Cytotoxicity, Antibacterial And Antifungal Effects Of Silver Nanoparticles Synthesized From The Aavi Leaf Extract

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ABSTRACT

In the present investigation, we have studied the clinical significance of the silver nanoparticle synthesized from the leaf extracts of the tree "Aavi" which is found to be rich in phytochemical constituents, thereby offering pharmacological effects. The medicinally active compounds present in the leaf extract reduce the silver ions to form silver nanoparticle, which would be further used for therapeutic applications. Cellular toxicity of the silver nanoparticles were tested on chick liver cells, embryonic cells and bovine bone marrow cells respectively, to predict the 400µM minimum concentration which supports the viability of the cells. Besides, the antibacterial and antifungal property of the silver nanoparticles, conferring the antimicrobial activity was studied by the zone of clearance produced by some pathogenic gram positive, gram negative bacteria and some pathogenic fungi respectively.

The appearance of brown colour, which is an indication of the silver nanoparticle formation, whose relative absorbance was found to be in between 425 nm to 435 nm, was justified by UV spectroscopy. This result was further supported by the characteriestic peak obtained for the presence of silver by EDAX analysis, which defines the elemental composition or the percentage purity of the synthesis.

Keywords: Antibacterial agents, Antifungal activity, Cytotoxicity, SEM analysis.

1. INTRODUCTION

In recent years, metal nanoparticles have received a considerable attention because of their unique potential properties in catalysis [1], plasmonics [2], optoelectronics [3], biological sensor [4,5] and pharmaceutical applications [6]. Their performance depends critically on their size, shape and composition. Chemical synthesis methods are available for the synthesis of metal nanoparticles, many of the reactants and starting materials used in these methods are toxic and potentially hazardous in concern with biological applications [7].

Literature survey states that silver nanoparticle synthesis are mediated through chemical methods such as reduction in solutions [8], electrochemical [9], sonochemical [10], chemical and photochemical reactions in reverse micelles [11], thermal decomposition of silver compounds [12] etc., are reported as established methods of nanoparticle synthesis. Besides physical, methods such as microwave assisted process [13] radiation assisted [14] nanoparticle synthesis are well described. Recently synthesis of nanoparticles through green chemistry route [15,16] and biosynthesis of nanoparticles using microorganisms are demonstrated.

In general, sillver nanoparticles (SNPs) are non-toxic to humans, but most effective against bacteria, virus and other eukaryotic micro-organism at low concentrations [17]. Moreover, several salts of silver and their derivatives are commercially manufactured as antimicrobial agents [18]. Aqueous solution of silver nitrate was treated with the plant leaf extracts for the reduction of Ag(+) to Ag(0). UV-visible spectroscopy was used to monitor the quantitative formation of silver nanoparticles. The antibacterial activity of synthesized silver nanoparticles showed effective inhibitory activity against water borne pathogens Viz., Escherichia coli and Vibrio cholerae. Silver nanoparticles 10microg/ml was recorded as the minimal inhibitory concentration (MIC) against E. coli and V. cholerae. Alteration in membrane permeability and respiration of the silver nanoparticle treated

bacterial cells were evident from the activity of silver nanoparticles [19].The long-term administration of AgNps synthesized by tea leaf extracts at the concentration of 10 microg significantly reduced the mortalities in F. indicus from V. harveyi infections .The AgNps synthesized by tea leaf extract may be an alternative to antibiotics in controlling V. harveyi infections [20].

In the present investigation, we had analysed the antibacterial and antifungal property exhibited by the silver nanoparticles, synthesized from the leaf extracts of Aavi. Aavi is found to be rich in phytochemical constituents, which offers pharmacological effects. The medicinally active compounds present in the leaf extract reduce the silver ions to form silver nanoparticle, which would be further used for therapeutic applications. The minimum inhibitory concentration (MIC) of silver nanoparticles against pathological strains are predicted by well diffusion assay,and the results were examined.

