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Medicinal aspects of Murraya koenigii mediated silver nanoparticles

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Abstract. The present work aimed to explore green approach via aqueous leaves extract of Murraya koenigii (ALEMk) for the synthesis of silver nanoparticles (AgNPsMk) in single step. The synthesis process was visualized with a color change and monitored by employing UV/Visible spectroscopy and a clear peak attained at 420 nm confirming the synthesis of AgNPsMk. The possible functional groups present in the extract which participated in the synthesis of AgNPsMk were identified with the help of FTIR spectroscopy. Further characterization using TEM images revealed the spherical shape of AgNPsMk with average particle size of 20 nm displaying well dispersion throughout the solution. Pronounced antioxidant activities of AgNPsMk at increased concentrations observed which evidencing strong radical scavenging ability. Moreover, AgNPsMk exhibited strong antibacterial behavior when tested against bacterial strains of Escherichia coli and Bacillus subtilis. Moving ahead, in vitro cytotoxicity work revealed potent cell viability loss appearing in AU565 and HeLa cancer cell lines on exposure to AgNPsMk at increased concentration. Finally, in vivo assessment carried out inside male Wistar rats indicated non toxic effect on examined liver tissues besides biochemical analysis including bilirubin, alkaline phosphtase (ALP) and serum glutamate pyruvate transaminase (SGPT) which found within the normal range when compared with control. The prior research work profoundly apprises the potential of green synthesized AgNPsMk to play a significant role in biomedical applications and formulations.
Keywords: antibacterial; antioxidant; biomedical; cell viability; characterization; cytotoxicity; green synthesis; nanoparticles

1. Introduction

As an outstanding field of science, nanotechnology is dealing with those objects having nanodimensions especially between 1 to 100 nm size range with exceptional behavior and vibrant features leading toward diverse applicability in multidimensional fields including food, sensors, optics, electronics, catalysis, agriculture, paints, cosmetics, batteries, and most importantly medicines (Kanipandian *et al*. 2014, Venkatesan *et al*. 2017). Reportedly, several amazing attributes are related with the noble metals nanoparticles but silver notably possesses unique place among them because of its high thermal and electrical conductivity, chemical stability, surface enhanced Raman scattering, strong catalytic ability as well as antimicrobial and antiviral characteristics (Jeyaraj *et al*. 2013). Owing to such remarkable medicinal features (Reddy *et al*. 2014) and properties researchers are enormously focusing on nanosilver behavior in the area of nanomedicine (Basavegowda *et al*. 2014).

For the synthesis of metal nanoparticles, numerous chemical and physical methods have been opted but are associated with certain drawbacks for instance, toxic chemicals involvement besides high pressure and temperature conditions (Liu *et al*. 2018). Apart from these, green chemistry approach is synergistic owing to its simple, eco-friendly, cost effective, non toxic, and easy perspective (Pethakamsetty *et al*. 2017) as synthesis of propitious size and shape of nanoparticles can be assessed with economically feasible way using biological means such as plants and their crude extracts (Uddin *et al*. 2017). In addition to this, plant's phytoconstituents have excellent reducing properties which participated in the reduction of metal ions into zero valent metallic nano form and ultimately contribute toward their stabilization (Thakkar *et al*. 2010). Moreover, the reduction of metal ions is mainly accompanied by the biomolecules present in the plants primarily include alkaloids, amino acids, proteins, alcohols, enzymes, vitamins, and polysaccharides (Makarov *et al*. 2014) therefore, the resultant nanoparticles have shown potential to treat and diagnose several life threatening diseases (Mittal *et al*. 2015, Ramar *et al*. 2015).

Researchers are focusing on specific formulations to develop such therapeutics which reduce the risks associated with the use of anticancer drugs as they cause severe side nanoparticles in combination with chemotherapeutic agents are in progress to produce anticancer effect (Rasheed *et al*. 2017). Notably, silver nanoparticles synthesized from plants extracts are considered to be important candidates in the sense that toxicity which might associated with the chemical and physical method can be mitigated especially while investigating medically for cancer therapy due to their pronounced biocompatibility with biological systems (Pirtarighat *et al*. 2019). Medicinal plants have unique place in the area of research for decades by reason of potent biomolecules they contain. Murraya koenigii commonly

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known as curry leaf or curry patta is a medicinal plant with characteristic aroma belonging to the family Rutaceae and cultivated throughout the summer, spring, and rainy season in the tropical regions (Singh *et al*. 2014) and originated from Pakistan, India, and China but also cultivated in United States and Australia as well. Studies have shown that different phytochemicals present in every poisonous bites and eruptions caused by animals can be cured by the roots and bark while dysentery, vomiting, diarrhea, inflammation, and itching type ailments are treated with the help of leaves (Jain *et al*. 2012). Virtually, there are several properties such as reducing cholesterol (Xie *et al*. 2006), antidiabetic (Srinivasan, 2005) antimicrobial (Rehman and Gray, 2005), positive inotropic (Shah and Juvekar, 2006), antioxidant (Gupta and Singh, 2007), antiulcer (Patidar, 2011), and anticancer (Ito *et al*. 2006) exhibited by M. koenigii as well.

