"Biosynthesis of silver nanoparticles from *Leea indica* plant extract and evaluate its Antimicrobial and Anticancer activity against prostate cancer cell line"

ABSTRACT

Environmental-friendly and economical production of silver nanoparticles (Ag-NPs) is achieved through green synthesis, utilizing *Leea indica* plant extract. These nanoparticles (NPs) are then tested for their Antimicrobial Activity measured using Minimum Bactericidal Concentration (MBC) and Minimum Inhibitory Concentration (MIC) of Ag-NPs against Staphylococcus aureus bacteria. Verification of the produced Ag-NPs is carried out using UV-vis spectroscopy Analysis using Fourier-transform infrared spectroscopy (FTIR) indicates the existence of hydroxyl and carbonyl groups in *leea indica* plant extract, and hydroxyl and organic material in the Ag-NPs. The size range of the NPs is revealed by scanning electron microscopy (SEM) to be 150-300nm, with a tetra-angular shape. The purity of the Ag-NPs is confirmed through X-ray diffraction. Then the Ag-NPs were tested for the Anticancer activity against prostate cancer cell line (PC-3).

Key words: *Leea indica (LI)*, antibacterial, Ag-NPs, anticancer, antibacterial

INTRODUCTION

Nanoparticles, ranging from 1 to 100 nanometres, have unique properties like enhanced conductivity and catalytic efficiency. They're used in fields like medicine, environmental remediation, and catalysis, with ongoing research driving advancements in nanotechnology [1]. Metal nanoparticles (NPs) like copper, silver, and gold, produced through chemical, physical, or biological processes, offer unique properties. These NPs hold potential in textiles, agriculture, medicine, and diagnostics due to their distinct physicochemical characteristics [2]. Silver (Ag), known for its white metallic luster, is prized in jewellery, coinage, and cutlery. Despite being scarce, its antimicrobial properties have historically made it ideal for storing water and wine to prevent microbial growth. [3].

Silver nanoparticles have recently been synthesized using natural materials and their derivatives, such as starch, natural, rubber, green tea (Camellia sinensis), neem (*Azadirachta indica*), leguminous shrubs (Sesbania drummondi), various leaf extracts, aloe vera extract,

and lemongrass leaf extract [4]. Silver nanoparticles (Ag-NPs) offer exceptional physical, chemical, and biological properties. Their nanoscale structure enhances performance, making them valuable in water disinfection, optoelectronics, diagnostics, and medical applications like antibacterial and anticancer therapies.[5]. Ag-NPs possess strong antibacterial properties, making them valuable in medical applications like surgical instruments, clothing, and wound dressings. Their effectiveness as antimicrobial agents come from their ability to target multiple microbial structures simultaneously [6]. Green synthesis is a more efficient, eco-friendly method for producing nanoparticles, offering lower costs, reduced failure rates, and safer production compared to traditional physical and chemical methods. It uses plant extracts, minimizing toxicity.[7]. *Leea indica*, a tropical evergreen shrub found in Asia, is traditionally used for its anticancer, antidiabetic, and antispasmodic properties. However, limited studies have explored its antioxidant and anticancer effects, particularly in prostate cancer [8].

Leea indica is used to treat headaches, vertigo, ulcers, colic, diarrhoea, muscle spasms, and various ailments. Its leaves and roots are also employed for diabetes, heart conditions, fever, and as a remedy for obstetric pain and contraceptive purposes [9]. It has been also possessing strong analgesic, anti-inflammatory, CNC depressant, antibacterial, antifungal, and cytotoxic properties. The CNS depressant effect may be associated with benzodiazepine receptor involvement. Flavonoids and steroids are the primary phytoconstituents responsible for these effects [10]. The *Leea* genus includes several medicinally important species found worldwide, the limited knowledge of some species offers significant potential for further research [11].

AIM AND OBJECTIVES

AIM

Study of the anticancer and antimicrobial activity of synthesized silver nanoparticles using *Leea indica* plant extract.

OBJECTIVE

To investigate the anticancer and antimicrobial activity of silver nanoparticles synthesized using *Leea indica* extract, specifically targeting their effectiveness against prostate cancer cell.

Materials and Methods

Solvents and Reagents

All the solvents and chemicals used were analytical grade and were obtained from Hi-media, India.