2. EXPERIMENTAL DETAILS

2.1 Synthesis of silver nanoparticles

The silver nitrate (AgNO3) was purchased from Sigma-Aldrich chemicals. Aavi leaves were collected from chengalpet. The leaf extract was used for the reduction of Ag+ ions to Ag°. 20g of finely cut leaves were thoroughly washed, dried and immersed in 100 ml of distilled Water contained in a500 ml Erlenmeyer flask. The mixture was boiled for 15 to 20 minutes in a hot plate. Then the extract was further filtered through Whatman No. 1 filter paper and stored at 4°C for further experiments.

1mM aqueous AgNO3 solution was prepared. 10 ml of the leaf extract was added to 90 ml of 1 mM AgNO3 (aqueous) solution and kept in dark for 48 hours for the formation of silver nanoparticles.

2.2 Characterization of silver nanoparticles

2.2.1 UV-Vis spectral analysis

The bioreduced aqueous extract of Aavi containing silver nanoparticle was confirmed by taking an aliquot of 100µl of the extract for measuring the absorbance. The absorbance maxima are scanned in beween 300 – 800 nm wavelength on Perkin – Elmer Lambda spectrophotometer.

2.2.2 EDAX Analysis

The prepared silver nanoparticle was subjected to EDAX, for identifying the percentage elemental composition of the sample.

3 APPLICATIONS

3.1 Antimicrobial activity

Clinical isolates of *Bacillus cereus ATCC* 10987, *Bacillus subtilis MTCC* 1133, *Staphylococcus aureus MTCC* 96, *Micrococcus luteus ATCC* 4698, *Vibrio cholerae ATCC* 14035, *Escherichia coli MTCC 118, Salmonella typhi MTCC 733, Klebsiella pneumoniae MTCC 109* were collected from KMCH, Coimbatore (India). Each test strain was inoculated in Mueller Hinton liquid medium (broth) and incubated in a temperature controlled shaker (120 rpm) at 30 °C overnight. Fungal cultures *Candida albicans MTCC 183, Candida parapsilosis MTCC 2509, and Candida tropicalis MTCC 184.*

Used for the experimental analysis were maintained on potato dextrose agar (PDA) at 28°C. Reference antibiotics such as Eryhtromycin and Flucanazole used for the antibacterial and antifungal analysis respectively, were purchased from Sigma Aldrich, Bangalore, India and Merck Limited, Mumbai, India.

8 petriplates respectively for 8 different bacterial species, containing Nutrient agar were prepared. 24 hours growing culture of *Bacillus cereus ATCC* 10987, *Bacillus subtilis MTCC* 1133, *Staphylococcus aureus MTCC* 96, *Micrococcus luteus ATCC* 4698, *Vibrio*

cholerae ATCC 14035, *Escherichia coli MTCC 118*, *Salmonella typhi MTCC 733*, *Klebsiella pneumoniae MTCC 109* were swabbed on it. Five wells (10mm diameter) namely A, B, C, D, E on each plate were made by using cork borer. Well A was loaded with 25µg/ml concentration of "Erythromycin" (reference antibiotic). Well B was loaded with 25µg/ml concentration of silver nanoparticles. Well C and D were loaded with 50µg/ml and 100µg/ml concentration of silver nanoparticles respectively. Well E was loaded with 25µl of distilled water which will serve as a blank. The plates were then incubated for 24 hours at 37 º C. The zone of inhibition (in diameter) was measured.

Similaly, 3 petriplates respectively for 3 different fungal strains, containing potato dextrose agar were prepared. *Candida albicans MTCC 183, Candida parapsilosis MTCC 2509, and Candida tropicalis MTCC 184* were swabbed on it. Five wells (10mm diameter) namely A, B, C, D, E on each plate were made by using cork borer. Well A was loaded with 25µg/ml concentration of "flucanazole" (reference antibiotic). Well B was loaded with 25µg/ml concentration of silver nanoparticles. Well C and D were loaded with 50µg/ml and 100µg/ml concentration of silver nanoparticles respectively. Well E was loaded with 25µl of distilled water which will serve as a blank. The plates were then incubated for 24 hours at 37 º C. The zone of inhibition (in diameter) was measured.

