and finasteride treatment. Administration of biogenic AgNPs significantly increased body weight and serum testosterone level. Liver function parameters appear normal while nephrotoxicity appears to be present.

Conclusion Biogenic AgNPs is beneficial in the treatment of BPH. Safety and long term effects should however be properly evaluated.

Keywords Benign prostatic hyperplasia · Silver nanoparticles · Testosterone · Bitter leaf · Biogenic synthesis

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1 Introduction

Nanotechnology may be described as the science of producing and utilizing nano-size particles, which are objects ranging in size from 1 to 100 nm [1]. Nanotechnology is being utilized for a variety of applications, including food processing, electronics, information technology, environmental remediation, fuel and energy production, and biotechnology, among others [1, 2].

The therapeutic use of nanoparticles, as obtained in nanomedicine, has received considerable attention in recent times [3–8]. For example, nanotechnology has been of great impact on pharmaceutical improvement through the production and optimization of drugs. As drug delivery tools, nanoformulations can be used to enhance the site-specific, targeted delivery of drugs. This typically results in significant reductions in side effects and concomitant improvements in efficacy [9]. Metallic nanoparticles are also known for their wide range of potentials in pharmaceutical applications such as anti-cancer, anti-parasite, bactericidal and fungicidal agents [9, 10].

Although the synthesis of nanoparticles (NPs) through conventional physical and chemical methods is still popular, the use of these classical techniques in nanoparticles synthesis has some drawbacks such high energy requirement, high cost and involvement of toxic compounds, which are hazardous to human health and environment. Hence, the physical and chemical synthesis of nanoparticles are less attractive for biomedical applications [11–13]. Consequently, biogenic or green synthesis of NPs has been largely accepted as it provides an alternative pathway to the production of metallic nanoparticles that is cheap, biocompatible and environmentally friendly [11].

Biogenic synthesis of NPs involves the use of fungi, bacteria, plant and microorganisms in reducing metallic nanoparticles to stable forms [1, 14–16]. For example, biogenic synthesis of NPs has recently been reported with use of *Trigonella foenum-graecum* (fenugreek) seed extract [17], *jujube* core extract [18], *Alstonia venenaa* leaf extract [19], aqueous extracts of the leaves and stem bark of *Grewia lasiocarpa* [20] and Pyrus betulifolia Bunge extract [21]. Silver nanoparticles (AgNPs) are of interest due to their unique physicochemical properties and potential medical applications. AgNPs have been recognized as having anti-inflammatory, antibacterial, antifungal and cytotoxic activities against cancer cells [2, 8, 21–24]. Green synthesis of AgNPs is therefore important in view of their pharmacological activities [25].

Benign prostatic hyperplasia (BPH) is a condition that is characterized by non-cancerous enlargement of the prostate gland in adult male [26, 27]. Although the pathological process of BPH is poorly understood, available evidence suggests that hormonal and inflammatory changes could be responsible [28–30] as numerous growth factors linked with cellular growth, differentiation, cell death and epithelial/stromal interface are involved in the pathophysiology of BPH [31, 32].

Medical management of BPH currently involves the use of α1-adrenergic receptor antagonists and 5α-reductase inhibitors which reduce BPH associated lower urinary tract symptoms [33, 34]. These drugs are however not without their side effects, which include reduced libido and erectile dysfunction. Hence the continued search for improved therapy for the management of BPH [35]. Therefore, the aim of the present study is to synthesize AgNPs from the aqueous extract of the medicinal plant, *V. amygdalina* and to assess the ameliorative effect of the synthesized biogenic AgNPs on testosterone-induced BPH in Wistar rat.

2 Materials and Method

2.1 Preparation of Plant Extract

Fresh leaves of *V. amygdalina* were obtained from Ogori Mangogo Local Government Area (7° 28′ 15.42″ N, 6° 09′ 47.90″ E) of Kogi State, Nigeria. A taxonomist identified and confirmed the plant in the Biological Science Department of Kogi State University Anyigba while voucher sample was deposited in the same department. *V. amygdalina* leaves were air-dried at ambient temperature for 10 days and powdered with an electric grinder under sterile conditions; approximately 30 g of the powdered sample was soaked in 300 mL of distilled water and subjected to agitation on an orbital shaker for 24 h. Thereafter, filtration of the extract was carried out using Whatman No.1 filter paper and the resultant filtrate lyophilized into dry power and kept in a tightly stopped centrifuge tube at 4 °C prior to the biosynthesis of AgNPs.

2.2 Green Synthesis Silver Nanoparticles

Plant extract solution (10 mL, 20 mg/mL in water) was mixed with a solution of silver nitrate ($AgNO_3$) (Chem Lab, South Africa), 100 mL, 2 mM at ratio 1:10 (v/v). The resultant mixture, which was light-yellowish in colour, was shielded from light by means of aluminium foil to avoid auto-reduction of the $AgNO_3$. Stirring was continuously done for about 8 h at ambient temperature. The bio-reduction of Ag^+ to Ag^o was ascertained through the physical colour change from colourless ($AgNO_3$) to a brownish-dark (AgNPs) (Fig. 1a). The precipitate was separated by centrifugation at a speed of 8000 rpm for 10 min using a centrifuge machine (Model 0508-I). The precipitate was rinsed

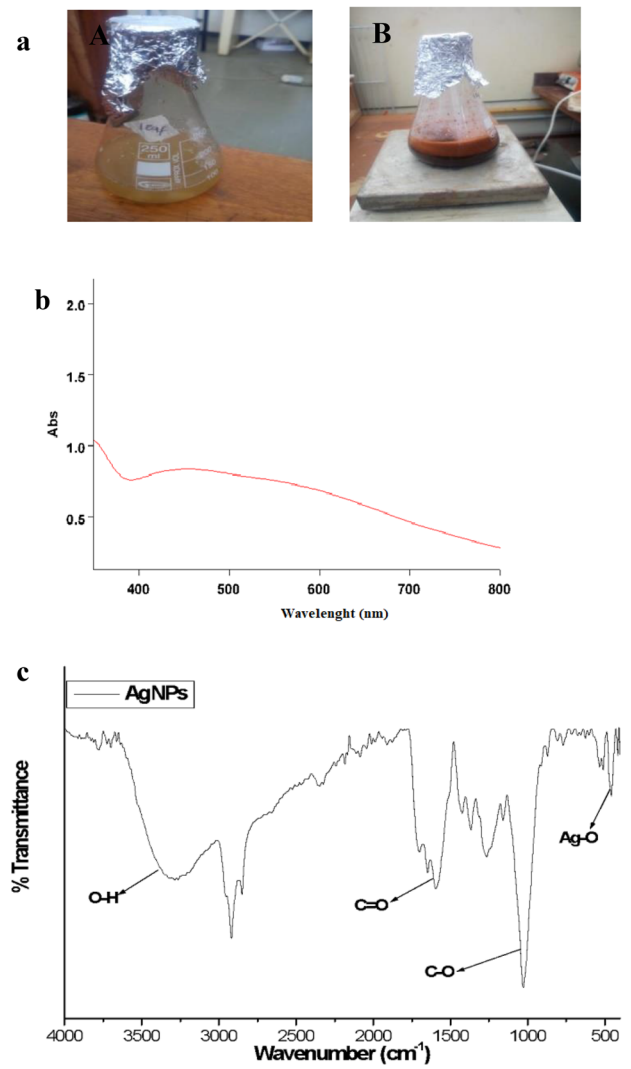

Fig. 1 Bioreduction of $AgNO_3$ to AgNPs; **a** colour change of *V. amygdalina* leaf extract with the addition of 2 mM $AgNO_3$ solution (A) before and (B) after the formation of AgNPs. **b** UV–vis absorption spectrum of the *V. amygdalina* synthesized AgNPs. **c** FTIR spectrum of the *V. amygdalina* synthesized AgNPs

three times with deionized water and dried up in an oven at 50 °C for 24 h.