The present study was spawned prospect to synthesize stable silver nanoparticles from the ALEMk which further leading toward characterization. In vitro antioxidant and antibacterial activities were carried out using different concentration of AgNPsMk and a comparison was made with extract and standard. Cytotoxicity approach was assessed to check in vitro anti-proliferative role of AgNPsMk on cancer cell lines of AU565 and Hela and ultimately analyzed in vivo biocompatibility within the biological system.

2. Materials and methods

2.1 Materials

Silver nitrate ($AgNO_3$), Ascorbic acid, 2,2, Diphenyl-1-picrylhydrazyl (DPPH), Hydrogen peroxide (H_2O_2), Butylated hydroxyl toluene (BHT), Sodium nitroprusside, Chloramphenicol, Mueller Hinton Agar (MHA), Dulbecco's Modified Eagle Medium (DMEM), Fetal bovine serum (FBS), Penicillin, Streptomycin, 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl-tetrazolium bromide, DMSO, Doxorubicin, Cisplatin, Formalin, and de-ionized water. All chemicals and reagents used in this study were of analytical grade and used without any purification.

2.2 Collection of plant material and extract preparation

Fresh leaves of *M. koenigii* were collected from the botanical garden of University of Agriculture, Faisalabad during the month of June and July and washed with tab water and finally with double distilled water, dried, and ground to fine powder. The ALEMk was prepared according to the method mentioned by Ramesh *et al*. (2018) with some modification. The powdered leaves (5 g) were heated in 100 mL de-ionized water for approximately 15 min followed by filtration at room temperature and resulting filtrate stored at 4°C used within two days.

2.3 Synthesis of AgNPsMk

10 mL of the ALEMk was added into 1mM freshly prepared $AgNO_3$ (50 mL), the resulting mixture stirred for 5 min and placed in dark chamber at room temperature. The visual inspection of color was continuously monitored during incubation. The UV/Visible absorption spectra of the AgNPsMk solution, ALEMk, and $AgNO_3$ solution were processed in the range of 200 to 800 nm and compared (Adur *et al*. 2018). The synthesis process, characterization and applications of AgNPsMk are illustrated in Fig. 1.

2.4 Characterization

2.4.1 UV visible spectroscopy

Double beam spectrophotometer (SP 2000 UV) was used to monitor the AgNPsMk synthesis at a scan of 100 per minute with 0.3 s integration of time (Pinto *et al*. 2017).

2.4.2 FTIR spectroscopy

The FTIR spectroscopy was employed to determine the possible phytoconstitiuents present in the ALEMk which participated in the reduction process along with the synthesized AgNPsMk. FTIR spectra obtained with the transmittance mode and the selected range was 4000 to 500 cm^{-1} with $4cm^{-1}$ resolution using Shimadzu IR Prestige FTIR spectrometer. 1% sample was taken and mixed with powdered KBr with thin layer slice and placed in oven (Prabhakar *et al*. 2017).

2.4.3 Transmission Electron Microscopy (TEM)

The TEM micrograph images were used to determine the structure and morphology of synthesized AgNPsMk using Gatan Erlangshen CCD model 100 CX II with 200kV high resolution. The sample preparation was involved sonication of AgNPsMk solution for approximately 20 min and the obtained suspension (2 to 3 drops) then evenly distributed onto the carbon coated grid and dried (Balashanmugam *et al*. 2016).

2.5 Antioxidant potential

2.5.1 DPPH assay

The DPPH activity was determined according to the method used by Mohanty and Jena (2017). The AgNPsMk, ALEMk, and ascorbic acid (standard) with different concentrations such as 25, 50, 100, 250, 500, and 1000 µg/mL were mixed with 5mL freshly prepared methanolic solution of DPPH (0.1M). The solution placed in the dark for half an hour after which absorbance was measured at 517 nm. Radical scavenging activity was calculated according to the Eq. (1):

$$\text{Inhibition (\%)} = \frac{\text{AC}-\text{AS}}{\text{AC}} \text{ x } 100 \qquad (1)$$

where AC is absorbance of control and AS absorbance of sample or standard.

2.5.2 H_2O_2 assay

H_2O_2 activity was calculated followed by Kanipandian *et al*. 2014 and the AgNPsMk and extract (200 µL) at different concentration (100, 200, 300, 400, 500, and 600 µg/mL) were individually mixed with H_2O_2 solution (5 mM) and incubated at room temperature for 30 min. The optical density of the solutions was recorded at 610 nm.