3.2 Cytotoxicity Assay

Using *Lucas aspera* leaf extract as a reducing agent, silver nanoparticles were synthesized and it was subjected to chick liver cells, chick embryonic cells and bovine bone marrow cells for studying its cytotoxic effects. 1M stock concentration of the silver nanoparticles was diluted with DMEM medium to obtain a working concentration of 100 µM to 500 µM concentration respectively. The cytotoxicity assays were carried out using 1 ml of cell suspension containing 1,000,00 cells ,which were seeded in a 96-well microtitre plate.

This forms the monolayer of the seeded cells which serve as cell control (CC) was shown in Fig.21. After 24 hours from seeding of cells, fresh medium containing 100 µM to 500 µM concentration of the silver nanoparticles (AgNps) were added to all the wells except (CC) cell control.The microtitre plate was incubated for 48 hrs at 37°C in a 5% CO_2 enriched atmosphere. Eight wells (duplicates) for each test concentration (100 µM to 500 µM of AgNps) against the above said three cells were analysed.

The Morphology of the cells were inspected day by day (from 24 hrs to 48 hrs) and observed for microscopically detectable alterations. The viability of the cells were analysed through standard MTT assay.The absorbance value so obtained for the drug treated cells with respect to the control was determined and plotted.

4. RESULTS AND DISCUSSION

4.1 FORMATION OF SILVER NANOPARTICLES

When 1mM $AgNO_3$ solution was added to the leaf extract of Aavi pale yellow coloured broth gradually changes to brownish red coloured solution, which indicates the formation of silver nanoparticles. The formed nanoparticles were found to be stable and are uniformly distributed throughout the solution. Similar type of methodology was followed for synthesizing nanoparticles from ocimum leaf extract was already reported [21].

4.2 UV SPECTROPHOTOMETRY ANALYSIS

A characteristic peak was observed at 430nm confirmed the presence of silver nanoparticle formation. The broadening of the peak indicates the uniform distribution and polydispersion of silver nanoparticles raised from the leaf extract of Aavi. The size and shape of the nanoparticle was determined by the frequency and width of the surface plasmon absorption produced.This might also influenced by the dielectric constant of the metal itself

and the surrounding medium . The surface Plasmon resonance (SPR) at 430nm indicates the stable silver nanoparticle formation, using the seed extracts of Jatropha curcas were confirmed by UV–vis spectral analysis [22].

4.3 EDAX ANALYSIS

Irrespective of the elements present in the sample (nanoparticle), silver holds the highest percentage composition, which was clearly depicted from the peaks obtained from the graph.

4.4 ANTIMICROBIAL PROPERTIES

4.4.1 ANTIBACTERIAL EFFECTS OF SILVER NANOPARTICLES SYNTHESIZED FROM AAVI PLANT

Silver nanoparticle(25µg/ml) , silver nanoparticle (50µg/ml) and combination silver nanoparticle and erythromycin(50µg/ml) showed 13mm, 15mm and 15mm diameter of zonal expansion respectively against *B.cereus* ATCC 10987 (Fig.9), on well diffusion assay. Similarly, with respect to B.*subtilis* MTCC 1133 (Fig.10), silver nanoparticle (25µg/ml and 50µg/ml) and combination exhibited 14mm and 15mm diameter of inhibitory zone respectively.

For 25µg/ml and 50µg/ml (MIC) concentration , *S.aureus* MTCC 96, *M.luteus* ATCC 4698, *V. cholerae* ATCC 14035 ,*E. coli* MTCC 118, and *S. typhi* MTCC 733, showed 24mm, 20mm and 22, 14mm and 17mm, 25mm, 15mm, 25mm diameter of zone of inhibition. Combined effects of the silver nanoparticle and erythromycin pose equal effects as

that of nanoparticle.All the results obtained were compared with the control in order to check the effecieny of silver nanoparticles.