2.3 Characterization of Synthesized Silver Nanoparticles

UV – VIS absorption spectrophotometer (Perkin-Elmer Universal) was used to obtain information on the optical properties of the synthesized material by scanning from 200 to 800 nm wavelength range. Fourier transformed infrared spectrophotometer (FTIR) (Perkin-Elmer Universal ATR 100) was employed for the determination of vibrational frequencies of *V. amygdalina* synthesized AgNPs and to ascertain the functional groups that might be responsible for capping and stabilization. Measurement of the vibrational spectra of the NPs was achieved via a scanning range of 4000–440 cm^{-1}. The Perkin-Elmer Universal ATR 100 machine, used to determine the functional groups of the biosynthesized nanoparticles, requires no sample preparation. It was manufactured to overcome the disadvantages of KBr pellets and liquids cells in the conventional transmission mode. All types of samples (solids, liquids, powders, pastes, pellets etc.) are placed undiluted on the Attenuated Total Reflectance (ATR) crystals, a clamping arm with a pressing anvil applies pressure to the solids for uniform contact with the internal reflective element pressed towards the ATR crystals (e.g. diamond).

Scanning electron microscope (SEM)/electron diffraction spectrophotometer (EDS) analysis were carried out on the same instrument (JEOL JSM-6490A) by placing the synthesized AgNPs on a two-sided carbon coated stub at a voltage of 15–20 keV and observing at different magnifications. SEM and EDS provided information on the surface morphology and composition of NPs respectively. Transmission electron microscope (TEM) operated via JOEL 1210 operating at 100 kV was used for the structural analysis of AgNPs. AgNPs were dissolved in DMSO (Merck, Germany) and sonicated for 20 min after which a drop from the suspension was placed on a carbon-coated-copper grid and left to dry at room temperature before analysis. To confirm the crystallinity phase of biogenic AgNPs, X-ray diffraction (XRD) (Bruker D8 advanced x-ray diffractometer) was employed and operated at 45 kV.

2.4 Animals

Thirty Wistar rats (n = 30), adult males weighing about 120 g–180 g, were raised and kept in the Animal House of the College of Health Sciences, Kogi State University, Anyigba. Research was conducted according to the guidelines on animal use by College Research and Ethics Review of the College of Health Sciences, Kogi State University, Anyigba. The animals were left to adapt for 14 days prior to the commencement of the experiment, and they were also exposed to food and water at will under normal laboratory conditions of light and temperature. Animals were housed in a well partitioned wooden cage.

2.5 Experimental Design

Thirty Wistar rats were divided into 6 groups (A-F) of 5 animals each by random selection. The experiment which lasted for 14 days was designed as follows; Group A, the placebo, was supplied with top grower feed and distilled water. They were also administered with Goya extra virgin olive oil (0.05 mL) subcutaneously. Group B was given subcutaneous injection of testosterone propionate (TP) at 3 mg/

kg/day and 50 mg/kg/day of biogenic AgNPs dissolved in Goya extra virgin olive oil. Group C was administered with 3 mg/kg/day TP by subcutaneous injection and 25 mg/kg/day of biogenic AgNPs dissolved in Goya extra virgin olive oil. Group D was given subcutaneous injection of 3 mg/kg/day TP only. Group E was administered with only 25 mg/kg/day of biogenic AgNPs dissolved Goya extra virgin olive oil. Group F was administered with 3 mg/kg/day of TP by subcutaneous injection and 0.7 mg/kg/day of finasteride orally by the use of oral gavage. Animals were weighted on the first day (day 0) and on the last day of the experiment (day 14) before sacrifice.

Animals were euthanized at day 14 of the experiment using chloroform sedation by putting the animals in closed glass container with chloroform-soaked cotton wool for 10 s. A 3 mL blood sample was earlier obtained via cardiac puncture into sterile bottles and allowed to clot for two hours, defabrinised and spun at 4000 rpm. Thereafter, serum was pipetted into sterile bottles, labelled accordingly and stored at 4 °C before running liver function tests, hormone and urea/creatinine assays. The prostate gland, liver and kidney were also harvested, carefully weighed and preserved without delay in 10% formal saline prior to histological analysis. The prostate index or prostate weight/body weight ratio (PW/BW) of each rat was determined by dividing prostate weight by body weight and multiplied by 1000.

2.6 Histological Analysis

Fixed organs were transferred from fixative into varying concentrations of ethanol (70%, 80%, 90% and 100%). Organs were then treated with acetone and cleared in three changes of xylene for 30 min to boost tissue transparency after which impregnation and embedding in paraffin wax was carried out. Tissues were then sectioned at 5 μm thickness with a microtome, placed on glass slide and processed for hematoxylin and eosin (H&E) staining. Sections were examined under light microscope for structural changes and photomicrographs taken.

2.7 Determination of Serum Testosterone, Estrogen, Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Prostate Specific Antigen (PSA) Levels

ELISA kit (Monobind Inc., USA) was used to measure the serum levels of testosterone, estrogen, FSH, LH and PSA according to the manufacturer's instruction. 10 μL of serum sample was pipetted into an assigned microplate well and 50 μL of working testosterone enzyme reagent (estrogen enzyme reagent, FSH enzyme reagent, LH enzyme reagent or PSA enzyme reagent as the case may be) added to all wells. The microplate was agitated lightly for 20–30 s to mix well before adding testosterone biotin reagent (estrogen biotin reagent, FSH biotin reagent, LH biotin reagent or PSA biotin reagent as the case may be) to all wells. Microplate was again swirled gently for 20–30 s. Wells were then covered and incubated for 60 min at ambient temperature. Contents of the microplate was discarded by decantation and the microplate blotted dry with absorbent paper. Wash buffer of about 350 μL was added to the microplate and then decanted; this procedure was repeated three times. Instruction on how to wash the microplate was followed strictly based on the manufacturer manual. Working substrate solution (100 μL) was pipetted into all the wells. To curb reaction time differences reagents were added in the same order. The plate was left to stand after the addition of substrate. It was then incubated at ambient temperature for 15 min. Afterwards, stop solution of 50 μL of was appended to each well and lightly mixed for 15–20 s. To curtail reaction time differences between wells the stop solution was added in the same order. Absorbance reading at 450 nm was recorded in each well via a reference wavelength of 620–630 nm to curtail irregular reading of absorbance wavelength in the microplate well. Reading of the solution was recorded within 30 min of stop reagent addition.

2.8 Serum Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST)

Determination of ALT and AST levels was carried out using Cobass C111 automatic analyser (Roche). Measurement against reagent blank; two test tubes were cleaned and set up as reagent blank and sample test and 0.5 mL of the Reagent 1 (R1) (Buffer) was put into the two tubes. 100 μL of distilled water was pipetted into the reagent blank while an equal volume serum was added to the sample test tube. The two test tubes were vortexed and incubated for 30 min at 37 °C. After incubation, 0.5 mL of Reagent 2 (R2) was added to both the reagent blank and sample test tubes, they were well agitated on a vortex machine to obtain a homogenous mixture and thereafter, incubated for 20 min at a temperature of about 20 °C–25 °C. 5 mL of sodium hydroxide was then pipetted into the two test tubes and mixed properly, after which the absorbance of the sample tube was taken against reagent blank at 546 nm after 5 min.

Measurement against sample blank; two test tubes were cleaned and set up as sample blank and sample test and 0.5 mL of Reagent 1 added to each tube respectively. 100 μL of sample serum was added to the sample test tube and the two test tubes swirled for proper mixing and incubated for 30 min at 37 °C. 0.5 mL of Reagent 2 was put into each sample blank and sample test and 100 μL of serum sample was added to the sample blank test tube. The two test tubes were thoroughly mixed and incubated for 20 min at a temperature of about 20 °C–25 °C. Five (5 mL) of sodium hydroxide

was pipetted into both test tubes after incubation, The two tubes were vortexed and absorbance of the sample test tube recorded against the sample blank at 546 nm after 5 min.