Preparation of precursor solution for the synthesis of silver nanoparticles

For the experiment 1mM $AgNO_3$ was used as precursor solutions for the synthesis of silver nanoparticles.

Collection of plant

The *Leea indica* plant was collected from dandeli, which is situated in north Karnataka's western Ghats. Identification and authentication of the plant were done by Dr.K. Kotresha of the Department of Botany at Karnataka Science College in Dharwad, Karnataka. After a thorough water wash and air drying, the fresh plant material was ground into a fine powder. The powder was kept for later usage at 4°C in sealed containers.

Preparation of Plant extract

The chosen plant material in the current investigation was extracted rapidly of utilizing distilled water as a solvent in a 1:10 ratio.250 millilitres of distilled water used to extract 25grams of plant material. Following extraction, the filtered extract was utilized as a capping and reducing agent in the production of silver nanoparticles.

Synthesis of Silver nanoparticles

1ml of an aqueous plant extract was added to 10mL of 1Mm $AgnO_3$ solution. To avoid any unintended photochemical reactions, the procedure was carried out at room temperature in the dark. Following the reaction period, the mixtures colour changed shifting from colourless to dark brown, signifying the creation of nanoparticles-containing mixture was centrifuged for 22min at 4000rpm.Centrifugation and redispersion in double-distilled water were carried out multiple times to eliminate any remaining plant extract from the nanoparticles. After that, the resultant nanoparticles were left to dry in China dish and turn into a powder.

Bulk reaction

The process was repeated for bulk nanoparticle production. A total of 50 mL of plant extract was mixed with 500 mL of 1mM silver nitrate. The reaction conditions were kept the same as in the pilot experiment to ensure consistency.

The total yield obtained after the synthesis of the silver nanoparticles is 10g

Characterization of newly synthesised nanoparticles

UV-visible Spectroscopy

The optical properties of biosynthesized silver nanoparticles samples were analysed at room temperature using (Shimadzu UV-1601) UV -vis spectrophometer. The instrument operated within a wavelength range 200-800 nm with a resolution 1nm [12].

Fourier Transform Infrared Spectroscopy (FTIR)

The biomolecules responsible for ion reduction in plant extract and the synthesis of Ag-NPs were identified using BRUKER's FTIR device [13].

Scanning Electron Microscopy (SEM)

After biosynthesis, Ag-NPs were dried in an incubator and ground into powder. The morphology of the Ag-NPs was analysed using a JSM-IT500 scanning electron microscope (SEM) [14].

X-ray diffraction (XRD)

The analysis of plant extract derived AG-NPs was collected in an incubator they were subsequently measuring using the XRD (Smart Lab SE) instrument. XRD was done to check the purity of the compound [15].

Antibacterial Activity using Minimum Bactericidal Concentration (MBC) and Minimum Inhibitory Concentration (MIC)

The antimicrobial efficacy of silver nanoparticles (Ag-NPs) was assessed using the standard broth dilution method. The minimum inhibitory concentration (MIC) was determined in BHI broth with Ag-NPs concentration ranging from 0.132mg/ml to 10mg/ml and bacterial concentration of 1x 10^8CFU/ml (0.5 McFarland's standard). Controls included positive control with bacteria in BHI broth and a negative control with uninoculated broth. The MIC, defined as the lowest Ag-NPs concentration inhibiting 99% of bacterial growth, was determined by visual turbidity and confirmed in six replicates. After MIC determination, 50µl aliquots from clear tubes were plated on Ag-NPs free BHI agar and incubated at 37°C for 24 hours. The minimum bactericidal concentration (MBC) was recorded as the lowest Ag-NP concentration that eliminated 99.9% of the bacterial population [16].

CYTOTOXICITY ASSESSMENT- IN-VITRO ASSAY

Maintenance of cell lines

The National Centre for Cell Sciences provided the PC-3 cell lines, and their compatibility with the ATCC profile was confirmed using STR profiling. The cells were aseptically stored in a Class II cabinet at 5% CO_2 after being cultivated in 100 millilitres of complete media (MEM, 10% FBS, and 1% antibiotics). Cells were trypsinized (TCL007) and subculture in accordance with normal protocols after achieving 85% confluence.

