Potential in vitro anti-breast cancer activity of green-synthesized silver nanoparticles prepartion against human MCF-7 cell lines.

R. Ananthi and R. Anuradha

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Abstract

The present study was aimed to investigate the dose of aqueous extract of fresh leaves of *Aegle marmelos* was used for the synthesis of silver (Ag) nanoparticles. UV–Vis spectroscopy studies were carried out to assess silver nanoparticles formation within 5 min. Scanning electron microscope was used to characterize shape of the Ag nanoparticles, X-ray diffraction analysis confirmed the nanoparticles as crystalline silver and face centered cubic type and Fourier transform Infra-Red assessed that shows biomolecule compounds which are responsible for reduction and capping material of silver nanoparticles. The anti cancer activity of silver nanoparticle was tested against the breast cancer cell line, MCF7. The approach of plant-mediated synthesis appears to be cost efficient, eco-friendly and easy method.

Key words: *Aegle marmelos*, anticancer activity, characterization, silver nanoparticles

INTRODUCTION

Nanotechnology deals with production, manipulation and use of material ranging in nanometers (Kavitha *et al.,* 2013). Nanotechnology has got direct impact on human life (Jannathul Firdhouse *et al.,* 2012). Nanotechnology mainly deals with the nanoparticle synthesis having a size of 1-100 nm in one dimension and is used significantly concerning medicinal chemistry, atomic physics, and all other known fields too (Amudha Murugan *et al.,* 2014). Nanomaterials are the atomic and molecular building blocks (~0.2 nm) of matter. Nanoparticles belong to a wider group B of nanomaterials having amorphous or crystalline form and their surfaces can act as carriers for liquid droplets or gases (Buzea, 2007). Richard Feynman was the first person who gave a talk on Nanoparticles in the year 1959. It later on inspired the conceptual foundations of nanotechnology.

Nanoparticles produced by plants are more stable and the rate of synthesis is faster than that in other cases of other organisms. The reduction and stabilization of silver ions by combination of biomolecules such as proteins, amino acids, enzymes, polysaccharides, alkaloids, tannins, phenolics, saponins, terpinoids and vitamins which are already established in the plant extracts having medicinal values and are environmental benign, yet chemically complex structures. In each and every steps of the experiment, sterility conditions were maintained for the effectiveness and accuracy in results without contamination (Sahayaraj *et al.,* 2011). Silver nitrate ($AgNO_3$) was used. 5mL leaf broth was added to 100mL 10-3 M silver nitrate and allowed to react at ambient conditions. The observed colour change of reaction mixture from transparent yellow to dark brown indicates the formation of Silver nanoparticles. The suspension of Silver nanoparticles was allowed to settle and the excess liquid was removed. The particles were then rinsed to remove any organic residue and resuspended in 95% ethanol for further characterization (Kasthuri *et al*., 2009).

In this study, an attempt was made to the green synthesis of AgNPs from *Aegle marmelos* and was characterized. The same was also used to assess their effect on cytotoxic activity against breast cancer cell line MCF-7. *Aegle marmelos* belonging to family Rutaceae, is commonly known as Bael in indigenous system of medicine and has been regarded to possess various medicinal properties (Chopra *et al.,* 1956; Orwa *et al*., 2009). The bael is one of the sacred trees of the Hindus. Leaves are offered in prayers to Shiva and Parvathi since ancient times (Rajasekaran *et al*., 2008). This is generally considered as sacred tree by the Hindus as its leaves are offered to Lord Shiva during worship. According to Hindu mythology, the tree is another form of Lord Kailashnath (Purohit and Vyas,

R. Anuradha

email: *mathianuradha@gmail.com*

Assistant Professor, Department of Biochemistry, S.T.E.T. Women's College (Autonomous), Sundarakkottai, Mannargudi - 614 016, Tamil Nadu, India.

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2004). Leaves, fruit, stem and roots of this tree at all stages are used as ethno-medicine against various human ailments (Shankar and Majumdar, 1997; Kunwar *et al.,* 2009, 2010; Dhankar *et al*., 2011; Dutta *et al*., 2014; Jhajharia and Kumar, 2016). The Indian medicinal plants are considered a vast source of several pharmacologically active principles and compounds which are commonly used in home remedies against multiple ailments (Balick and Cox, 1997; Bhattarai, 1997; Shankar and Majumdar, 1997; Anonymous, 1985; Agarwal, 1990, 1997; Atal and Kapur 1997; Akhtar *et al*., 2005; Ansary, 2005; Kala *et al.,* 2005).