2.9 Determination of Serum Urea and Creatinine Levels

To determine serum urea levels, we used Cobass C111 automatic analyser (Roche). Briefly, three separate test tubes labelled as standard, blank and sample were set up. Working reagent of 1000 µL was filled into each of the three test tubes. The standard test tube was filled with 10 µL of the standard reagent after which another 10 µL of the serum sample was added to it. Contents of the three test tubes were vortexed and incubated for about 5 min at 37 °C. Thereafter, 1000 µL of colour reagent was pipetted and added to each of the three test tubes, mixed properly by swirling and incubated for 5 min at 37 °C, thereafter, addition of deionized water of 1000 µL to each of the three test tubes was done. The test-tubes were thoroughly vortexed and absorbance of serum sample and standard were measured alongside the reagent blank. Concentration of urea was calculated according to the expression below;

$$\text{Urea conc (mg/dL)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 40$$

Creatinine level was determined by labelling two test tubes; a sample blank and test sample respectively. 1000 µL of working reagent was added to each of the sample blank and test sample tubes while 100 µL of standard reagent was added to the sample blank and 100 µL of serum added to the test sample. The concoctions were vortexed and the Optical Density (T_1) recorded at 60 s after the serum sample or standard addition had been added. Precisely 1 min after the first reading, a second reading (T_1) was taken. Creatinine concentration in the sample was then calculated according to the expression below;

$$\text{Creatinine Concentration (mg/dL)} = \frac{T_2 - T_1 \text{ of Sample}}{T_2 - T_1 \text{ of Standard}} \times 2$$

2.10 Gas Chromatography/Mass Spectrometry Analysis

Agilent gas chromatograph (GC) (6890N) attached with a 5973 mass selective detector (MSD) and HP-5MS column (30 m × 250 µm × 0.25 µm) was employed for compound identification and separation of aqueous extract of *V. amygdalina* leaves. The carrier gas used was Helium at 1 mL/min flow rate whereas the average velocity and nominal initial pressure was programed at 26 cm/sec and 13 psi respectively. The ion source and quadruple temperatures were 230 °C and 150 °C respectively while the acquisition scan mass range from 50 to 500 amu. Starting temperature was set at 70 °C (2 min hold) and conditioned at 30 °C/min to 300 °C, thus giving a total runtime of 49.67 min. The aqueous extract (1 µL) was injected in a splitless mode at 250 °C with a 50 mL/min purge flow [36].

2.11 Detection of the Constituents

Crude aqueous extracts components of the leaves of *V. amygdalina* were detected from the GC–MS chromatogram. Analysis of the components of the extracts was carried out by comparison of their retention times (RT) with homologous series of *n*-alkanes in the NIST library 2014. Identity of the mass fragmentation patterns and calculated retention times of each compound were checked and compared with those available in the databases [37–39].

2.12 Qualitative Phytochemical Screening

Aqueous extract of *V. amygdalina* leaves was screened qualitatively for the detection of phytochemical components present in the extract by using the techniques of Harborne [40] and Evans [41]. Qualitative screening of the various phytochemicals was carried out by means of Mayer's and Wagner's reagents (alkaloids). Additional tests carried out include the modified foam test (saponins), Salkowski and Liebermann Burchard's tests (steroids and triterpenoids), ferric chloride test for (phenols and tannins) and lead acetate test for (flavonoids).

2.13 Statistical Analysis

Statistical analyses of data were performed using the SPSS version 14.0 for Windows (SPSS Inc., Chicago, IL). All data were expressed as Mean ± SEM. One-way analysis of variance (ANOVA) was used to assess differences between groups after which a post-hoc test, via Tukey's multiple comparisons analysis was employed to test least significant difference. Statistically significant difference of p-value < 0.05 was adopted.

3 Results

3.1 Spectrophotometric Evaluation of $AgNO_3$ Reduction

The most proficient and commonly used method to ascertain the synthesis of AgNPs at the preliminary stage of biosynthesis using plant is the UV–vis absorption spectrophotometer. An alteration in colour of the aqueous leaf extract from light yellow to deep brown after the addition of $AgNO_3$

solution validates the reduction of the $AgNO_3$ to AgNPs (Fig. 1a). UV–vis absorption spectrum of the *V. amygdalina* synthesized AgNPs is shown in Fig. 1b.

FTIR absorption peak observed in the biosynthesized AgNPs is 480 cm^{-1} assigned to the Ag–O band confirming the successful synthesis of AgNPs. Other vibrational peaks noticed at 1100, 1720 and 3300 cm^{-1} were allocated to C–O, C=O and O–H stretches respectively indicating the functional group of the different phytochemicals present in the plant part which are responsible for the reduction of $AgNO_3$ (Fig. 1c).

3.2 Morphological Characterization of Biogenic AgNPs with Electron Microscopy

Electron microscopy of the biosynthesized AgNPs with transmission electron microscope (TEM) shows that the shapes of the nanoparticles are spherical, rod-like and triangular with an average diameter of 34 nm (Fig. 2a). AgNPs appear to be well dispersed with minute evidence of agglomeration, while they show cubical, triangular, spherical with a lot of irregular shaped structures upon examination with a scanning electron microscope (SEM) (Fig. 2b); which is a common morphology with AgNPs [42]. Interestingly, EDS result confirmed that the materials are only made of silver, carbon and oxygen with silver exhibiting about 88% of the overall composition of elements in the different materials (Fig. 2c).

3.3 XRD Signature of the Biosynthesized Nanoparticles

XRD pattern of the nanoparticles obtained in this present study is shown in Fig. 3. The diffraction peaks at 38.352 (111), 44.352 (200), 64.537 (220), 77.473 (311) established the crystallinity of the biogenic AgNPs, and the values stated above are