MTT cell viability Assay

During the MTT experiment was carried out using the 12-well plates, PC-3 cells were in the log phase of growth. Cells were exposed to three distinct treatments: curcumin, periodontal paste films, and nanoparticles, in addition to negative controls. There were 50,000 cells in 1 mL of medium each well. The films were taken off and the cells were cleaned with PBS following a 24-hour incubation at 5% CO_2. The cells were cultivated for a further twenty-four hours after a new media was added. After adding the MTT dye, the plates were wrapped in foil and left to incubate for four hours. After dissolving the formazan crystals in 500 µL of DMSO, the pH was adjusted using glycine buffer. After measuring the absorbance at 570 nm, the proliferative index was calculated using OD values.

RESULTS

1.X-Ray diffraction Analysis

Diffraction beam intensities and corresponding atomic locations of silver nanoparticles derived from L. indica leaf extract were measured using X-ray diffractometry. By examining the plane-indexed peaks of the pattern of diffraction in Figure 3, the presence of crystalline silver nanoparticles could be confirmed. Eleven distinct diffraction peaks were detected at 2θ=24.16°, 26.36°, 29.06°, 33.1°, 35.64°, 40.8^o, 49.54^o, 54.14^o, 62.38^o, 63.98^o and 75.32^o respectively. It resembles the diffraction peaks found in metallic silver Cubic close packed structure at (1 1 1), (2 0 0), (2 0 0), (2 1 1), (2 1 1), (2 2 0), (2 2 2), (3 2 1), (3 3 1), (4 2 0) and (5 1 0). The pattern's peaks also correlated with the fcc structures reference from the Powder Diffraction Standard Joint Committee (JCPDS) Card No-087-0720. Maximum intensity peak appeared when the crystallographic (2 1 1) plane matched the Bragg diffraction criterion, average crystalline size of the produced samples was determined using the Scherrer formula,

which is contained in the subsequent equation. The crystalline size has been estimated by Eq. (1) which results in 32.3 nm, respectively**.**

------(1)

Where 'D' is the crystalline size

λ is the wavelength of X-ray (λ=0.154056 nm)

β is the full width at half maximum (FWHM) of the Braggs peak (in radians)

θ is the diffraction angle of the reflection.

2.FTIR Analysis

The FTIR spectra of Ag-NPs synthesized using *L. indica* extract. Peaks at 3657, 3211, 2729, 2004, and 1592 cm^{-1} indicate the presence of hydroxyl, aldehyde, alkyne, alkene, and amine groups. These functional groups suggest that biomolecules in the extract are responsible for the reduction and stabilization of Ag-NPs**.**

3.Scanning Electron Microscopy

The SEM images of *L. indica* nanoparticles reveal the formation of flower-like structure form and polydisperse with a typical diameter that ranges from 150-250 nanometre, these size effects due to the numerous factor impact on the nanoparticles including pH value, environmental condition, precursor material and concentration.

4.UV-Analysis

UV– analysis of the synthesized Ag-NPs with extracts of *L. indica*. The formation of Ag-NPs synthesized by L. indica extracts showed the absorbance band at 812 and 874 nm**.**

Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) of 1.25 mg/ml indicates the concentration needed to eliminate bacteria, whereas the Minimum Inhibitory Concentration (MIC) of 0.64mg/ml represents the lowest concentration required to prevent microbial growth. The MBC of 1.25mg/ml reflects the concentration essential for bacterial eradication.

Cytotoxicity assay

The MTT assay results show that the percentage of PC-3 cell viability was assessed at various concentrations of *Leea indica* derived Ag-NPs (250, 200, 150, 100, 50, and 10 µg/mL). According to the results, the IC_{50} value for *L. indica* Ag-NPs was 185.9µg/mL, whereas IC_{50} for cisplatin, standard positive control used 5.23 µg/mL. It shows a bar graph that illustrates the relationship between *L. indica* silver nanoparticles concentration and cell viability, it highlights the inverse relationship, which shows that cell viability increases as *LI*-Ag-NPs concentration decreases. Furthermore, as it illustrates, *L. indica* Ag-NPs kill the PC-3 prostate cancer cell line.

