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Comparative Study of Cytotoxic Activity of Nano Silver Against A549 and L929 Cell Lines

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Studies in recent years are focussed on anticancer drugs which can selectively induce cell death with less toxicity to normal cells. The present work therefore aims at exploring the potential of nano silver as selective anticancer drug by comparing its cytotoxic activity against human lung carcinoma cell line (A549) and mouse normal fibroblast cell line (L929) *in vitro*. Nano silver was synthesized by both chemogenic (AgNP-C) and biogenic (AgNP-B) method and characterized by using PXRD, SEM and TEM. In order to assess the molecular mechanism involved in cytotoxicity, apoptosis inducing effect of nano silver was assessed by Annexin V/PI staining, cell cycle analysis and caspase-3 expression study. From the results, it was confirmed that A549 cells treated with nano silver showed decreased cell viability (AgNP-C: 173.5 ± 2.51 µg/mL, AgNP-B: 29.2 ± 0.22 µg/mL) compared to L929 cells (AgNP-C: 317.2 ± 3.43 µg/mL, AgNP-B: 622.3 ± 1.6 µg/mL), indicating lower toxicity of nano silver towards normal cells. Apoptotic study, cell cycle analysis and caspase-3 studies showed decreased expression of Bcl-2 and increased expression of Bax mitochondrial genes facilitating release of cytochrome c (cyt c) into cytosol by disrupting mitochondrial membrane potential indicating induction of cell death in A549 cells through mitochondrial mediated intrinsic apoptosis pathway. Present investigation provides conclusive evidence for application of biogenic nano silver as a potential candidate for anticancer drug development.

Keywords: Nano silver, Human lung carcinoma cell line, Mouse normal fibroblast cell line, Caspase-3.

INTRODUCTION

Cancer has become prominent genetic disease around the world leading to increased mortality [1,2]. Among the various cancer types affecting male and female, lung cancer is one of the leading cause for death [3-5]. Usage of many anticancer drugs for treatment of cancer has been restricted due to less efficacy, side effects, resistance to therapeutic agents, expensive, *etc*. Hence, there is a need for new antineoplastic drugs which are target specific, biocompatible and also cost-effective [6]. Advancements in nanotechnology has provided promising results in developing new anticancer drugs. Nanomaterials have the ability to circulate in the blood stream without being detected by the immune system causing less side effects compared to conventional anticancer therapies and agents [7-9].

Among various metal nanoparticles, nano silver, a multifunctional material due to its unique physico-chemical properties such as high electrical conductivity, optical, thermal and biological properties, has been used for variety of applications in biomedical field, food industry, textile industry, cosmetics, *etc*. as a potent and broad spectrum antibacterial and antifungal agent [10-13]. In addition to above, it finds application in biomedical field as anticancer agents, in drug delivery, diagnostics [14-17]. Further, nano silver is also known to possess antiviral, antiplatelet, antiangiogenesis properties [18,19]. Nogueria *et al.* [20] and Castro-Aceituno *et al.* [21] have reported the ability of nano silver to cause induced cytotoxicity, DNA damage and apoptosis in various types of cancer cell lines due to enhanced ROS levels. Similarly, Durai *et al.* [22] observed the apoptotic effects of nano silver on HT29 cell lines (colon cancer).

Various methods have been employed to synthesize nano silver of desired size for specific applications. Among them, physical and chemical methods are the most common methods. However, these methods have several disadvantages such as being expensive, presence of toxic and hazardous chemicals on the surfaces leading to potential and harmful biological stakes in biomedical field [15,23,24]. By contrast, biological methods are cost effective, less toxic and eco-friendly [6]. Green synthesis method has therefore been used as an alternative to overcome the limitations of other methods [25]. Plant extracts and phytochemicals are commonly used for biosynthesis of nanoparticles [2].

Nano silver has been synthesized using various plant extracts such as *Rubus fairholmianus* roots [2], *Gossypium hirsutum* leaves [26], *Panax ginseng* Meyer leaves [21], *Phoenix dactylifera* fruits [27] and fruit peels of citrus [28], *Punica granatum* [29], Cavendish banana [30] *etc*. However, comparative mechanistic studies of cytotoxicity of synthesized chemogenic and biogenic nano silver are hitherto unexplored. Present study was therefore performed to evaluate the cytotoxic effects of synthesized chemogenic (AgNP-C) and biogenic silver (AgNP-B) nanoparticles against human lung carcinoma cell line (A549) and mouse normal fibroblast cell line (L929) *in vitro*. Cell viability, apoptosis, cell cycle and caspase-3 expression study were analyzed using *in vitro* assays to understand the mechanism of cytotoxicity.

EXPERIMENTAL

Analytical grade chemicals were used for synthesis of nano silver. Silver nitrate ($AgNO_3$; 99.9 %), sodium citrate tribasic dihydrate ($C_6H_5Na_3O_7{\cdot}2H_2O$; 99 %) and sodium borohydride ($NaBH_4$; 98 %) were procured from Sisco Research Laboratories Pvt. Ltd, Mumbai. Cell culture medium: DMEM high glucose medium (#AL111), fetal bovine serum (#RM10432), MTT reagent ($C_{18}H_{16}BrN_5S$; # 4060 – 5 mg/mL) were purchased form HiMedia laboratories Pvt. Ltd, Mumbai and Cisplatin ($Pt(NH_3)_2Cl_2$– 99.9 %; trade name: Platinol® and Platinol®-AQ - #PHR 1624) from Sigma-Aldrich Pvt. Ltd, Mumbai. Apoptosis detection kit (Cat No: 556547), cell cycle analysis kit (Cat No: 550825) and Caspase – 3 assay kit (Cat No: 560901) were procured from Becton Dickinson (BD) Biosciences India Pvt. Ltd, Bangalore. Human lung carcinoma (A549) and Mouse normal fibroblast cell lines (L929) were procured from National Centre for Cell Science (NCCS), Pune, India. Preparation of aqueous extract and other solutions were carried out using double distilled water.