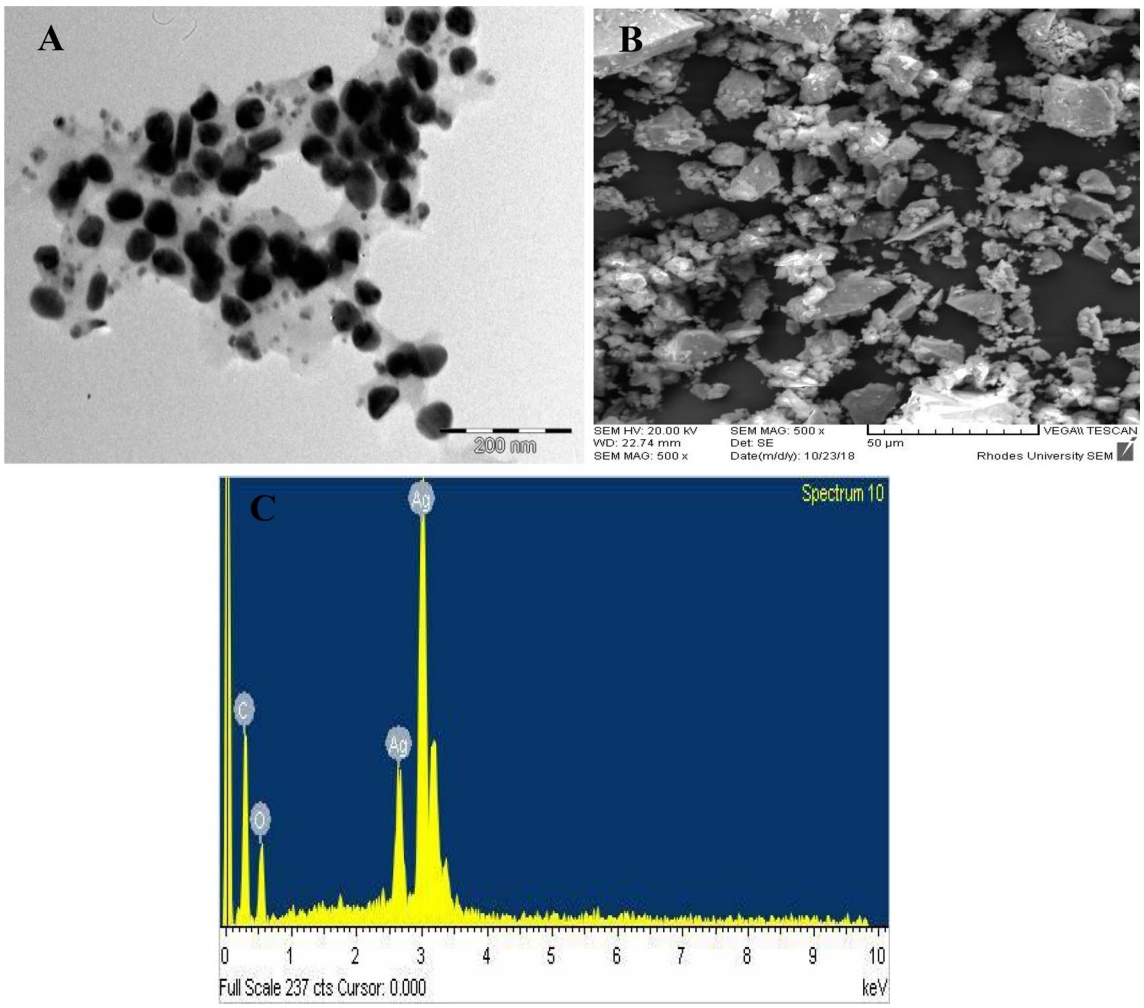

Fig. 2 Images of AgNPs under **A** TEM, **B** SEM, and **C** EDS

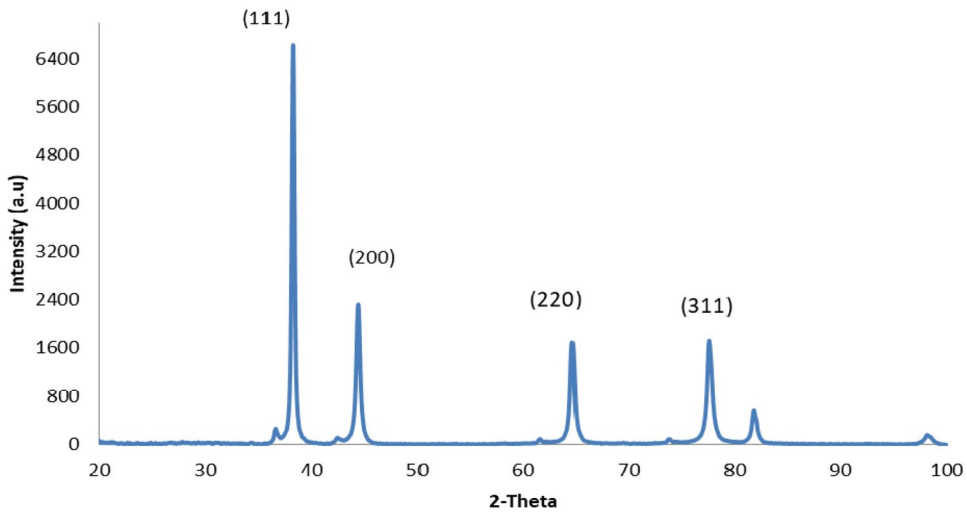

Fig. 3 XRD signature of bio-synthesized AgNPs

in harmony with those accounted for by the Joint Committee on Powder Diffraction Standards (JCPDS) file no: 04-0783.

3.4 Effect of Biogenic AgNPs on Body Weight and Prostatic Index of Wistar Rats

Figure 4 shows the distribution and changes in animal body weight and prostate weight. At the expiration of the 14-day experiment, body weight of rats given 50 mg/kg/day (group B) and 25 mg/kg/day (group C) of biogenic AgNPs were significantly higher than that of control (group A) at $p \leq 0.005$ while the weight of group B administered with high dose was significantly higher than group C, the low dose, testosterone only group (group D), biogenic AgNPs only group (group E) and finasteride treated group (group F) at $p \leq 0.0001$. Also, weight of the low dose group (group C) was significantly higher than that of finasteride treated group (group F) at $p \leq 0.005$ although there is no significant weight difference among the control and finasteride treated group.

The prostate weight of groups B and C were significantly different from A and F with both B and C higher than A and F but group B was found to be higher than C. Changes in prostatic index, which is a more accurate measure of changes in prostate weight as it takes into account the body weight, shows that rats in group C have the highest prostatic index followed by group D, group F, group B and control with significant difference present at $p \leq 0.0001$ between group B and control. Although the group with low dose of biogenic AgNPs has a higher prostatic index compared to the testosterone only group, the difference is not significant.

3.5 Administration of Biogenic AgNPs Reduced Epithelial Cell Hyperplasia, Increased Glandular Atrophy and Stroma Width in the Prostate of Wistar Rats Induced with BPH

As indicated in Fig. 5, normal histological features of prostatic tissue were recorded upon histological analysis of tissues from the animals in group A (placebo), which were given Goya olive oil and feeds The columnar epithelial cell of the control group recorded a mean height 3.1 mm with normal glandular histoarchitecture and stroma width. Group B animals, administered with 3 ml/kg/day of testosterone propionate (TP) and 50 mg/kg/day of biogenic AgNPs, shows high atrophy of the prostate gland, with an average height of 3.7 mm and wide stroma. Group C which was given 3 mL/kg/day of TP and 25 mg/kg/day of biogenic AgNPs shows columnar epithelial cell height of 3.7 mm in average, high glandular atrophy and wider stroma, and group D which was administered with 3 mL/kg/day of TP only has an average epithelial cell height of 5.0 mm with severe glandular hypertrophy and no stroma observed. In group E animals, which were administered with only 25 mg/kg/day of biogenic AgNPs, the histological features show severe glandular hypertrophy (measuring an average of 5.8 mm in epithelial cell height) and no stroma observed. Group F animals which were administered with 3 mL/kg/day of TP and 0.07 ml/kg/day of finasteride show prostate histology with a mean epithelial cell height of 2.7 mm, high glandular atrophy and wider stroma.

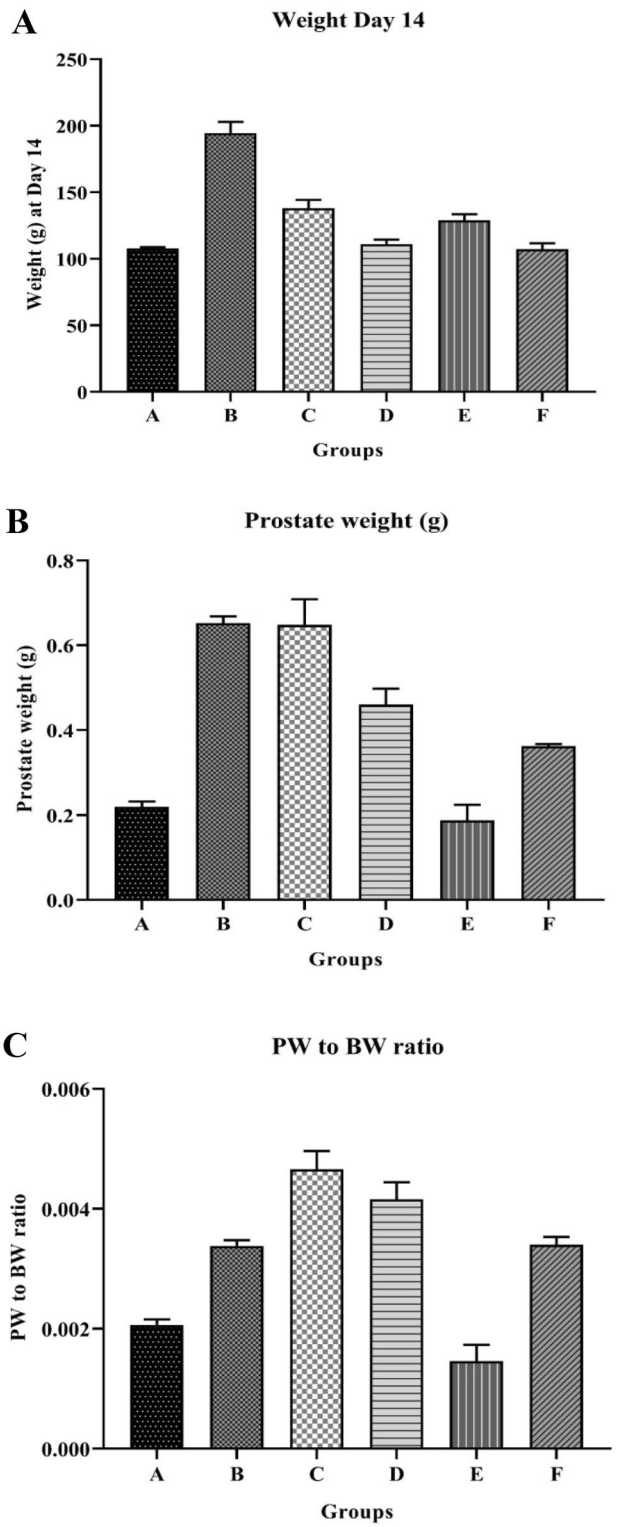

Fig. 4 Biogenic AgNPs affects the body weight and prostate weight of rats. **A** Body weight distribution. **B** Prostate weight distribution change. **C** Prostate index of animals at the end of the experiment. Bar A = control rats given water and feed only, B = rats given 3 mg/kg/day of TP and 50 mg/kg/day of biogenic AgNPs, C = rats given 3 mg/kg/day of TP and 25 mg/kg/day of biogenic AgNPs, D = rats given 3 mg/kg/day of TP only, E = rats given 25 mg/kg/day of biogenic AgNPs only and F = rats given 0.7 mg/kg/day of finasteride