DISSCUSSION

L. indica was extracted using distilled water and ethanol, with muslin cloth to prevent photochemical reactions. The extract was dried at 37°C. As reported by ***Ghagane et al. (2017),*** Soxhlet extraction with solvents like methanol, ethyl acetate, and chloroform was used for effective separation of polar and nonpolar compounds**.** A UV-visible spectrophotometer (150–300 nm) was used to measure the formation of Ag-NPs, and extracts from L. indica showed absorbance at 812 and 874 nm. In the 300–800 nm range [17], ***Sreeshna et al. (2024)*** also found maxima for ethanol and aqueous extracts at 410 and 415 nm [18]. X-ray diffraction confirmed the crystalline nature of Ag-NPs synthesized from *L. indica* leaf extract, showing 11 peaks at 2θ values (24.16°–75.32°) corresponding to FCC silver planes like (111), (200), (211), and others. The highest intensity was observed at the (211) plane. Peak patterns matched JCPDS Card No. 087-0720. The average crystallite size was calculated using the Debye-Scherrer formula**. *Deepika, S., Selvaraj et al. (2020)*** confirmed the FCC structure of Ag-NPs via XRD. Aqueous extracts showed peaks at 27.7°, 32.6°, and 46.6°, while ethanol extracts showed peaks at 38.2°, 44.4°, and 77.4°. Unassigned peaks suggested plant residue. Crystallite sizes were 58.4 nm (aqueous) and 31.0 nm (ethanol) [19]. SEM images showed polydisperse, flower-like *L. indica* Ag-NPs sized 150–250 nm, influenced by factors like concentration, pH, and precursor**. *Jinu et al. (2017*** reported that Ag-NPs exhibited various shapes in SEM images and caused significant damage to the DLA cell line. Cytotoxicity was linked to interactions between silver atoms and phytoconstituents, affecting DNA phosphate groups, nitrogen bases, and protein functional groups [20]**.** FTIR analysis of *L. indica* Ag-NPs showed peaks indicating O-H, C-H, C≡C, and C=C groups, suggesting plant biomolecules aided in silver ion reduction and nanoparticle formation. ***Rana A et al. (2023)*** reported FTIR peaks indicating various functional groups like CH (aromatic), carboxylic acids, ketones, ethers, amines, and phenols. These

phytochemicals aid in the reduction, stabilization, and formation of Ag-NPs [21]. Silver nanoparticles were synthesized from *L. indica* showed cytotoxicity against prostate cancer cells with an IC_{50} of 5.23 µg/mL, indicating potential anticancer properties**. *Firdhouse et al. (2013)*** synthesized Ag-NPs from *Alternanthera sessile* with an IC_{50} of 6.85 mg/mL, showing cytotoxicity against PC3 prostate cancer cells, suggesting anticancer potential [22]**.**

CONCULSION

The present study successfully synthesized the silver nanoparticles via a green synthesis technique using an extract from the *L. indica* plant. Applying different scientific techniques, this analysis confirmed the nanoparticles and structural and physical characteristics Ag-NPs have demonstrated significant antibacterial efficacy against the common bacterial pathogen *Staphylococcus aureus*. Additionally, they have demonstrated cytotoxic effects on PC-3cells, indicating that they could help treat cancer by preventing the growth of malignant cells. These nanoparticles may be helpful in biological applications, such as the creation of novel medications or therapies, due to their combined antibacterial and anticancer qualities. This process is environmentally benign and offers a sustainable substitute for conventional nanoparticle manufacturing methods. Consequently, it is an interesting field for additional study, especially to comprehend the molecular processes underlying these nanoparticles and their possible uses in therapeutic settings.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Fig 1. Powder of ***Leea indica***