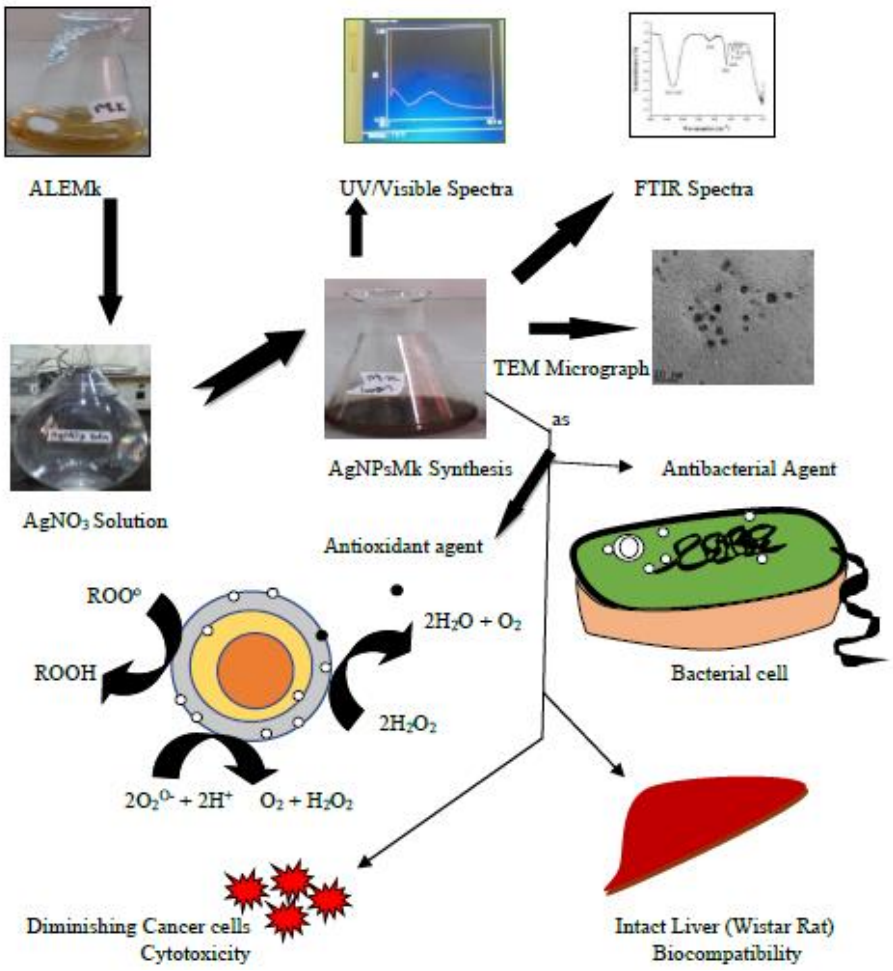


Fig. 1 Schematic for simply-supported CNTs

Inhibition (%) was calculated according to Eq. (1) where ascorbic acid used as standard.

2.5.3 Nitric Oxide (NO) assay

NO radical scavenging potential was determined taking different concentration (25, 50, 100, 150, 200, and 250 µg/mL) of ALEMk and AgNPsMk as measured by Geetanjali and Pawar, (2018) with BHT as standard. Each (200 µL) was mixed with equal volume of 20 mM freshly prepared sodium nitroprusside and the resultant solution incubated for 90 min at room temperature. The optical density was measured at 546 nm and radical scavenging activity calculated according to Eq. (1).

2.6 Antibacterial activity (Agar well diffusion method)

Antibacterial activity was evaluated according to the (Rajesh *et al*. 2018) with minor modification. From the microbiology Department, University of Agriculture, Faisalabad, pure cultures of *Escherichia coli* and *Bascillus subtilis* were collected and MHA (2%, pH 7) employed to prepare plates which extended 10 mL of the ALEMk was added into 1mM freshly prepared $AgNO_3$ (50 mL), the resulting mixture stirred for 5 min and placed in dark chamber at room temperature. The visual inspection of color was continuously monitored during incubation. The UV/Visible absorption spectra of the AgNPsMk solution, ALEMk, and $AgNO_3$ solution were processed in the range of 200 to 800 nm and compared (Adur *et al*. 2018).