Reported pharmacological activities include antibacterial activity (Rajasekaran *et al*., 2008), antihistamic activity (Nugroho *et al.,* 2010), hepatoprotective activity (Singh *et al*., 2016), insecticidal activity (Kumar *et al.,* 2008), hypogiycemic and antioxidant activity (Upathya *et al.,* 2009), immuno modulatory activity (Rajadurai *et al.,* 2005), myocardial infarction (Rajadurai *et al.,* 2005), testicular activity (Das *et al*., 2006), cardiotonic activity (Dama *et al*., 2010), anxiolytic and antidepressant activity (Kothari *et al*., 2010), wound healing activity (Jaswanth *et al.,* 2000), anticonvulsant activity (Sankari *et al*., 2010), anti stress and adaptogenic activity (Duraisami *et al.,* 2010) and antifertility activity (Joshi *et al*., 2009).

Hence the present study was carried out to synthesize and characterize silver nanoparticles and to examine the cytotoxic effect of green synthesized AgNPs against MCF-7 breast cancer cell line.

MATERIALS AND METHODS

Collection of plant materials

The leaves of *Aegle marmelos* were collected from Thanjavur, Tamil Nadu, India. The collected leaves were washed several times with distilled water to remove the traces of impurities from the leaves. Healthy leaves were spread out in a plain paper and shade dried at room temperature for about 10 days and a fine powder was made using grinder mixture. The powdered materials were used for further studies.

Chemicals and reagents

Silver Nitrate (e" 99.5 % purity) were acquired from Sigma Aldrich (St Louis, MO, USA). Dulbecco's modified Eagles medium (DMEM), phosphate buffered saline (PBS), 3–(4,5 dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), fetal bovine serum (FBS) and dimethyl sulfoxide (DMSO) were acquired from HiMedia Lab Ltd., Mumbai, India. The primary antibodies for caspase- 3 and 9 were obtained from Sigma Aldrich, USA Antibodies were purchased from Santa Cruz Biotech, USA.

Preparation of AgNPs extract

The weighed 10g plant powder was put into a beaker with 100ml distilled water. The mixture was heated for 20 minutes at 60^0C while stirring occasionally and then allowed to cool at room temperature. The mixture was filtered using the Whatman 42 filter paper and then centrifuged for 20 minutes. The extract was stored in the refrigerator for further use to synthesize Ag nanoparticles from $AgNO_3$ precursor solution.

Synthesis of silver nanoparticles

For the Ag nanoparticles synthesis, 5 ml of aqueous extract of *Aegle marmelos* was added to 45 ml of 1 mM aqueous $AgNO_3$ solution in a 250 ml Erlenmeyer flask. The flask was then incubated in the dark for 5 hours (to minimize the photo activation of silver nitrate), at room temperature. A control set up was also maintained without plant extract. The Ag nanoparticle solution thus obtained was purified by repeated centrifugation at 10,000 rpm for 15 minutes followed by re- dispersion of the pellet inde-ionized water. Furthermore, the mixture was analysed for characterization studies.

Characterization of Silver Nanoparticles UV-Visible Spectroscopic analysis

The synthesized AgNPs monitored by measuring the UV-Vis spectrum of the reaction medium after diluting a small aliquot of the sample into distilled water. The colour change in the reaction mixture was recorded through visual observation. The UV spectral analysis was done by using Shimadzu Spectrophotometer at the wavelength of 200 to 800 nm.

Fourier Transform Infrared (FTIR) Spectroscopy analysis

AgNPs were subjected to FTIR spectroscopic analysis to assess the functional groups present. AgNPs were lyophilized and 2mg of lyophilized AgNPs powder is mixed with 200mg of KBr pellet and the mixture were placed on the FTIR sample holder. The samples were assessed at the spectroscopic range of 400-600 $cm^{"1}$ (FTIR Spectrum 2000, Perkin Elemer, USA).