Fig 2. Mixture containing plant extract with 10 mL of a 1mM $AgNO_3$

RESULTS

1.X-Ray diffraction

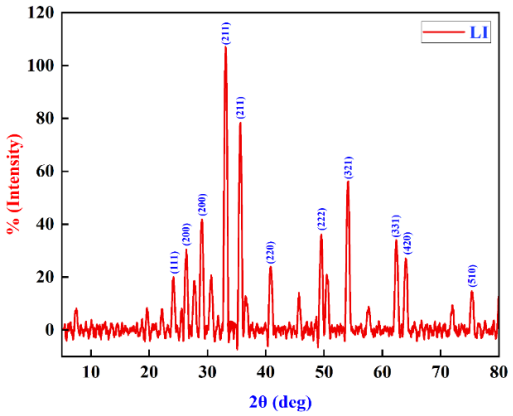


Fig.3 X-ray diffraction patterns of Ag nanoparticles

2. Fourier Transform Infrared Spectroscopy

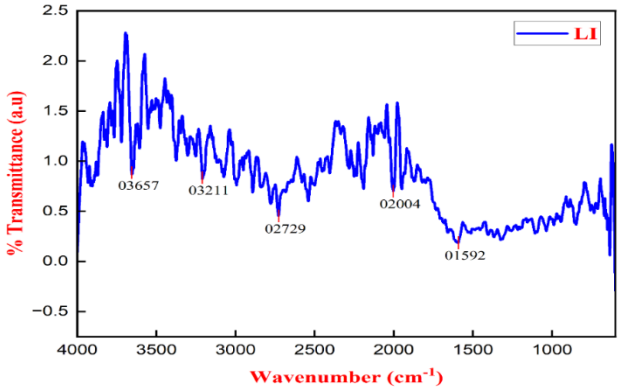
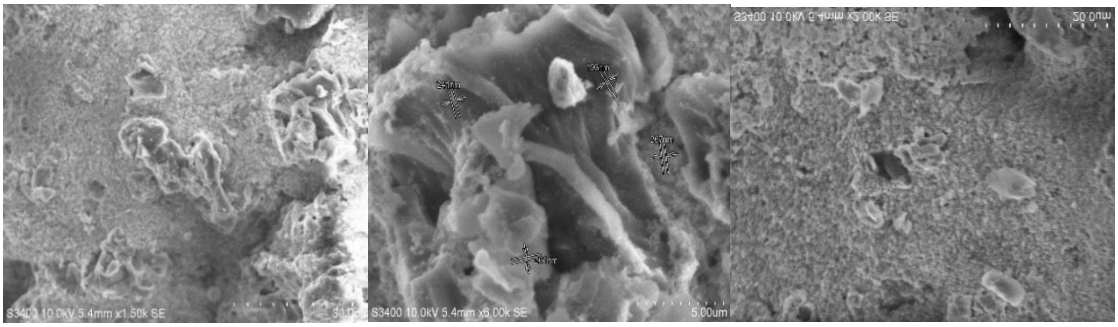