2.7 In vitro cytotoxicity analysis of AgNPsMk

2.7.1 Cell culture and cell line maintenance

In vitro cytotoxicity work was performed in Hussain Ebrahim Jamal (HEJ) research institute of chemistry. The Hela cells and AU565 breast carcinoma cells were individually employed to assess cytotoxicity of synthesized AgNPsMk. DMEM having pH 7.2 was implemented for cells growth along with FBS (5%), 100 IU/mL penicillin and 100 µg/mL streptomycin taken in 75 cm^2 which further incubated with 5% CO_2 atmosphere at room temperature (Piug *et al*. 2011).

2.7.2 MTT assay

The MTT assay was used for cytotoxicity assessment of AgNPsMk based on colorimetric detection and 96 well flat bottom micro plate integrated with cultured cells (100 µL/well) having 5×10^4 cells/mL concentration. Medium was removed with 200 µL fresh medium after overnight incubation and to this, 200 µL of AgNPsMk with different concentrations (30 to 100 µL) added and the resulting medium again incubated for 2 days. Insoluble crystals of

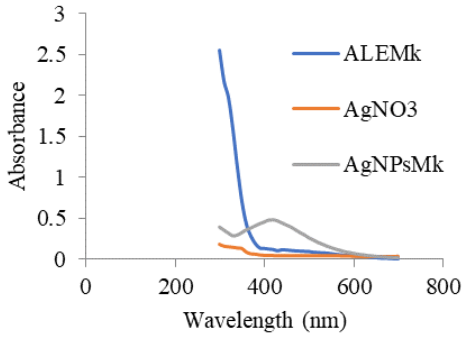


Fig. 2 UV/Visible absorption spectra of ALEMk, $AgNO_3$ solution and synthesized AgNPsMk

formazan were formed after addition of 200 µL of MTT (0.5 mg/mL) into this medium which further incubated at 37°C for four hours. Multichannel well plate used to remove excess bulk medium without disturbing formazan crystals. These crystals were dissolved by addition of 100 µL DMSO in each well which further 15 to 20 min agitated followed by immediate measurement of absorbance at 570nm with 650nm reference wavelength. Doxorubicin and Cisplatin were used as standard whereas cells (without sample) served as positive control (Rolim *et al*. 2019). % Inhibition determined from the Eq. (2) as:

$$\text{Inhibition (\%)} = 100 - \frac{\text{MAS}-\text{MAC}}{\text{MAC}} \text{ x } 100 \qquad (2)$$

where MAS is the mean of absorbance of test sample, MAC is mean of absorbance of positive control.

2.8 In vivo biocompatibility assessment

2.8.1 Animal selection and maintenance

The green synthesized AgNPsMk were subjected to cytotoxicity study inside the living organs using Wistar albino male rats having body weight between 100-150 g by following day and night controlled conditions including commercial balanced diet throughout the experiment. National Biosafety Committee (NBC) University of Agriculture, Faisalabad provided the approval for the study and all necessary measures were put into consideration for the respective study. Animals were placed in animal house, University of Agriculture, Faisalabad and any injury or illness in animals was monitored (Ramar *et al*. 2015).

2.8.2 Experimental work

All animals were weighed before and after the completion of dose period (16 days dose period including one, 6th, 11th and 16th day). Two groups were made each consisted of 4 animals from which one group served as control (only distill water without any dose) while other treated (injected 50 µg/mL dose of AgNPsMk) group. Animals were weighed and blood collected from them and dissection performed on the twenty first day and liver coefficients taken which preserved in 10% formalin (Rojas *et al*. 2017). After collection, blood was centrifuged at 1000 rpm for 20 min and liver function was performed such as bilirubin (total, direct, indirect), ALP and SGPT for biochemical analysis. Histopathological study of the excised liver tissues was carried out and for this purpose, specimens were fixed in 10% formalin followed by embedding and staining using paraffin and dying by hematoxylin and eosin dye. The optical microscope was used to examine the slides at 40× photograph (Karthick *et al*. 2014).

2.9 Statistical analysis

The results were expressed as mean ± S.E (at least 6 runs) and statistical analysis was performed using Students't-test to estimate the level of significant differences. Difference was considered significant when $p < 0.05$ (Javed *et al*. 2017).