4.4.2 ANTIFUNGAL EFFECTS OF SILVER NANOPARTICLES SYNTHESIZED FROM AAVI PLANT

For 25µg/ml, 50µg/ml concentration of silver nanoparticle, combinations (silver nanoparticle and flucanazole) exhibited 15mm diameter of inhibition zone against *C.albicans* MTCC 183 (Fig.17), which was found to be half of the effect of the control. But, for *C.parapsilosis* MTCC 2509 and *C tropicalis* MTCC 184 species, control become ineffective. No characteristic zone of inihibition was observed for the selected reference antibiotic. But, silver nanoparticle(25µg/ml , 50µg/ml), exhibit a pronounced effect of 14mm and 18mm diameter of increase in the fold area of inibition zone against *C.parapsilosis* MTCC 2509 (Fig.18), and *C tropicalis* MTCC 184 (Fig.19), species respectively. This inferred the antifungal property of silver nanoparticles over broad range of pathogens tested. All the results obtained were comparatively analysed with the control.

Not only does the silver nanoparticle serve as a good antibacterial agent, but also capable of using as a potential antifungal agent against broad spectrum of bacterial and fungal pathogens taken under clinical investigation.

4.5 Cytotoxicity analysis of silver nanoparticles on chick liver cells, chick embryonic cells and bovine bone marrow cells

Chick liver cells, chick embryonic cells and bovine bone marrow cells does not show any toxicity upto 400 µM concentration, beyond which the number of viable cells started decreasing. This was proved by the MTT assay, wherein which the decrease in the absorbance at 400 µM concentration was directly proportional to the decrease in the cellular proliferation at 24 hrs and 48 hrs of incubation repectively.

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6. FIGURES

Fig.1 Aavi leaf broth

Fig.2 Formation of silver nanoparticles

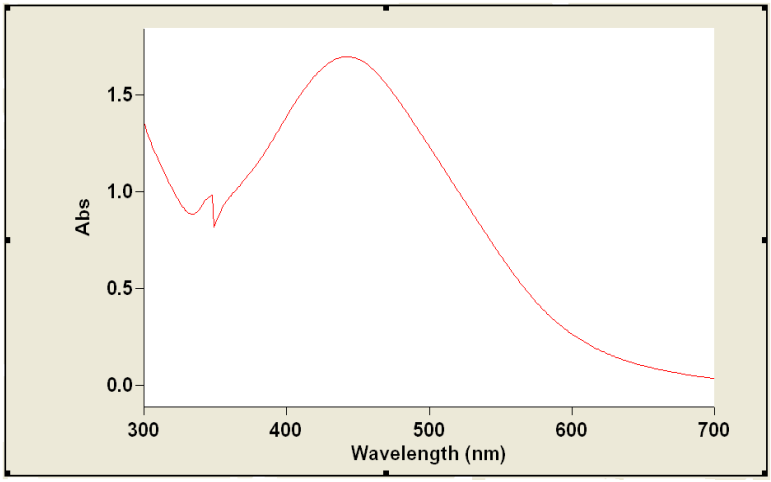

Fig.3 UV - Spectrophotometry results showing maximum absorption peak at 430nm indicates the formation of silver nanoparticles