Fig 4. FTIR spectra of *L. indica*

3.Scanning Electron Microscopy



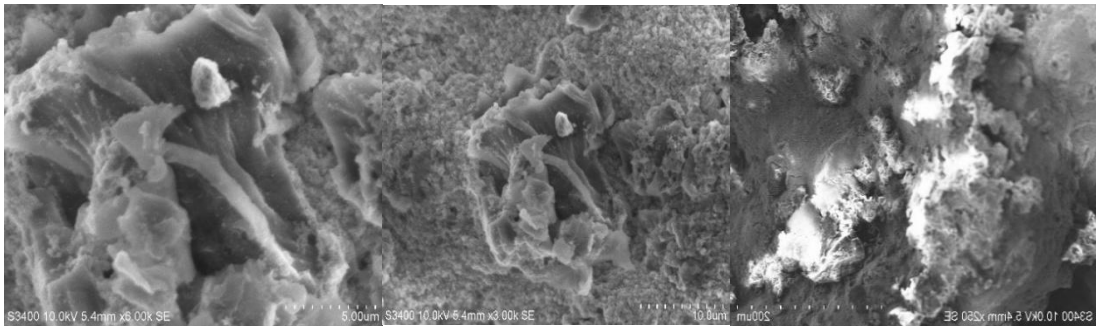


Fig.5 SEM micrographs of Ag-NPs

4.UV-Analysis

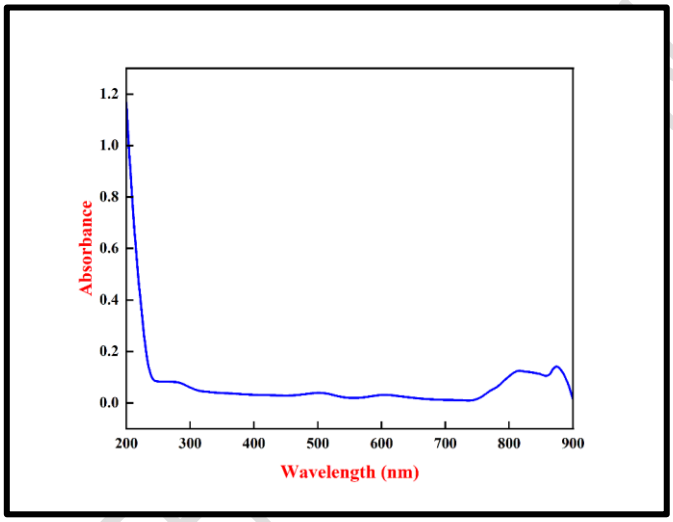
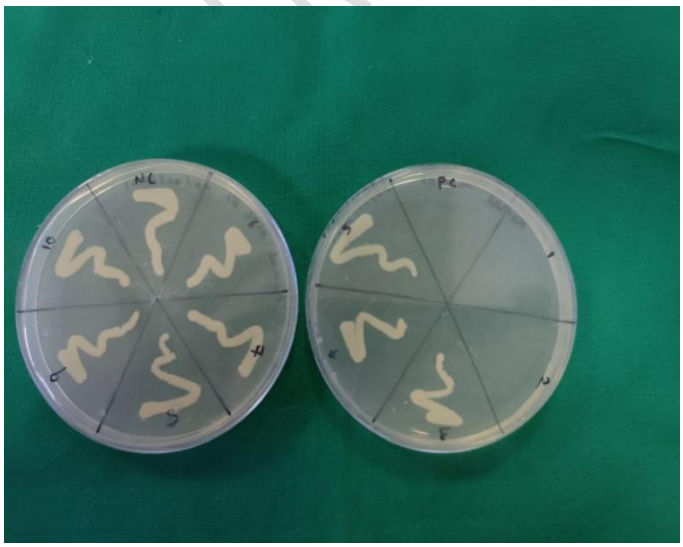


Fig 6. UV- spectroscopy of silver Ag-NPs

Minimum Bactericidal Concentration (MBC)