3.6 Changes in Reproductive Hormones and PSA Following BPH Treatment with Biogenic AgNPs in Wistar Rats

Figure 6 shows the profile of reproductive hormones and PSA. Blood testosterone level of group B (administered with a high dose biogenic AgNPs) is significantly higher relative to that of control (group A), group E (biogenic AgNPs only) and finasteride treated group (group F). Serum testosterone of group C, which got the lower dose of biogenic AgNPs, is significantly higher than that of E and F at $p < 0.0005$. Furthermore, the testosterone level of group C, is not significantly different from both groups A and F (Fig. 6A). Serum FSH level of group C is significantly higher than those of groups A and F (Fig. 6B) while the serum LH level of group E is the highest followed by group C, group A and group B in that order. The LH level of group D was significantly higher than control at $p < 0.0001$. Groups B and C had LH levels that were not significantly different from that of group A, however the LH values of both group B and group C were significantly higher than that of group F (Fig. 6C). Estrogen values of group D, which is the highest, is significantly higher than that of control, group B, group C and group E while that of group F is significantly higher than group B at $p < 0.0005$ (Fig. 6D). PSA shows no appreciable change across groups.

3.7 Effect of Biogenic Silver Nanoparticles on the Liver Enzymes ALT and AST in Wistar Rats

The highest expression of ALT was recorded in group F, and it is significantly higher than that of every other group at $p < 0.0005$. Following after group F is group D, which is significantly higher than groups A and B at $p < 0.0005$. Group E ranks third in ALT expression and it is significantly higher than groups A and B at $p < 0.0005$ although its difference with D is not significant. Group B has the lowest ALT value. While B was seen to be lower than A, Group C was seen to be higher than A. Group F also expressed the highest AST values, which is significantly higher than those of other groups at $p < 0.0005$ except group C. Group B has a higher AST value than control which is not significant. Group B is however the least in AST expression among the rest of the groups (Fig. 7).

3.8 Assessment of Urea/Creatinine Level in Wistar Rats

Group F has the highest urea level and it is significantly higher than all the other groups at $p < 0.0005$. All groups are notably higher than that of control (Fig. 8) and there is

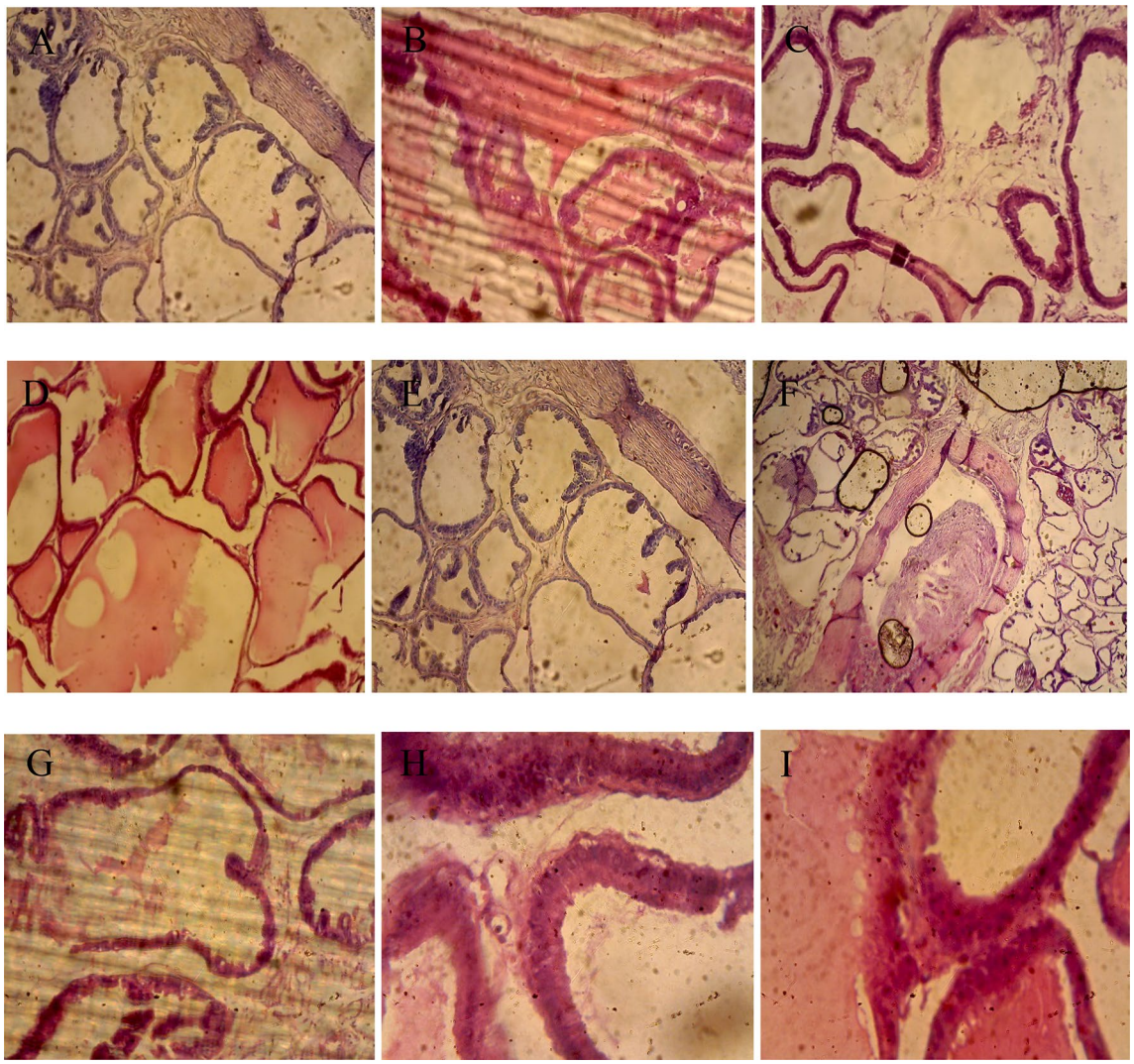

Fig. 5 Biogenic AgNPs reduces prostate hypertrophy in Wistar rats with testosterone-induced BPH. Representative pictures of H&E stained prostate tissues (magnification ×100) in; **A** control (placebo) group (mean epithelial cell height = 3.1 mm; normal stroma). **B** High biogenic AgNPs plus TP administered group (mean epithelial cell height = 3.7 mm; wide stroma). **C** Low biogenic AgNPs plus TP administered group (mean epithelial cell height = 3.7 mm; wider stroma). **D** TP only group (mean epithelial cell height = 5.0 mm; no stroma). **E** Biogenic AgNPs only group (mean epithelial cell height = 5.8 mm; no stroma). **F** Finasteride plus TP administered group (mean epithelial cell height = 2.7 mm; wider stroma). **G** Group B at magnification ×400. **H** Group C at magnification ×400. **I** Group D at magnification ×400. *AgNPs* silver nanoparticles, *TP* testosterone propionate

no significance difference in both urea and creatinine levels between the high dose biogenic AgNPs (group B) and low dose biogenic AgNPs (group C).