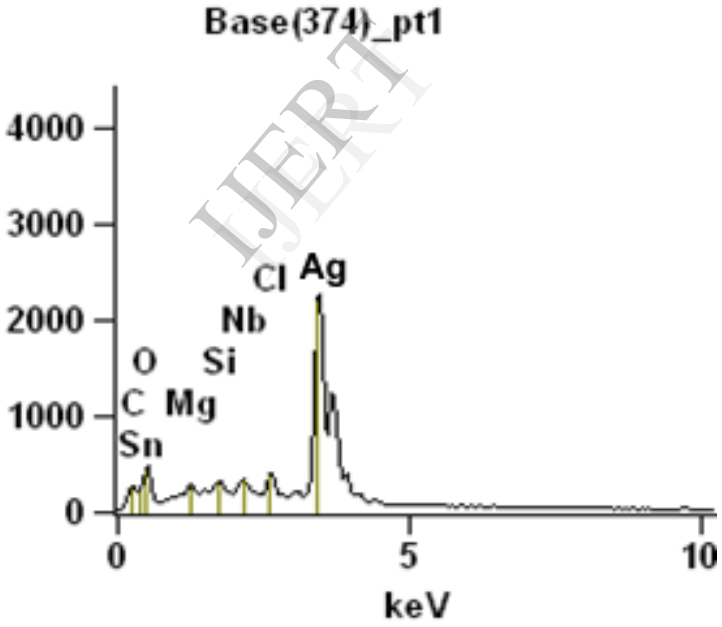

Fig.4 EDAX analysis depicting the percentage composition of the silver in comparison with the other elemental composition

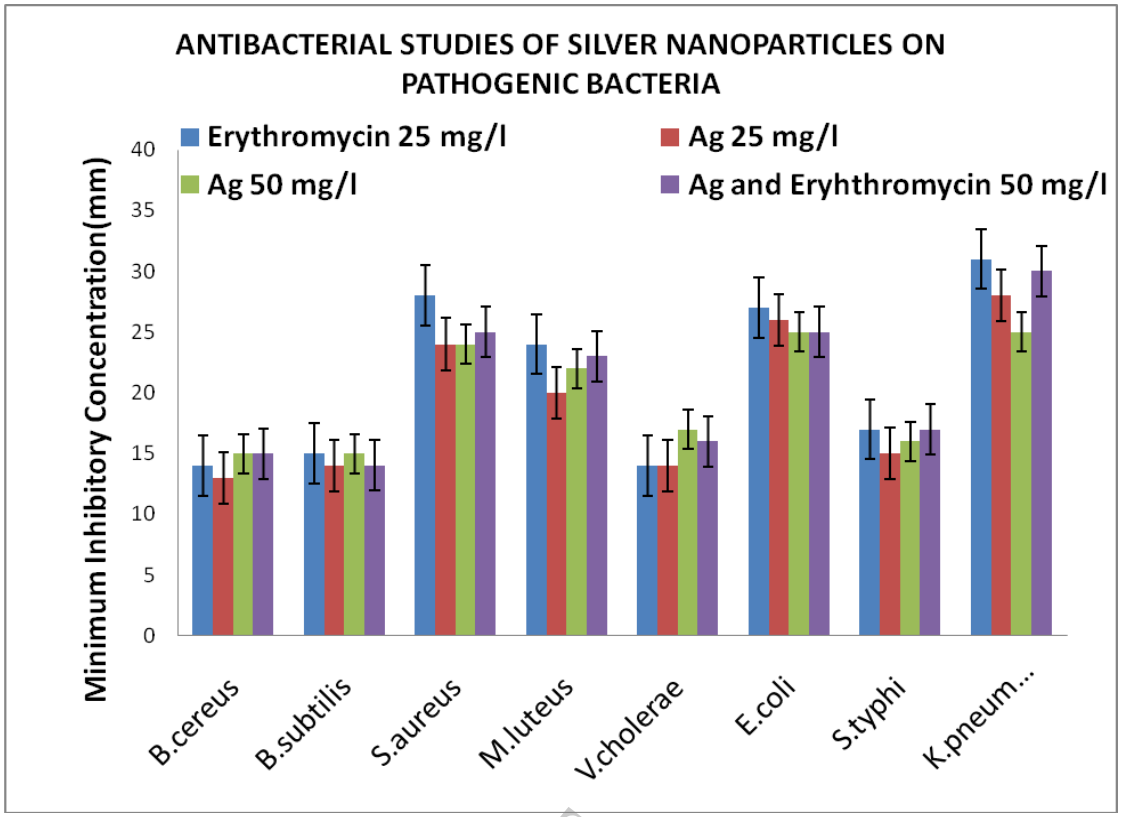

Fig.5 Antibacterial effects conferred by the silver nanoparticle on various pathogenic gram positive and gram negative bacteria