1.10mg/ml of Ag-NPs

2.5mg/ml of Ag-NPs

3.2.5mg/ml of Ag-NPs

4.1.25mg/ml of Ag-NPs

5.0.62mg/ml of Ag-NPs

6.0.31mg/ml of Ag-NPs

7.0.15mg/ml of Ag-NPs

8.0.07mg/ml of Ag-NPs

9.0.03mg/ml of Ag-NPs

10.0.01mg/ml of Ag-NPs

Fig.7 Determination of MBC

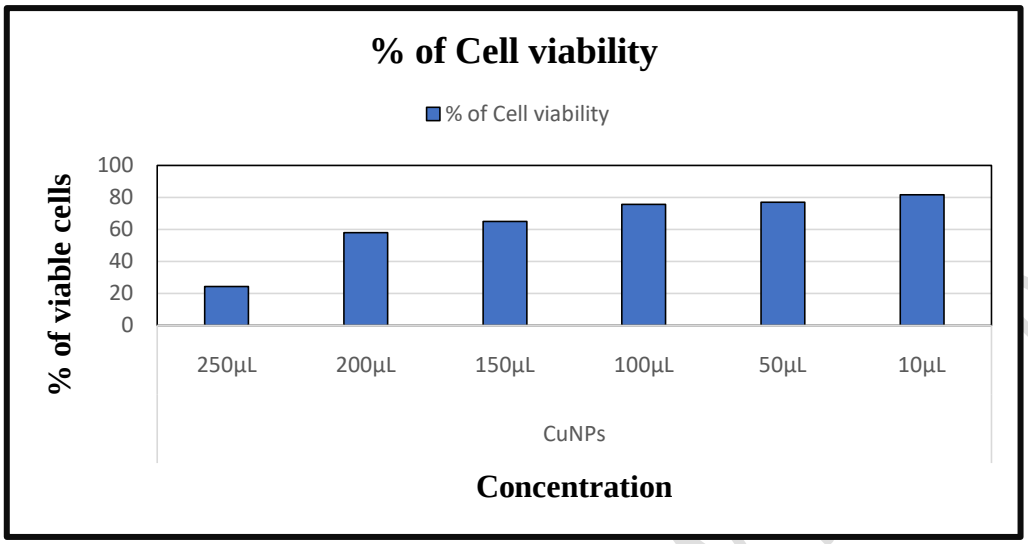


Fig.8 % of viable cells vs concentration of *LI* -Ag-NPs

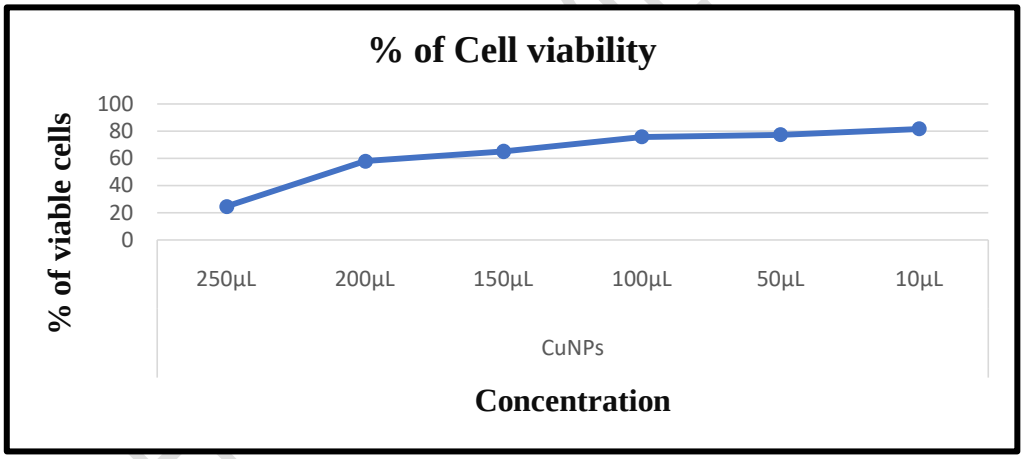
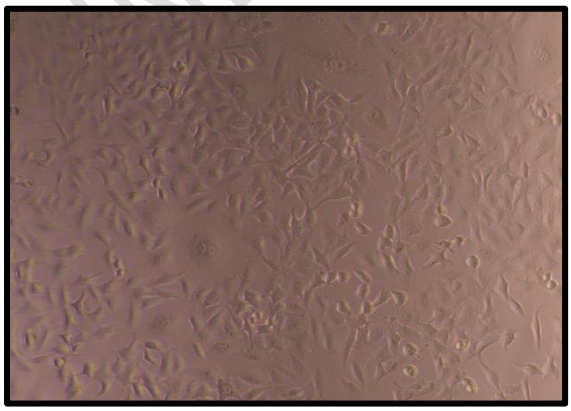
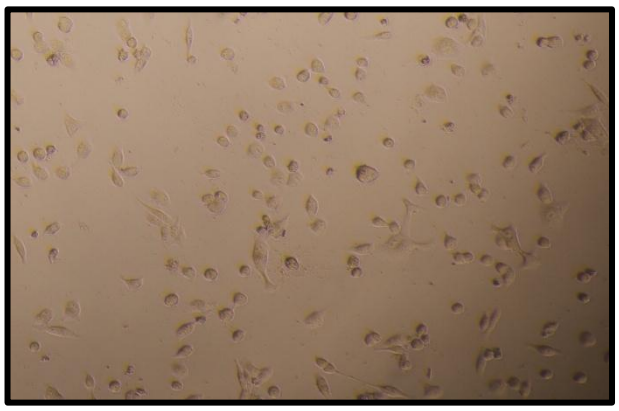
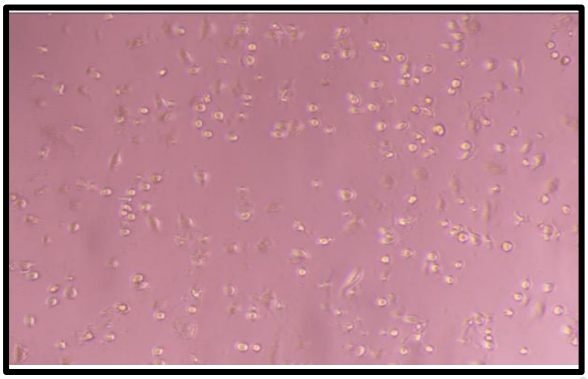


Fig.9 %cell viability of *LI* Ag-NPs on PC-3 cell line





a. Untreated (Negative Control) **b. *LI* treated on PC-3 cell line**



c. positive control

Fig.10 Influence LI Ag-NPs on PC-3 cell line

Concentration µg /mL	Cell viability%
250	24.48
200	57.86
150	64.98
100	75.51
50	77.15
10	81.60

Table 1. IC_{50}(µg /mL) value for MTT assay

Sl no	Samples	IC_{50}
1	Ag-NPs	185.9
2	Cisplatin	5.23

Table 2. % of viable cells vs concentration of *LI* -Ag-NPs