3.9 GC–MS Result of the Aqueous Extract of *V. amygdalina*

In an attempt to determine the composition of the aqueous extract of *V. amygdalina* leaves, we performed GC–MS analysis. The GC–MS components alongside their retention time (RT), percentage composition, molecular weight, molecular formula with the class of the chemical components of *V. amygdalina* aqueous extract are shown in Table 1. Forty-one components were identified by comparison of their retention times (RT) with homologous series of n-alkanes in the NIST library 2014, the extract showed a complex blend of several chemical classes such as hydrocarbon, ketone, saturated fatty acid, monounsaturated fatty acid, polyunsaturated fatty acid, monoterpenoid, diterpenoids, esters, sterols etc. The main phyto-constituents found in the aqueous leaf extract of this plant are α-linolenic acid (30.23%), hexedecanoic acid (18.48%), hexane (10.67%), squalene (6.59%), phytol (4.54%), heneicosane (5.31%), while those appearing in significant amounts are isopropyl linolenate (2.96%), 11,

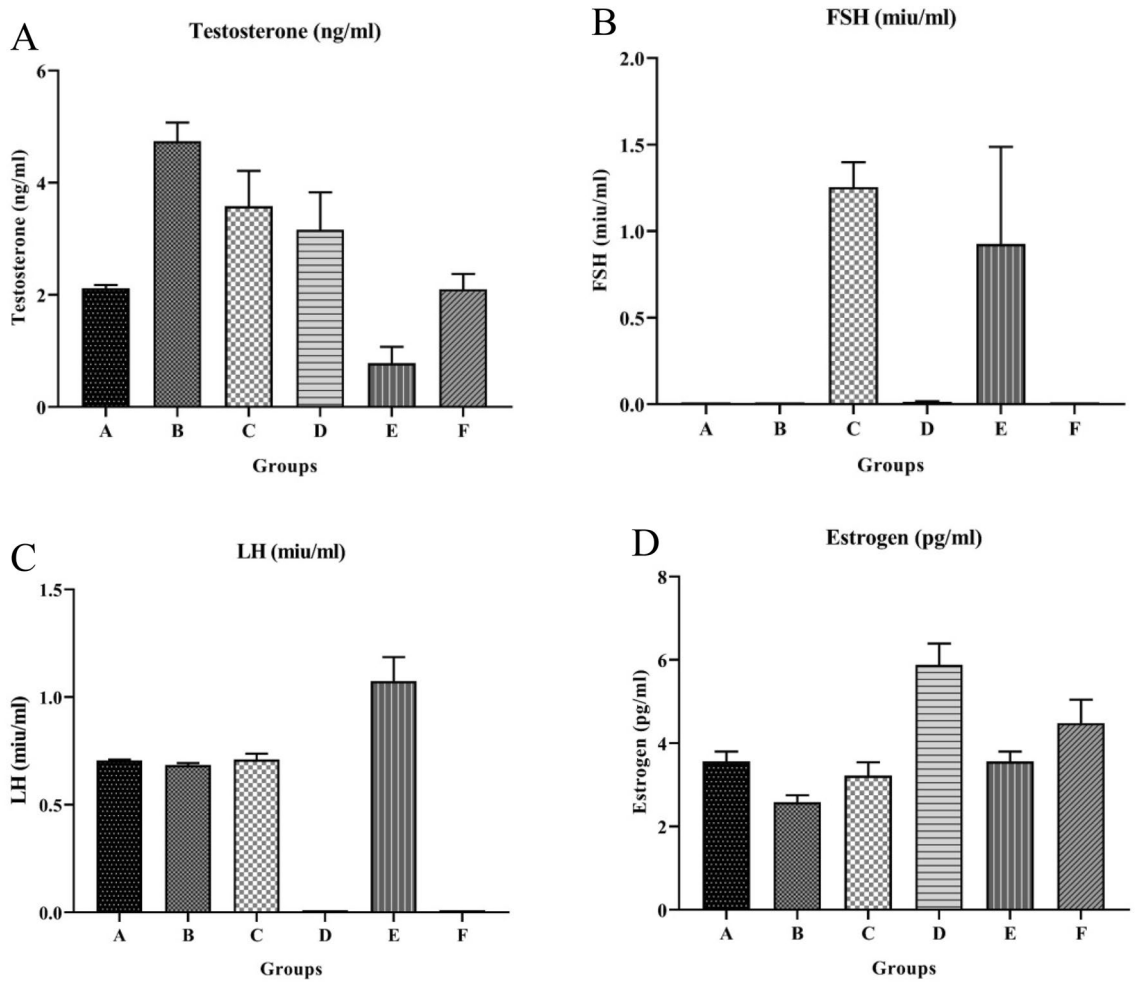

Fig. 6 Effect of biogenic AgNPs on reproductive hormones in rats with testosterone-induced BPH. **A** Testosterone. **B** Follicle Stimulating Hormone (FSH). **C** Lutenizing Hormone (LH). **D** Estrogen. Bar A = control rats given water and feed only, B = rats given 3 mg/kg/day of TP and 50 mg/kg/day of biogenic AgNPs, C = rats given 3 mg/kg/day of TP and 25 mg/kg/day of biogenic AgNPs, D = rats given 3 mg/kg/day of TP only, E = rats given 25 mg/kg/day of biogenic AgNPs only and F = rats given 0.7 mg/kg/day of finasteride

13-dimethyl-12-tetradecen-1-ol acetate (1.86%), phytyl tetradecanoate (1.69%), eicosanoic acid (1.54%) and vitamin E (1.31%).

3.10 Phytochemical Screening

The secondary metabolite study of the aqueous plant extract showed the presence of different bioactive compounds such as alkaloids, saponins, steroids, and triterpenoids, flavonoids, phenols, and tannins (Table 2).

4 Discussion

Advances in nanotechnology and medicinal plant research have led to the development of reliable strategies to generate nanoparticles ranging from 1 to 100 nm from plant extracts that are eco-friendly and more suitable for clinical applications [16]. In this study, AgNPs with an average diameter of 34 nm were successfully synthesized using aqueous extract of *V. amygdalina*. The AgNPs were spherical, rod-like and triangular in shape. Similar particle size of 34 nm was reported to have been obtained with the use of *Azadirachta indica* aqueous leaf extract in the synthesis of AgNPs [43]. Also comparable with our results is that of *Alternanthera sessilis* extract with AgNPs particle size of less than 50 nm [44], *Sinapis arvensis* seed exudates with particle size of 1–35 nm [45], *Butea monosperma* leaf extract with particle size of 5–35 nm [46], *Quercus infectoria* extract with particle size of 40 nm [47], *Aegle marmelos* (Bael) fruit extract with particle size of 34.7 nm [48] and *Sargassum longifolium* with particle size of 30 nm [49]. The quantity of plant extract and $AgNO_3$ concentration are said to be critical factors to a successful generation of biogenic AgNPs [50].

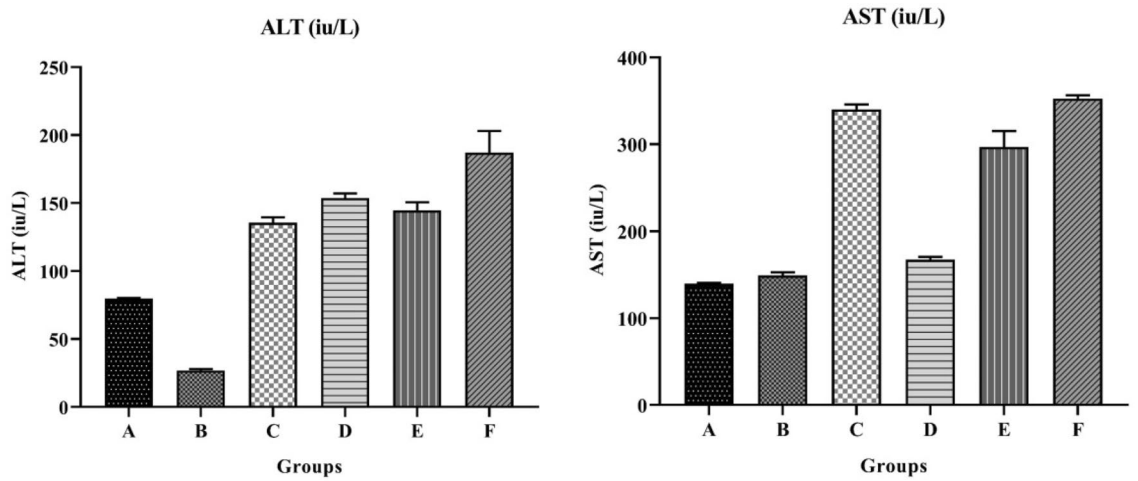

Fig. 7 Effect of biogenic AgNPs on the liver enzymes ALT and AST in testosterone-induced BPH rats. Bar A = control rats given water and feed only, B = rats given 3 mg/kg/day of TP and 50 mg/kg/day of biogenic AgNPs, C = rats given 3 mg/kg/day of TP and 25 mg/kg/day of biogenic AgNPs, D = rats given 3 mg/kg/day of TP only, E = rats given 25 mg/kg/day of biogenic AgNPs only and F = rats given 0.7 mg/kg/day of finasteride