Scanning Electron Microscope (SEM) analysis

Morphology of the samples was observed by scanning electron microscope (Vega 3SB, TESCAN, Czech Republic) at 5 kV. A drop of sample suspension was placed onto the glass plate and then air dried. After that, the dried samples were sputtered by Au (thickness ~ 2 nm) and then visualized

Transmission Electron Microscopic (TEM) Analysis

The morphology and the nature of the biosynthesized AgNPs were assessed with TEM. 5µl of AgNPs was placed on the copper grids coated and dried at room

temperature. The dried samples were then scanned at voltage of 120 kV, in which the sample is subjected to beam of electron and the image, was captured (JEOL 1200EX, Japan). The captured image was assessed with Image J software.

X-RAY Diffraction method (XRD)

The phase evolution of calcined powder as well as that of sintered samples was studied by X-ray diffraction technique (Philips PAN analytical, The Netherland) using Cu Ká radiation. The generator voltage and current was set at 35 KV and 25 m A respectively. The ZnO and Ag samples were scanned in the 2è ranges 15 to 700^{0}C range in continuous scan mode. The scan rate was 0.04 o /sec. Phases present in the sample has been identified with the search match facility available with Philips X'pert high score software.

Cell culture

MCF-7, breast cancer cell line was purchased from NCCS, Pune, India. The cells were seeded in DMEM medium supplement with 10% FBS, 1Xpenicillin and streptomycin. The cells were maintained at 37^{0}C in a humidified atmosphere with 5% of CO_2 and 95% of air incubation.

***In vitro* Cytotoxicity assay by MTT**

The cytotoxicity of AgNPs was evaluated by employing 3-(4,5-dimethyl thiazol- 2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay based on the reduction of MTT by the action of mitochondrial reductase enzyme which presents in viable cells. The amount of reduction of MTT to formazan in AgNPs treated and untreated cells were directly proportional to the number of viable cells, it was assessed by taking absorbance values by dissolving the formazan with DMSO. In brief, 1 × 10^5 cells were plated into 96-well plates and incubated for 24 h at 37 °C in a humidified condition. After complete incubation the old medium was aspirated without disturbing cells then the cells were treated with different concentrations (10, 20, 40, 60, 80 and 100µg/ml**)** of AgNPs dissolved DMEM, the plate was kept in same incubation condition. After incubation, 100 µL of MTT reagent (5 mg/mL in PBS) was subsequently added into each well further, the plates were incubated for 24 h at 37°C. The resulting formazan was dissolved by adding 100 µL of DMSO and the absorbance of the reactant solution was recorded at 595 nm wavelength using a multi well plate reader (Tecan Multimode Reader, Austria). The concentrations of the test sample which showed 50% of cell death was calculated (Gunaseelan *et al*., 2017).

Measurement of intracellular ROS generation

Intracellular ROS production of AgNPs treated and untreated MCF-7 cells were estimated by non-fluorescent probe DCFH-DA that can easily enter into the intracellular cell matrix. There it was oxidized into fluorescent dichlorofluorescein (DCF) by the action of produced ROS. Accordingly, the fluorescence strength was relatively proportional to the level of ROS production (Jesudason *et al.,* 2008). The $HepG_2$ cells were inoculated (1X10^6 cells/well) into 6-well plate, treated with 44.µg/ml (IC_{50}) concentration of AgNPs and placed in CO_2 (5%) incubator for 24 h. After incubation, cells were exposed to 100 µl of DCFH-DA for 10 min at 37°C. Fluorescence depth was estimated with excitation and emission filters set at 485 and 530 nm, respectively (Shimadzu RF-5301 PC spectrofluorometer). The results showed an increased percentage of fluorescence depth.

Determination of mitochondrial membrane potential

The mitochondrial membrane potential of treated and untreated MCF-7 cells was estimated by staining the cells with Rhodamine-123 (Rh-123), lipophilic cationic dye (Johnson *et al.,* 1980). The cells (3X10^4 cells/well) were cultured in 6 wells plate and treated with 44.93µg/ml (IC_{50}) concentration of AgNps for 24 h. Then Rhodamine-123 fluorescent dye was added into treated and untreated cells and left for 30 minutes. The Mitochondrial membrane potential was qualitatively examined under a Floid cell imagingstation (Invitrogen, USA). Subsequently, the cells were harvested by Trypsinization of cells and the fluorescence strength was estimated at 485/530 nm wavelength under Spectrofluorometer (Schimadzu, USA). The results were compared with positive control which were maintained without treatment.