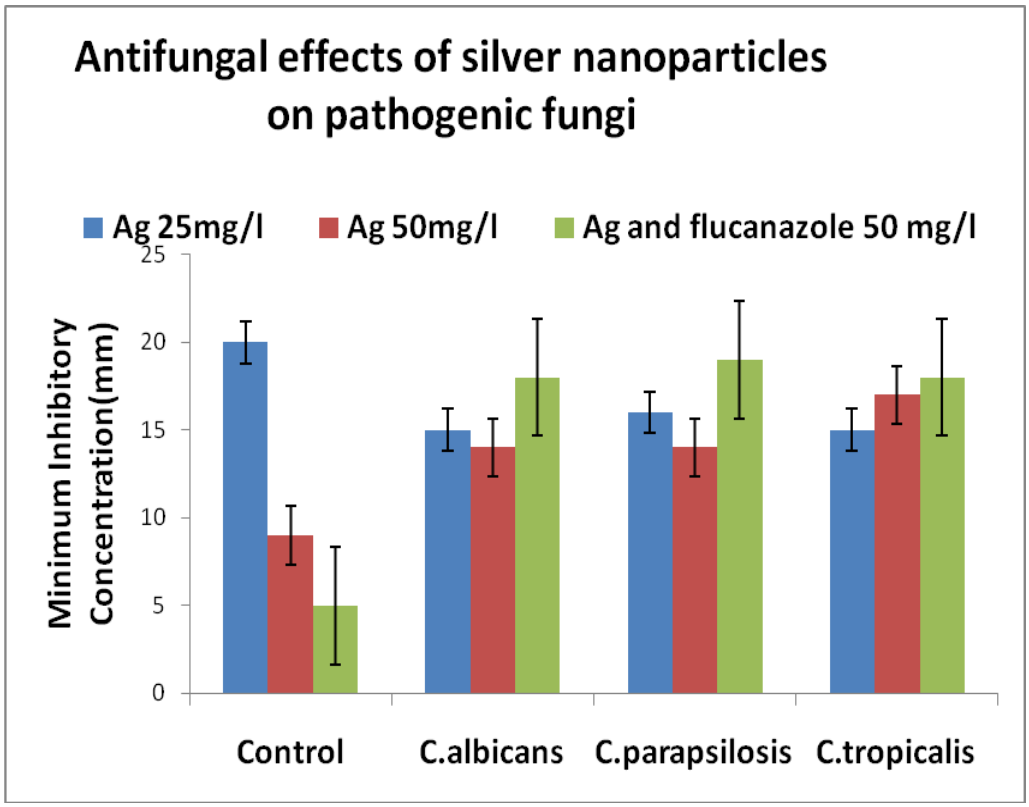

Fig.6 Antifungal effects conferred by the silver nanoparticle on various pathogenic fungi

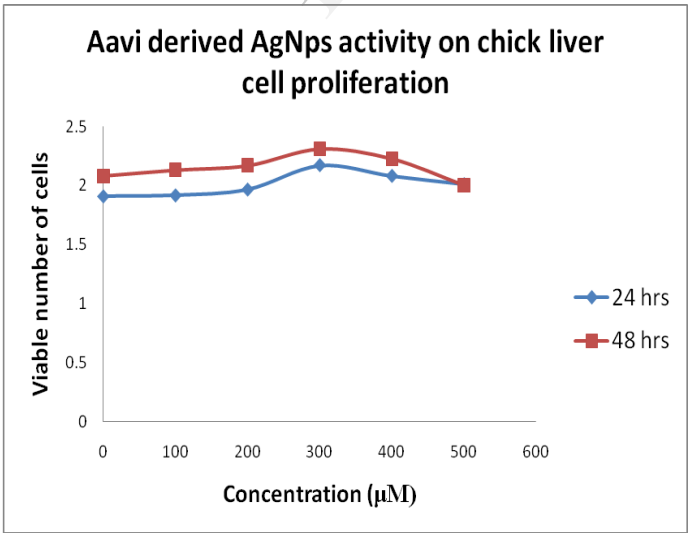

Fig.7 Aavi derived silver nanoparticles activity on chick liver cell proliferation