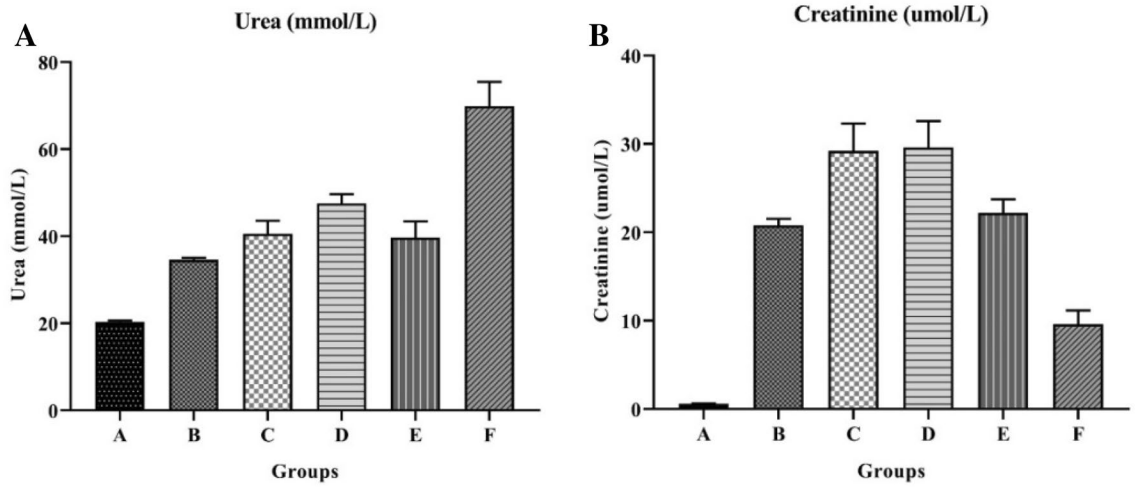

Fig. 8 Effect of biogenic AgNPs on kidney function. **A** Urea. **B** Creatinine. Bar A = control rats given water and feed only, B = rats given 3 mg/kg/day of TP and 50 mg/kg/day of biogenic AgNPs, C = rats given 3 mg/kg/day of TP and 25 mg/kg/day of biogenic AgNPs, D = rats given 3 mg/kg/day of TP only, E = rats given 25 mg/kg/day of biogenic AgNPs only and F = rats given 0.7 mg/kg/day of finasteride

These factors are also important in the size control of the synthesized AgNPs [50].

The use of nanoparticles in targeted drug delivery, stem cell therapy, cancer treatment, vaccines and as anti-microbial agents is well documented [6]. Although nanomaterials may be produced using physical and chemical methods we have utilized the aqueous extract of *V. amygdalina* in reducing metallic silver ion. The choice of AgNPs was due to its well documented therapeutic activity such as antibacterial, anti-fungal, anti-inflammatory and cytotoxic properties against cancer cells [2, 22–25, 44, 45, 51–54]. This is in addition to its large surface-to-volume ratio, non-toxic, non-allergic and environmentally friendly properties [11]. The biological activity of inorganic nanoparticles such is known to be influenced by factors that include size distribution, morphology, surface charge, surface chemistry and capping agents [2, 55]. Moreover, working with AgNPs is relatively cheaper compared with AuNPs, for example. In this study, aqueous extract of *V amygdalina* was used as a reducing and stabilizing agent in the green synthesis of AgNPs because liquids that use water solvents are considered to be much safer for health and the environment than chemical solvents [56, 57]. Several studies have established the ethno pharmacological basis for the use of aqueous extracts of plants in

Table 1 Bioactive constituents of aqueous extract of *Vernonia amygdalina*

S/no.	RT (min)	Bioactive constituents in (*Vernonia amygdalina*)	Molecular weight (MW)	Molecular formula (MF)	Area (%)	Class of compound
1	3.28	*n*-Hexane	86	C_6H_{14}	10.67	Hydrocarbon
2	6.03	*trans*-β-Ionone	192	$C_{13}H_{20}O$	0.13	Ketones
3	6.18	Dodecanoic acid	200	$C_{12}H_{24}O_2$	0.34	Saturated fatty acid
4	6.24	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	180	$C_{11}H_{16}O_2$	0.39	Bicyclic heterocycle
5	6.36	Caryophyllene oxide	220	$C_{15}H_{24}O$	0.13	Bicyclic Sesquiterpene
6	6.42	*trans*-Chrysanthenol	152	$C_{10}H_{16}O$	0.08	Monoterpenoid alcohol
7	6.55	1,6,6-Trimethyl-7-(3-oxobut-1-enyl)-3,8-dioxatricyclo [5.1.0.0(2,4)] octan-5-one	236	$C_{13}H_{16}O_4$	0.17	Ketones
8	6.65	Tetradecanoic acid	228	$C_{14}H_{28}O_2$	0.36	Saturated fatty acid
9	6.73	E-15-Heptadecenal	252	$C_{17}H_{32}O$	0.16	Aldehyde
10	6.80	Loliolide	196	$C_{11}H_{16}O_3$	0.13	Monoterpenoid lactone
11	6.83	Neophytadiene	278	$C_{20}H_{38}$	0.26	Hydrocarbon
12	6.85	2-Pentadecanone, 6,10,14-trimethyl-	268	$C_{18}H_{36}O$	0.28	Ketone
13	6.88	Pentadecanoic acid	242	$C_{15}H_{30}O_2$	0.17	Saturated fatty acid
14	6.93	3,7,11,Trimethyl-8,10-dodecedienylacetate	266	$C_{17}H_{30}O_2$	0.23	Ester
15	7.02	Hexadecanoic acid, methyl ester	270	$C_{17}H_{34}O_2$	0.12	Fatty acid methyl ester
16	7.07	Palmitoleic acid	254	$C_{16}H_{30}O_2$	0.16	Monounsaturated fatty acid
17	7.13	*n*-Hexadecanoic acid	256	$C_{16}H_{36}O_2$	18.48	Saturated fatty acid
18	7.32	Heptadecanoic acid	270	$C_{17}H_{34}O_2$	0.78	Saturated fatty acid
19	7.43	Phytol	296	$C_{20}H_{40}O$	4.54	Diterpenoid
20	7.55	α-Linolenic acid	278	$C_{18}H_{30}O_2$	30.23	Polyunsaturated fatty acid
21	7.78	Isopropyl linolenate	320	$C_{21}H_{36}O_2$	2.96	Ester
22	7.92	Shogaol	276	$C_{17}H_{24}O_3$	1.18	Others
23	7.96	Stigmasta-4,6,22-trien-3.alpha.-ol	410	$C_{29}H_{46}O$	0.79	Sterol
24	8.00	Eicosanoic acid	312	$C_{20}H_{40}O_2$	1.54	Saturated fatty acid
25	8.05	11, 13-Dimethyl-12-tetradecen-1-ol acetate	282	$C_{18}H_{34}O_2$	1.86	Ester
26	8.34	Pentacosane	352	$C_{25}H_{52}$	0.48	Saturated hydrocarbon
27	8.43	Cis-13-octadecenoic acid	282	$C_{18}H_{34}O_2$	0.24	Monounsaturated fatty acid
28	8.49	Bis (2-ethylhexyl) phthalate	390	$C_{24}H_{38}O_4$	0.51	Ester
29	9.00	Heptacosane	380	$C_{27}H_{56}$	0.08	Saturated hydrocarbon
30	9.43	Heneicosane	296	$C_{21}H_{44}$	5.31	Saturated hydrocarbon
31	9.56	Squalene	410	$C_{30}H_{50}$	6.59	Triterpenes
32	9.78	α-Tocospiro	462	$C_{29}H_{50}O_4$	0.22	Others
33	9.95	Nonacosane	408	$C_{29}H_{60}$	3.88	Saturated hydrocarbon
34	9.99	Cis-1-chloro-9-octadecene	286	$C_{18}H_{35}Cl$	0.21	Hydrocarbon
35	10.13	Trans-geranyl geraniol	290	$C_{20}H_{34}O$	0.40	Diterpenoid alcohol
36	10.54	Triacontane	442	$C_{30}H_{62}$	0.64	Hydrocarbon
37	11.38	Nonacos-1-ene	406	$C_{29}H_{58}$	0.31	Hydrocarbon
38	11.73	Vitamin E	430	$C_{29}H_{50}O_2$	1.31	Vitamers
39	13.18	Stigmasterol	412	$C_{29}H_{48}O$	0.27	Sterols
40	13.88	Stigmasta-7,16-dien-3-ol, (3β,5α)-	412	$C_{29}H_{48}O$	0.90	Sterol
41	17.25	Phytyl tetradecanoate	506	$C_{34}H_{66}O_2$	1.69	Ester
Total bioactive constituents (99.18%)						

the treatment BPH [58–63]. Studies have also shown that *V. amygdalina* is rich in phytochemicals including flavonoids [64–67]. This can be confirmed by the presence of carbon and oxygen as shown by the EDS spectra in the biogenic AgNPs, which could be attributed to plant-derived bioactive compounds present in the formed nanoparticles. Moreover,