3. Results and discussion

3.1 Nanoparticles synthesis and characterization

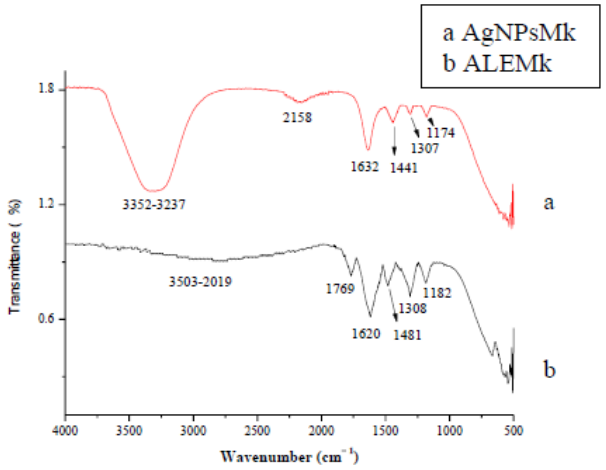
3.1.1 UV/Visible study

The AgNPsMk synthesized from the ALEMk were monitored with the help of UV/Visible spectroscopy and resulting spectra is given in Fig. 2. The preliminary evidence of the synthesis process was obvious as the solution color started to change just after mixing the colorless $AgNO_3$ solution and pale colored ALEMk which got intensified as the reaction time increase and maximum wavelength obtained at 420 nm mainly attribute to the surface plasmon resonance (SPR) exhibiting by free electrons which are present in the metal and get excitation when light interact with the metal surface producing combined oscillation as examined by Benakashani *et al*. (2016) and others. It is observed that there is no such peak formation was appeared in case of ALEMk or $AgNO_3$ solution when their UV/Visible spectra were taken.

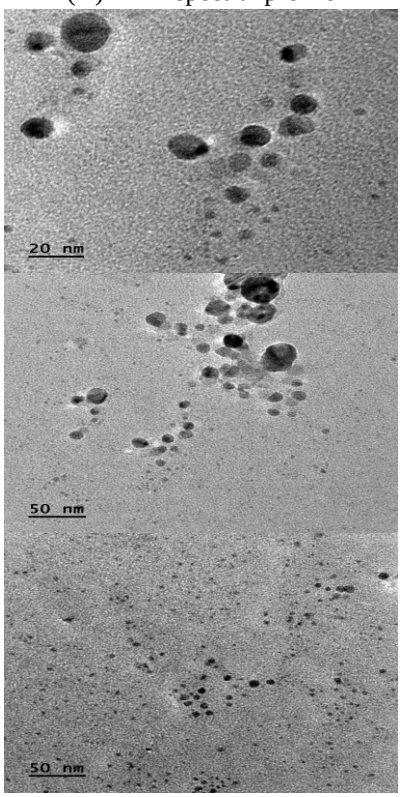
The change in solution color and peak appearance in the specific region are the key evidences of the fruitful synthesis of silver nanoparticles as there exists the SPR phenomenon behind these. It is reported in numerous studies that the emergence of peak in the UV/Visible spectra confirms the synthesis process.

3.1.2 FTIR analysis

When ALEMk subjected to FTIR analysis, the peaks were found in the different regions of the spectrum. A broad band is analyzed at 3503-2019 cm^{-1} which might be corresponded to the phenol functional group, N-H/C-H/O-H stretching bonding of amines, amides, —COOH, C-N groups. The presence of carbonyl functional groups is supposed to be attribute due to small peak at 1769 cm^{-1} while a strong peak at 1620 cm^{-1} possibly due to stretching of C=C groups. There are some other peaks particularly seen at 1481, 1308, 1182, and 663 cm^{-1} possibly relating with the aliphatic ethers. The functional groups participated in the synthesis process of AgNPsMk were also evaluated by FTIR investigation. The large band from 3352-3237 cm^{-1} possibly arisen due to primary amine N-H stretching and O-H stretching due to alcohol. A prominent peak at1632 cm^{-1} might be linked with C=C stretching of alkenes, at 1441, 1307, and 1174 cm^{-1} peaks appeared because of O-H bending carboxylic groups, S=O stretching or aromatic



(A) FTIR spectral profile



(B) TEM images

Fig. 3 (A) FTIR spectra of (a) AgNPsMk with (b) ALEMk (B) TEM micrographs of AgNPsMk at different magnifications

ester as evidenced by Anand and Gokulakrishnan, (2012). The peaks are remarkably shifted from the origin of the ALEMk as seen in the FTIR spectra of AgNPsMk which subsequently found to be responsible for the reduction and stability as the difference and comparison can be made in the FTIR spectra of ALEMk and AgNPsMK in Fig. 3(A).

FTIR analysis is an important aspect to examine the synthesis process of silver nanoparticles with reduction process in which ionic silver (Ag^{1+}) is converted into metallic form (Ag^{o}). It is hypothesized that the phytochemicals present in the extract are responsible for reduction of metal by bestowing electrons. Additionally, these functional moieties also assist the synthesis process by providing encapsulation around the metal surface, thus giving stability.

3.1.3 TEM

The TEM micrographs for AgNPsMk have shown in Fig. 3(B) describing the nature of the synthesized nanoparticles. Most of the particles sizes analyzed to be 20 nm with spherical shape. The images also revealed the homogenous distribution of the nanoparticles corresponding to their very well stability even in the solution form without any agglomeration.