Caspase assay

Caspase-3 and -9 colorimetric assay kits (R&D Systems Inc., Minneapolis, MN, United States) were used to measure the caspase activity in the cell lysates. The cells were treated with AgNPs and incubated for 24 h, and then lysed in a buffer mixture [50 mM Tris-HCl (pH 7.4), 2 mM DTT, 1 mM EDTA, 10 mM digitonin, and 10 mM EGTA]. Ac-DEVD-pNA and Ac-LEHD-pNA were used as casepase-3 and -9 substrates for the incubation of the cell lysate at 37^{0}C for 1 h. Caspase activity and absorbance were measured using an Enzyme-Linked Immuno Sorbent Assay (ELISA) reader and OD at 405 nm.

Statistical analysis

All the experimental data were expressed as mean ± SD.

RESULTS AND DISCUSSION

The reacted mixture of *Aegle marmalos* leaves extract and silver nitrate solution are shown in Figure 1 as a

function of time using water as a solvent. Reduction of silver ion into Ag particles during exposure to *Aegle marmalos* extract could be followed by color change. AgNPs exhibit dark yellowish-brown color in aqueous solution due to the surface Plasmon resonance phenomenon (Figure. 1). A visible color change from transparent to light brown within 20 min indicates the more formation of AgNPs, which was confirmed by UV-Vis spectrophotometer and FT-IR analysis. After 70 min there was significant color change to dark brown due to increase in reaction time which enhances the growth of silver nanoparticles.

UV–Vis spectral analysis

Figure 2 shows the absorbance peak of synthesized AgNPs at various time intervals which was done by UV-Vis spectrometer. The peak centered at 420nm which is associated with absorbance of AgNPs. Absorption spectra of Ag nanoparticles formed in the reaction media has absorbance maxima at 420nm. A remarkable broadening of peak at around 380 nm to 480nm indicates that the particles are polydispersed. It was observed that the peak was blue shifted in the absorption spectrum from 380nm to 480 nm with increasing reaction time.

Absorbance spectroscopy is used to determine the optical properties of a solution. A Light is sent through the sample solution and the amount of absorbed light is measured. Then the wavelength is varied and the absorbance is measured at each wavelength. The optical measurement of UV-Visible spectrophotometer has different absorbance peak like 420nm when treated with the *Aegle marmlos* plant extract after addition of aqueous 1mM Silver nitrate solution. In case of *Aegle marmelos* get synthesized with Iron nanoparticles by the indication of suitable surface Plasmon resonance with high band intensities and peaks was found through UV-Visible spectroscopy at the range of 216-265 nm (Vasireddy *et al.*, 2012; Marslin *et al.*, 2018).

Fig. 1. Synthesis of Silver Nanoparticles

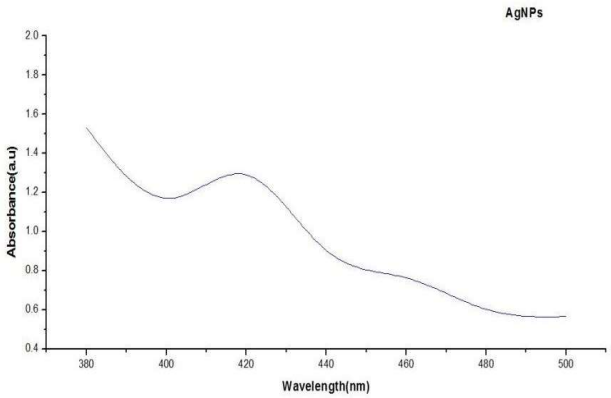

Fig. 2. UV-Vis spectral Analysis of generated silver nanoparticles by *Aegle marmelos* leaves extract

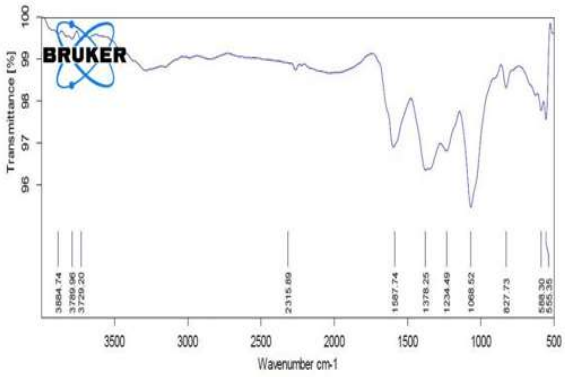

Fig. 3. FTIR analysis of AgNPs synthesized from aquous extract of *Aegle marmelos*