Table 2 Qualitative phytochemical analysis of aqueous leaf extract of *V. amygdalina*

Bioactive chemical components	Aqueous leave extract
Tannin	+
Saponins	+
Alkaloid	+
Flavonoids	+
Steroids	+
Phenols	+
Triterpenoids	+

+ present, − absent

our group has previously demonstrated the efficacy of aqueous extract of *V amygdalina* in the treatment of BPH [35]. Therefore, it is logical to perform green synthesis of AgNPs with aqueous extract of *V amygdalina* as this is likely to potentiate the ameliorative effect of AgNPs on BPH, at least in principle. Plant parts and their extracts are now used for nanoparticles production through green synthesis and their function is to reduce and stabilize the nanoparticles [54]. The reduction and stabilization of metallic ions by plant parts and their extracts is brought about by diverse plant metabolites like amino acids, alkaloids, tannins, saponins, flavonoids, enzymes, vitamins and terpenoids which have been established to possess therapeutic activity [54, 68]. All these phytoconstituents may have a synergistic effect in the bioreduction of the $AgNO_3$ solution.

In this study, the administration of biogenic AgNPs reduced prostate enlargement by inhibiting epithelial cell hyperplasia and glandular hypertrophy. Our findings indicate that biogenic AgNPs administered at a dose of 50 mg/kg/day and 25 mg/kg/day for 14 consecutive days ameliorated prostatic hypertrophy in rats with hormone-induced BPH indicating that biogenic AgNPs have a counteracting effect to that of testosterone in the prostate gland. However, animals administered with a low dose of biogenic AgNPs only in the absence of testosterone administration showed histological evidence of glandular hyperplasia and increased body weight as well as a significant reduction in prostate index compared to the placebo and finasteride treatment group. Interestingly, high dose of biogenic AgNPs appears to be more effective at achieving a lower prostate index than treatment with finasteride, a 5-alpha reductase inhibitor. Testosterone propionate, on the other hand, induced prostate enlargement characterized by hyperplasia of epithelial and stromal cells with the negative control showing normal histological features.

Biogenic AgNPs with the combination of exogenous testosterone increased serum testosterone level in a dose-dependent manner with 50 mg/kg/day, the high dose, producing a significantly higher testosterone level compared to control and finasteride treatment. Biogenic AgNPs alone induces a decrease in serum testosterone level as compared to control group. An earlier report indicates that gold nanoparticles elevate serum testosterone levels in mice [69]. Serum estrogen level on the other hand is reduced by the administration of biogenic AgNPs. Although this reduction in estrogen is not significantly lower than placebo, it is significantly lower than the values in rats administered with testosterone only as well as those treated with combination of finasteride and testosterone. This underscores the significance of hormonal milieu apart from the inflammatory processes that may be involved in the development of BPH [27, 70, 71].

Biochemical analysis involving the liver enzymes ALT and AST provide some insight into the functional integrity of liver cells, the hepatocytes and toxicity of administered biogenic AgNPs since liver injury results in spill over of these enzymes into the blood stream [72, 73]. Liver ALT is significantly lower in the biogenic AgNPs groups (with or without exogenous testosterone) compared to both control and finasteride treatment groups and a combination of exogenous testosterone with biogenic AgNPs produces a much lower ALT level compared to control group. Although AST values for the low dose AgNPs group is moderately high, that of the high dose group is surprisingly low, suggesting preservation of hepatocyte integrity and normal liver function. This is consistent with a previous report which found significant increase in body weight as well as no change in liver function due to nanoparticles treatment in male Wistar rats [74]. Significant increase in the levels of the renal biomarkers, urea creatinine, suggests that biogenic AgNPs may be toxic to the kidney [75, 76].

In the present study, α-linolenic acid was found to be the most abundant constituent of the aqueous extract of *V. amygdalina*. Interestingly, α-linolenic acid has been documented to trigger substantial increase in docosahexanoic acid (DHA), α-linolenic acid (ALA), docosapetaenoic acid (DPA), eicosapentaenoic acid (EPA) levels and ominously decrease arachidonic acid (AA) level in blood, liver and prostrate tissue as well as reduce prostate cancer growth in a mouse model [77]. Squalene, a polyunsaturated hydrocarbon is another major component of this plant extract, and it has also been shown to reduce oxidative damage in mouse [78].

A number of plants have been employed in the treatment of BPH. One of such plants is the African potato (*Hypoxis rooperi)* of the family Hypoxidaceae [79]. This plant is generally used in treating urinary tract infections, lung diseases, tuberculosis, arthritis and prostate cancer. The pharmacological action of African potato is linked to sterols particularly *β*-sitosterol and *β*-sitosterol glycoside, and lignans (rooperol). It acts by inhibiting cyclooxygenase-1 and cyclooxygenase-2, and obstruct the production of inflammatory intermediaries such as prostaglandins [80]. Another

plant of interest is *Serenoa repens*, commonly called *Saw palmetto*. *Saw palmetto* belongs to the genus Serenoa and it is used in the treatment of BPH [79, 81]. The therapeutic action of *Saw palmetto* against BPH is believed to be linked to its phytochemical constituents like free fatty acid, esters and glycerides, which act via the anti-androgenic pathway [82]. Its anti-inflammatory action is through the inhibition of cyclooxygenase production and pro-inflammatory genes among others [83–85]. Evidence from the literature indicates that plant extracts contain bioactive constituents comprising terpenoids, polyphenols, flavonoids, ascorbic acid, sterols, triterpenes, alkaloids, alcoholic compounds, saponins, β-phenylethylamines, tannins, polysaccharides, glucose, fructose, and proteins or enzymes, which might function as reductants for metal cations [70]. Although the bioactive components of plant extracts are numerous, thereby presenting a challenge with regards to identifying what is responsible for biological activity, they often work mutually to avert oxidative damage to cellular components [69]. A recent report indicates that flavonoids, phenols and alkaloids present in *Poa annua* could be functioning as reducing and stabilizing agents for AgNPs produced by green synthesis using *P. annua* extract [10] whereas glucose and ascorbate may bio-reduce gold and silver and ions to produce nanoparticles at higher temperatures [71, 72].

5 Conclusion

Biogenic AgNPs of approximately 34 nm size were successfully synthesized from the aqueous extract of *V. amygdalina*. Synthesized AgNPs administered for 14 days effectively ameliorated prostatic hyperplasia induced by testosterone in Wistar rats. Biogenic AgNPs show some evidence of nephrotoxicity with no hepatotoxicity. Further investigation is however needed to validate this result as well as the effect of long-term use of biogenic AgNPs.

Author Contributions RL conceived the idea; AA, RL, YA, NAU, and KA designed the study; AA, RL, YA, OOF, NAU, and JOB wrote this article and participated in performing the experiments; HA, SO, EA, EC, OSO, and UPI performed the experiments; RL, AA, NAU, JOB, and KA prepared the figures, analysed the data and revised the article.

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Availability of Data and Material Yes.

Code Availability Not applicable.

Declarations

Conflict of interest None.

Ethical Approval College Research and Ethics Review, Kogi State University, Anyigba.

Consent to Participate Not applicable.

Consent for Publication Approved by all authors.

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