It seems that the sintering of the nanoparticles is avoided due to the presence of phytochemicals in the extract which creating strong interaction with the surface of the nanoparticles resulting in the spherical shape. It is believed that the change in the volume of the extract can be attributed to the variation in the size and shape of the synthesized nanoparticles as illustrated by Bindhu and Umadevi, 2014 quite similar to our work.

3.2 Antioxidant activities

Antioxidant activities have been shown in Figs. 4(a)-4(c).

3.2.1 DPPH

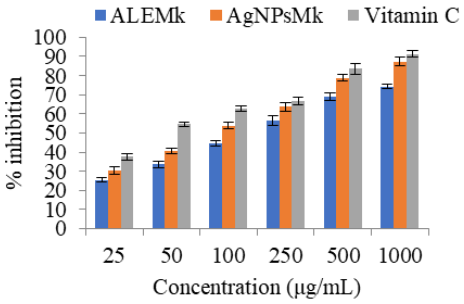
The DPPH radical scavenging activity was found to be ranged 25.42, 33.54, 44.46, 56.52, 69.12, and 74.24 % as well as 30.44, 40.41, 53.77, 63.68, 78.81, and 87.33 % for ALEMk and AgNPsMk with the increased concentration respectively while under similar conditions, the vitamin C showed 37.56, 54.53, 62.64, 66.61, 83.55 and 91.31% radical inhibition. There is minute difference in % inhibition shown by AgNPsMk and that of the standard. Free radicals such as DPPH having central nitrogen are able to accept hydrogen radical or electron during DPPH assay and the purple colored DPPH solution turns to yellow during the reduction of DPPH radical to nonradical form and absorption of this reduction measures at 517 nm (Rajan *et al*. 2017).

3.2.2 H_2O_2

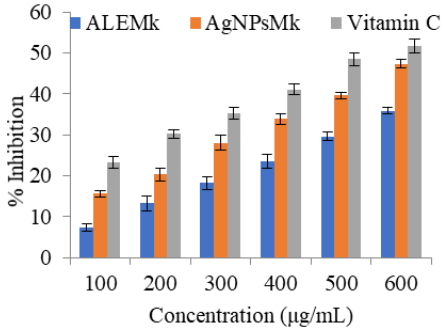
The inhibition of H_2O_2 is shown by ALEMk and AgNPsMk as 7.32, 13.23, 18.15, 23.48, 29.61, and 35.83% and 15.58, 20.25, 28.03, 33.81, 39.65, and 47.35% respectively with rise in concentration however, the H_2O_2 scavenging exhibiting by vitamin C as 23.24, 30.18, 35.22, 41.05, 48.43, and 51.65 %. The phenomena of aging and other similar disorders are associated with the generation of free radicals notably hydroxyl free radicals which cause accumulation of H_2O_2 in biological systems thus, disturbing the numerous energies producing systems. The nanoparticles possess strong tendency to scavenge these radicals and exhibit antioxidant ability which increase with increasing concentration as also analyzed by Sudha *et al*. (2017).

3.2.3 NO

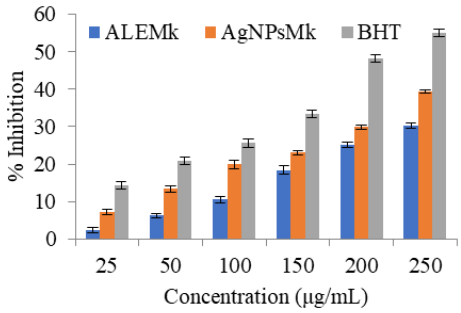
When NO activity was evaluated for ALEMk and AgNPsMk, % inhibition found as 2.44, 6.22, 10.52, 18.43, 25.05, and 30.18% and 7.17, 13.44, 19.88, 23.04, 29.85, and39.44% respectively while BHT represented 14.22, 20.77, 25.56, 33.52, 48.13, and 55.03%. NO involves in



(a) DPPH

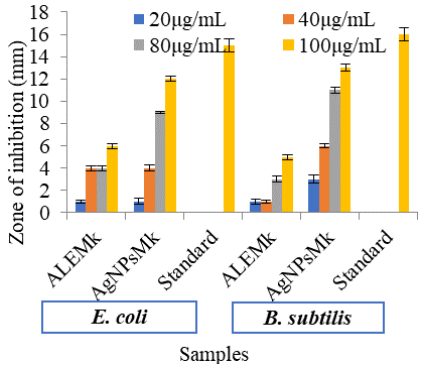


(b) H_2O_2

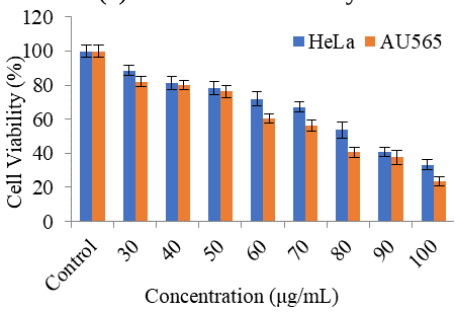


(c) NO

Fig. 4 Antioxidant activities exhibited by AgNPsMk, ALEMk and standard at different concentrations. Error bars representing the standard error of means of six replicated experiments ($p < 0.05$)