Table 1. FTIR Peak value of silver nano particles synthesized from aqueous extract of *Aegle marmelos*

S.No.	Group of Frequency cm-1 of the sample	Functional Group
1	3884.74 cm^{-1}	Unknown
2	3789.96 cm^{-1}	Unknown
3	3729.20 cm^{-1}	OH –Amide
4	2315.89cm^{-1}	C-N
5	1587.74cm^{-1}	Diketones
6	1378.25cm^{-1}	CH-CH2
7	1234.49cm^{-1}	Alkylketone,
8	1068.52cm^{-1}	Alkyl amide
9	827.73cm^{-1}	Aromatic
10	588.30 cm^{-1}	C-Br strong
11	555. 35cm^{-1}	C-CIHalogen

FT-IR analysis

FT-IR spectrum was performed to identify and to determine the different functional groups present in the AgNPs (Table 1 and Figure 3). The IR bands were

observed at 1587.74, 1378.25, 1234.49, 1068.52 ,827.73, The weak bands which appeared at 1587.74cm-1 are Diketone, the bands at 1378.25 cm-1 which is the CH and CH2 aliphatic banding group , 1234.49 cm-1 represents the alkyl ketone,1068.52 cm-1corresponding to the alkylamine, 827.73cm-1 showed the Aromatic compound. Fourier Transform Infrared [FTIR] spectroscopy measures infrared intensity versus wavelength of light. It is used to determine the nature of associated functional groups and structural features of biological extracts with nanoparticles. The calculated spectra clearly reflect the well-known dependence of nano particle optical properties. The green synthesized silver nanoparticle by employing various fruit extracts was analysed using Fourier Transform Infrared [FTIR] Spectroscopy and showed characteristic peaks (Amudha Murugan, 2014; Prasad *et al*., 2011).

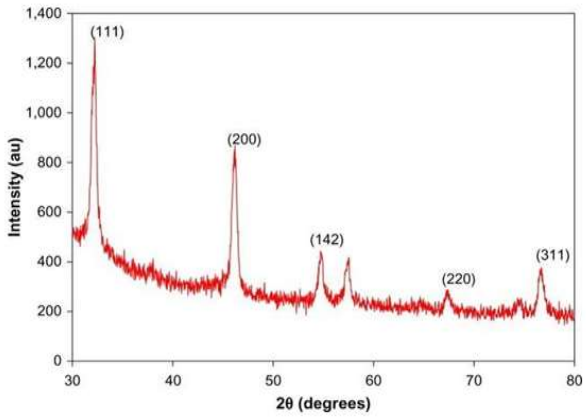

Figure 4: XRD analysis of synthesized of Ag NPs from aqueous extract of ***Aegle marmelos***

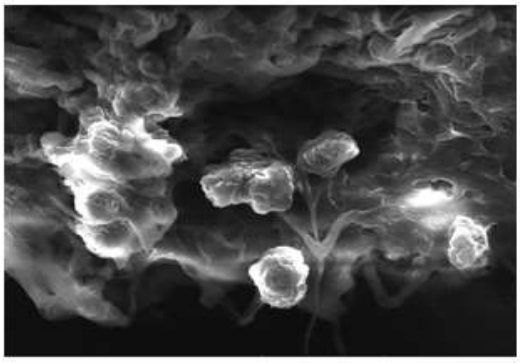

Fig. 5. SEM image of synthesis of silver nano particles from leaf extract of *Aegle marmelos*

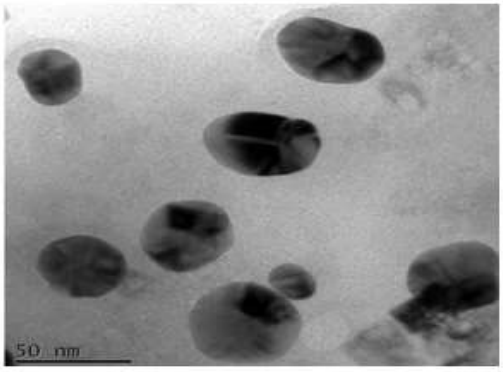

Fig. 6. TEM image of synthesis of silver nanoparticles from leaf extract of *Aegle marmelos*