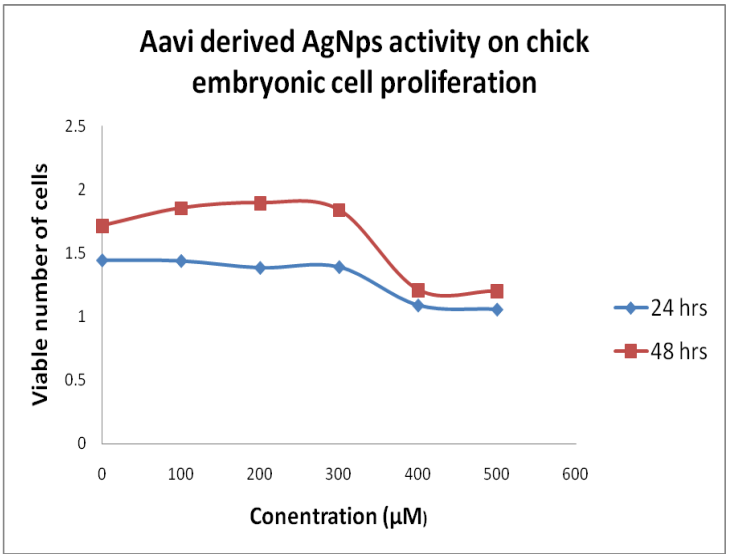

Fig.8 Aavi derived silver nanoparticles activity on chick embryonic cellular proliferation

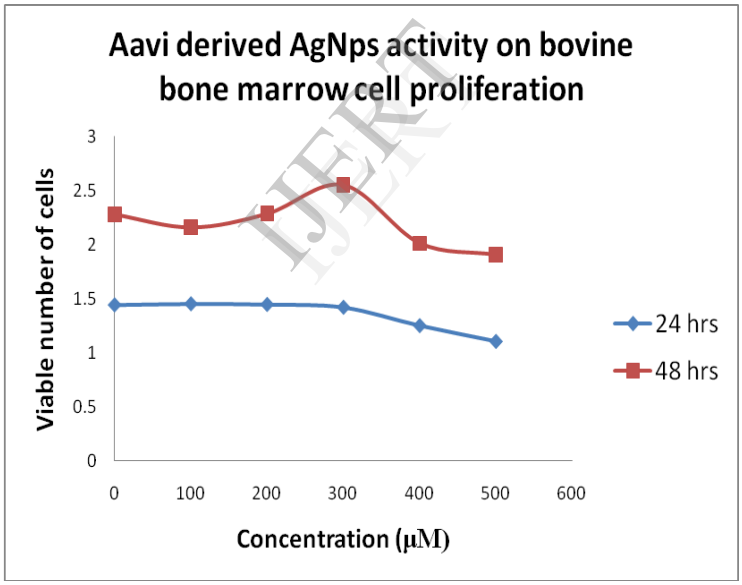

Fig.9 Aavi derived silver nanoparticles activity on bovine bone marrow cellular proliferation.

7. LEGENDS

Fig.1 Aavi leaf broth

Fig.2 Formation of silver nanoparticles

Fig.3 UV - Spectrophotometry results showing maximum absorption peak at 430nm indicates the formation of silver nanoparticles

Fig.4 EDAX analysis depicting the percentage composition of the silver in comparison with the other elemental composition

Fig.5 Antibacterial effects conferred by the silver nanoparticle on various pathogenic gram positive and gram negative bacteria

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Fig.7 Aavi derived silver nanoparticles activity on chick liver cell proliferation

Fig.8 Aavi derived silver nanoparticles activity on chick embryonic cellular proliferation

Fig.9 Aavi derived silver nanoparticles activity on bovine bone marrow cellular proliferation.