(a) Antibacterial activity



(b) In vitro cytotoxicity

Fig. 5 (a) Antibacterial against bacterial strains (b) *In vitro* cytotoxicity shown by AgNPsMk at different concentrations. Error bars representing the standard error of means of six replicated experiments ($p < 0.05$)

many biological functions including neuro transmission, antimicrobial, anticancer, smooth muscle relaxation in addition to regulation of blood pressure (Lone *et al*. 2013). The reaction of NO with superoxide produces peroxynitrite anion resulting in the peroxidation of lipids and DNA fragmentation, and therefore, responsible for oxidative damage (Ribeiro *et al*. 2014). Therefore, NO regularization should be managed to avoid its harmful effects, hence scavenging of it via nanoparticles examined to be quite significant in this regard (Omer *et al*. 2012).

In above studied assays, AgNPsMk displayed strong antioxidant potential in concentration dependent manner which remarkably comparable to the synthetic antioxidant standards which is mainly contributed by phytochemicals, besides large surface area of metals.

3.3 Antibacterial activity

Against E. coli and B. subtilis strain, the ALEMk and AgNPsMk showed zone of inhibition as 1, 4, 4, 6, 1, 1, 3, 5, 1, 4, 9, 12 and 3, 6, 11, and 13 mm at increased concentration respectively. At 100 µg/mL concentration, chloramphenicol exhibited 15 and 16 mm zone of inhibition against bacterial growth of E. coli and B. subtilis respectively (Fig. 5(a)).

The antibacterial activity possibly arises due to the small sized metal nanoparticles which either disturbing cellular functions, hindering DNA replication and protein synthesis, denaturing ribosomal units or creating binding with DNA molecule, thus ultimately leading toward the cellular breakdown. Another probable reason lies in the fact that during partial oxidation, metal ions are released in the solution causing toxic effect on the bacteria as reported by Chaloupka *et al*. (2010). It is envisaged that the plant extracts and nanoparticles synthesizing from them are strongly correlated to the antimicrobial attributes by inhibiting bacterial growth even at low concentration.

3.4 In vitro cytotoxicity

When AgNPsMk applied against the AU565 and HeLa cell lines as anti-proliferative agent, cell viability was gradually decreased as (82.13, 80.29, 76.31, 60.44, 56.25, 40.55, 37.66 and 23.55 IC50 47.61 µg/mL) and (88.71, 81.33, 78.14, 71.85, 67.23, 53.67, 40.66 and 33.33: IC50 55.35 µg/mL) % from 30 to 100 µg/mL increase inconcentration. More cell decline is observed in AU565 as compared to the HeLa cell lines (Fig. 5(b)) while without any treatment cells remained intact and showed 100 % cell viability. Cisplatin showed 97.25% decline in viability of these cells while Doxorubicin employed for AU565 cell lines diminished 98.82% cell viability. The current study

Table 1 (a) Average body weight and (b) Biochemical test of liver function of wistar rats

Samples	(a) Body Weight (g) (Mean ± S.E)		Change in Weight (g)
	Before Experiment	After Experiment	
AgNPsMk	122.8 ± 0.01	122.3 ± 0.03	0.5 ± 0.002
Control	100.3 ± 0.03	101.3 ± 0.06	1.0 ± 0.001

Samples	(b) Liver Function Test				
	mg/Dl			IU/L	
	S. Bilirubin (Total)	S. Bilirubin (Direct)	S. Bilirubin (Indirect)	S.G.P.T (ALT)	Alkaline Phosphatase
	Normal Range				
	Upto 1.1	0.0-0.3	0.0-0.8	09-43	80-306
AgNPsMk	1.0	0.3	0.2	33	193
Control	0.8	0.2	0.4	40	215