X-ray diffraction

An X-ray diffraction (XRD) pattern was recorded for the synthesized AgNPs (Figure 4) when showed a number of Bragg reflections corresponding to (111), (200), (142), (220) and (311) sets of lattice planes were observed, which may be indexed based on the structure of silver. The diffraction peaks at 2è= 38.04°, 45.12and 63.33° were indexed with the planes (111), (200), (142), (220) and (311) for the fcc lattice of obtained silver as per the Joint Committee on Powder Diffraction Standards (JCPDS) card no. 89- 3722 was matched with database. X-ray diffraction is a conventional technique for determination of crystallographic structure and morphology. There is an increase or decrease in intensity with the amount of the constituent. This Technique is used to establish the metallic nature of particles and gives information on translational symmetry size and shape of the unit cell from peak positions and information on electron density inside the unit cell, namely where the atoms are located from peak intensities. XRD patterns were calculated using X per Rota flex diffraction meter using Cu K radiation and =1.5406 Å. Xray diffraction analysis with various nanoparticles has been studied by various research workers to find the high crystallinity of the prepared sample (Wani *et al*., 2010).

SEM and TEM analysis

The nanoparticles had a spherical structure ranging from 20 to 60nm as confirmed by SEM and TEM (Figure 5 and 6). The average particle size was estimated at 30nm. The SEM image (Figure 6) has shown separate AgNPs as well as particle agglomeration. This indicates, the particle size is irregular and shape of the particles was spherical in morphology with an average size of 30 nm and that of TEM analysis is 50 nm.

The characterization of Scanning electron microscope analysis is employed to determine the size, shape and morphologies of formed nanoparticle SEM gives higher solution images of the surface of a sample that is desired. The scanning electron microscope works in the same principle as an optical microscope, but it measures the electrons scattered from the sample rather than photon. Because electrons can be accelerated by an electric potential, the wavelength can be made shorter than one of photons. This makes the SEM capable of magnifying images up to 200.000

Table 2. Cytotoxicity of *Aegle marmelos* synthesized AgNPs on MCF-7 cells expressed as percentage cell viability against different cincentrations.

Concentration	10µg	20µg	40µg	60µg	80µg	100µg
Average	76.31258	52.38095	33.57753	18.31502	11.72161	5.616606
SD	1.057418	2.039474	4.780652	2.287545	3.494283	2.438948

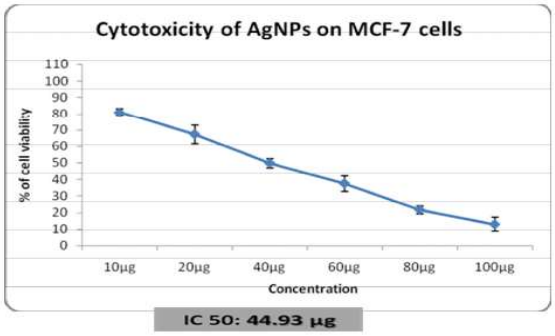

Fig. 7. Cytotoxicity of *Aegle marmelos* synthesized AgNPs on MCF-7 cells expressed as percentage cell viability against different cincentrations.