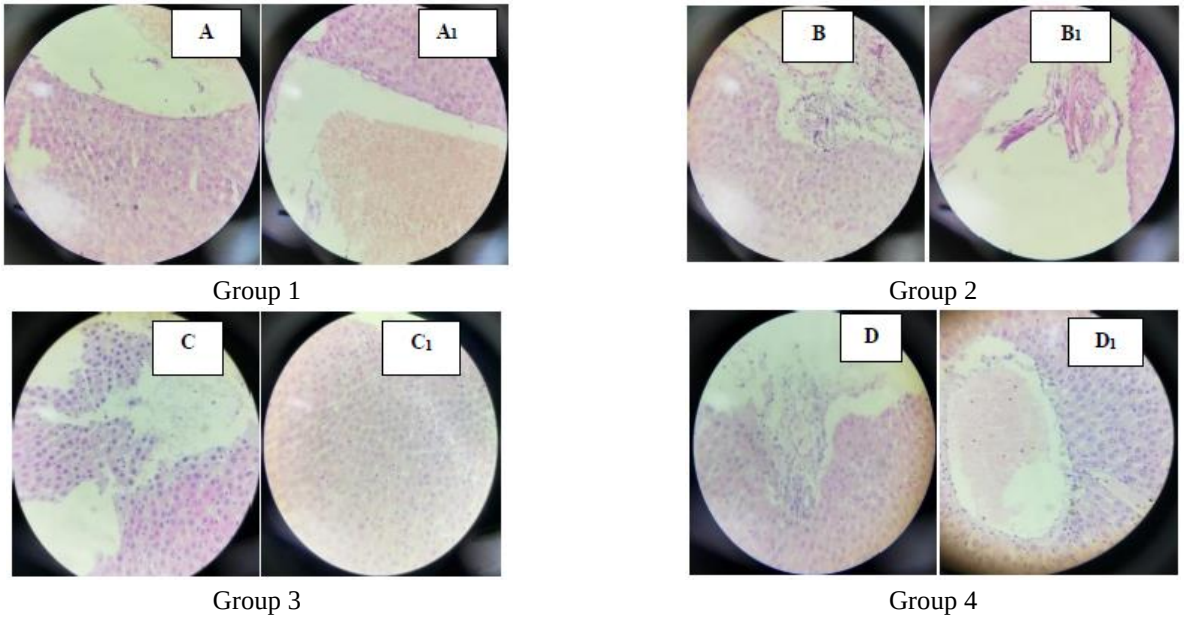


Fig. 6 Histopathological images of liver tissues of wistar rats (A-D) nanoparticles exposed tissues and (A_1-D_1) control group

substantiated that increased concentration of AgNPsMk enhances the cell viability loss in both cases but the extent observed to be different in the two cell lines indicating the more resistant nature of HeLa cell lines under similar conditions as worked out (Rasheed *et al*. 2017, Rajan *et al*. 2017). These results rationalize strong anticancer potential of the synthesized AgNPsMk for biomedical applications.

3.5 In vivo study

In vivo analysis of green synthesized AgNPsMk was carried out using male wistar rats using two groups (each with four animals) which were allocated as control and nanoparticles (treated) group in order to determine any possible toxicity. No change in body weight was observed after dose period (50 µg/mL) completion when compared with control (Table 1(a)).

Similarly, biochemical analysis revealed that the values of bilirubin (direct, indirect, and total), ALP, and SGPT were found within the normal range (Table 1(b)). Actually, the bilirubin (an antioxidant which is produced during heme metabolism), ALP (an enzyme which assists in the development of bone marrow as well as in the breakdown of proteins), and SGPT (an enzyme helps in the alanine cycle) are virtually essential biomarkers of liver which help in maintaining the normal functions of liver in the body. In Pan *et al*. (2012), observed non deleterious effect of nanoparticles as there was no inflammation or fibrosis seen in the liver.

The liver organs examined under light microscope showed no damage or harm caused by the application of AgNPsMk as the organs remain intact (Fig. 6). There are no pathologic lesions, edema or inflammations in the liver observed which eliminate the speculations about toxicity of these nanoparticles inside the living organs. No difference found between the treated and control group was observed during this careful study.

The reported results suggest that therapeutic field can assess simple green approach to formulate nanomedicines from nanoparticles of metals synthesized from plant extracts (Kanipandian *et al*. 2014).

4. Conclusions

The green synthesis is an ecofriendlier, low cost, and facile approach to synthesize AgNPsMk through ALEMk

• A band with absorption maximum at 420 nm has been confirmed the synthesis.

• These AgNPsMk were functionalized by the different functional groups present in the ALEMk which not only covered but also stabilize the metal surface as visualized through FTIR analysis while

• TEM micrographs revealed 20 nm sizes of nanoparticles with spherical shape.

• The remarkable attributes have been shown by these nanoparticles as studied from the antioxidant and antibacterial activities.

• *In vitro* cytotoxicity against HeLa and AU565 cell lines found to be excellent in dose dependent manner besides

• *In vivo* analysis showing biocompatibility of AgNPsMk inside the studied biological system

The contemporary exploration clearly illustrates that plant based metal nanoparticles are among the wonderful candidates for the formulation of anticancer drugs due to their unique outstanding features.

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