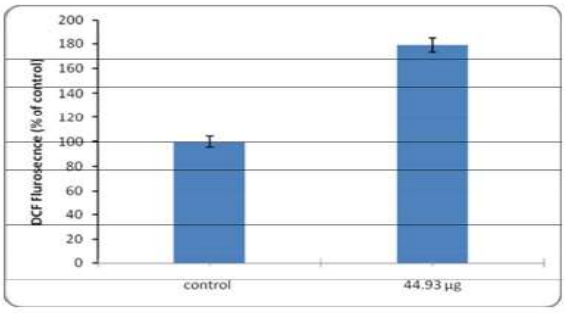

Fig. 8. Intracellular Ros Production of AgNPs Synthesized from MCF-7 cells

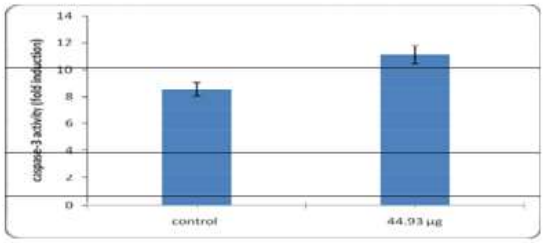

Fig. 9a. Caspase-3 activity of Ag NPs synthesized from MCF-7 cells

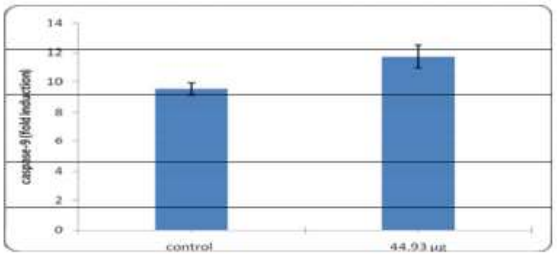

Fig. 9b. Caspase - 9 activity of AgNPs synthesized from MCF-7

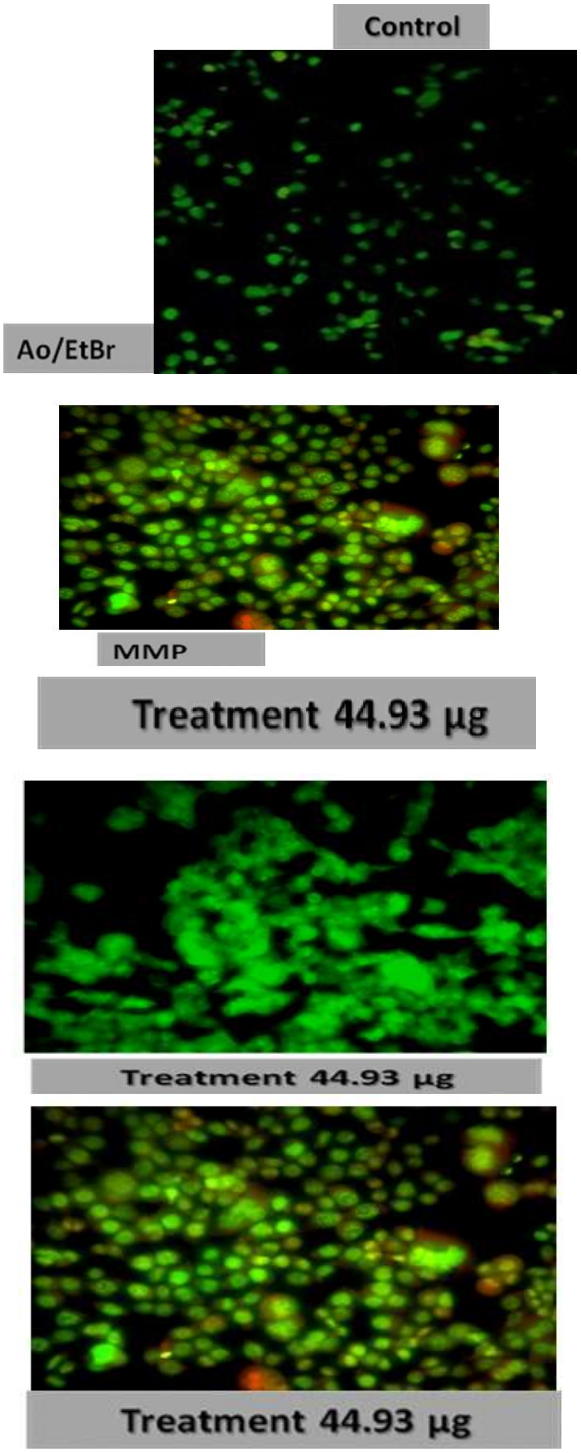

Fig. 10. *Aegle marmelos* synthesized AgNPs induced Apoptotic morphological changes and mitochondrial membrane potential lose in MCF-7 cells

times and to measures the particle size and characterization, Conductive or sputter coated sample involved and the sensitivity down to 1nm (Asim Umer *et al*., 2012).

Transmission electron microscopy is a microscopy technique in which a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device, such as a fluorescent screen, on a layer of photographic film, or to be detected by a sensor such as a CCD camera. TEM forms a major analysis method in a range of scientific fields, in both physical and biological sciences. TEMs find application in cancer research, virology, materials science as well as pollution, nanotechnology, and semiconductor research.

Cytotoxicity of silver nanoparticles (MTT assay)

The *in-vitro* cytotoxic activity of silver nanoparticles was evaluated against human ovarian cancer (MCF-7) cells line at different concentrations. The AgNPs exhibited potent cytotoxicity/anticancer activity in the tested cell lines. Results showed that at higher concentrations there is significant cell mortality. The inhibitory effect was observed after 24 h of incubation (Figure 7) and are shown by the changes in the percentage of inhibition in nanoparticles treated MCF-7 cells. The treatment of 10-100 µg/ml of sample with AgNPs significantly inhibited MCF-7 cells. The silver nanoparticles were able to reduce viability of the MCF-7 cells in a dose-dependent manner as shown in Table 2. Therefore these results indicate that the sensitivity of human cancer cell line for cytotoxic drugs.

The biomedical applications of silver nanoparticles are promising with their tremendous effects in the fields of medicine, drug delivery and anti angiogenic property of cancer (Gurunathan *et al*., 2009). In the present study the cytotoxic effect of biosynthesized AgNPs was tested against human Breast cancer cell line (MCF-7) using MTT assay. The present findings are similar to the observation of Nagajyothi *et al*. (2014) in which they reported antiproliferative activity of AgNPs against A549 human lung cancer cell line and MCF-7 human breast cancer (HTB-22) cell line. In another study, silver nanoparticles synthesized from *Iresine herbstii* showed potent cytotoxicity against HeLa cervical cell lines. Ih AgNPs induced over 88% death of HeLa cells at a treatment concentration of 300µg/ml (Dipankar and Murugan, 2012).

Intracellular ROS Production

The result obtained from ROS generation in MCF-7 cells exposed to AgNPs for 24h is shown in Figures 8. A statistically significant induction in ROS generation was measured in MCF-7cells exposed to AgNPs at 10-100µg/ml concentrations. As shown in Figures 8, an increase was observed in ROS generation at 44.93µg/ml, as compared to untreated control.

Caspase activity

The caspase family members participated in triggering different types of cell death. A significant increase in caspase 3 and 9 activity was observed between control and treatment after 24 hours (Figure 9a and 9b). The caspase 3 and 9 activity was comparatively higher at 100 µg/ml concentration after 24 hours. The fold induction value of caspase- 3 and -9 was recorded as 44.93µg.

Mitochondrial Membrane Potential (MMP)

Figure 10 illustrates the change in the MMP level. MCF-7 cells were treated for 24h at 10–100µg/ml of synthesized AgNPs. A significant induction in MMP level was found in MCF-7cells. The results of this study suggested that the integrity of mitochondrial membrane might be involved in AgNPs-induced MCF-7 cell death. It is well documented that the ROS generation at high level can lead to cellular damage by resulting in mitochondrial membrane damage, which can then induce toxicity.

The green synthesis of AgNPs using *Aegle marmelos* leaf extract was shown to be rapid, eco-friendly and produced nanoparticles are fairly uniform in size and shape. AgNPs began to form within 10min and the higher formation yield was at 70 minutes after addition of leaf extract to silver nitrate as shown by the UV-Vis spectrum at 400 nm. It was found that the formation of AgNPs was increased with time. The FT-IR spectrum ascribed the biological molecules which perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium. FTIR studies indicated the functional groups of the nano particles. The XRD peaks ascribed with FCC structure of silver. The synthesized AgNPs were spherical in shape and particle size found about 6.45 nm from XRD results in addition justified further by the SEM analysis. The results showed that biosynthesized AgNPs (ND-AgNPs) induced a concentration-dependent cytotoxicity in MCF-7 cells. ND-AgNPs were also found to induce oxidative stress as observed by the increase in ROS level. The increase in the intracellular ROS generation was found eventually to trigger the development of mitochondrial membrane damage and cell cycle alterations. This study also showed that ND-AgNPs have the capacity of inducing apoptosis and necrosis cell death of MCF-7 cells through SubG1 cell cycle arrest. Thus, our findings suggest the green synthesis is an effective and eco-friendly method of producing metal nanoparticles.

The anticancer activity of biologically synthesized silver nanoparticles was evaluated against MCF-7 showed significant results and could play an important role in the development of new therapeutic agent for the treatment of cancer.

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