Preparation of pomegranate peel extract (PPE): Pomegranate peels (*Punica granatum*) were washed thoroughly in distilled water to remove dirt and debris, dried in hot air oven at 50 °C overnight and ground to fine powder. The powdered sample was mixed with double distilled water in 1:10 ratio and refluxed for 45 min at 60 °C. The supernatant (dark brown extract) was obtained by centrifuging at 10000 rpm for 10 min and stored in air tight bottle for subsequent studies [31].

Synthesis of chemogenic and biogenic nano silver: Chemical reduction method was used for synthesis of chemogenic nano silver (AgNP-C). 0.1 M solutions of silver nitrate, sodium borohydride and trisodium citrate were utilized as metal precursor, reducing agent and stabilizing agent respectively [32]. Sodium borohydride and tri sodium citrate were added simultaneously drop-wise under constant stirring conditions to the silver nitrate solution till visible precipitate was formed. The obtained precipitate was washed and separated by centrifuging for 10 min at 10000 rpm using ethanol and dried at 60 °C overnight.

In case of biogenic nano silver synthesis (AgNP-B), 9 mL of pomegranate peel extract (PPE) was added drop-wise to 0.1 M silver nitrate solution under constant stirring conditions for 3 h to complete the reaction. After the reaction, the precipitate formed was washed and separated by centrifuging for 10 min at 10000 rpm using ethanol and dried at 60 °C overnight [33].

Characterization studies: Analytical techniques such as PXRD, SEM and TEM were used to characterize synthesized nano silver (AgNP-C and AgNP-B). Crystalline phases of synthesized samples were analyzed using Powder X-ray Diffractometer (PXRD, Rigaku Ultima IV, Japan) equipped with nickel filtered Cu-K_α radiation source wavelength of 0.1541 nm. The instrument was operated at 45 kV and 40 mA with a scanning rate of 1 °/min for scan angles of 0 to 90 °. Further, scanning electron microscopy (SEM, Carl Zeiss-ULTRA 55, Germany) was used to analyze the morphology and structure of samples. Since samples are insulating, gold sputtering was carried out to get clear images prior to electron micrographs. Analysis of synthesized nanoparticles size, shape and crystallinity was carried out using transmission electron microscopy (TEM, Jeol/JEM 2100, Japan) utilizing LaB_6 filament and field emission-tranmission gun at 200 kV.

***in vitro* anticancer activity:** *in vitro* anticancer activity of synthesized nano silver (AgNP-C and AgNP-B) were evaluated against human lung carcinoma cell line (A549) and mouse normal fibroblast cell line (L929) to analyze the dose-dependent effect.

Cell culture maintenance: Culturing of A549 and L929 cell lines were carried out using Dulbecco's modified eagle medium (DMEM) supplemented with fetal bovine serum (5 % v/v) and antibiotics such as penicillin (100 U/mL) and streptomycin (100 µg/mL) in 5 % CO_2 incubator maintained at 37 °C. Stock culture was maintained in 75 cm^2 tissue culture flask and sub cultured for every 3-4 days. Further, the revived and sub cultured cell lines were subjected to cell viability assay.

Cell viability assay: Assessment of cytotoxicity of synthesized nano silver (AgNP-C and AgNP-B) against A549 and L929 cell lines were carried out using MTT assay, a mitochondrial based cell viability assay. In this method, 200 µL of 2×10^4 cells/well A549 and L929 cells were seeded in microtitre plate and incubated in 5 % CO_2 incubator for 24 h at 37 °C. After 24 h, spent medium was discarded and subsequently treated with different concentration (12.5, 25, 50, 100, 200 µg/mL) of nano silver (AgNP-C and AgNP-B), standard drug (cisplatin - 25 µM) and untreated cells as control for 24 h at 37 °C in CO_2 incubator (5 %). Each well in microtitre plate was added with 100 µL of MTT reagent (0.5 mg/mL) and further incubated for 4 h. Post incubation, excess MTT reagent was discarded and 100 µL of solubilization solution (DMSO) was added to dissolve the resulting formazan crystals. Using a microplate reader samples were analyzed at a maximum absorbance

of 570 nm considering reference wavelength as 630 nm [34]. Further, the percentage cell viability was calculated using percentage ratio of absorbance of treated samples to that of control and half maximal inhibitory concentration (IC_{50}) value was determined graphically. At the end of the assay, the treated and untreated samples were visualized under inverted biological microscope for morphological changes.

Determination of apoptosis by Annexin V/PI assay: Annexin V-FITC/PI staining method was used to determine the apoptosis/necrosis in A549 and L929 cells treated with synthesized nano silver (AgNP-C and AgNP-B). Early stage apoptosis was assessed by phosphatidylserine externalization, a marker using Annexin V FITC (Annexin V fluorescein isothiocyanate), while binding of propidium iodide (PI) to nuclear DNA provided information about late stage apoptosis/necrosis due to extensive membrane leakage. In brief, using 6-well plates, A549 and L929 cells were seeded (3×10^5 cells per well) and treated with IC_{50} values of synthesized nano silver (AgNP-C and AgNP-B), cisplatin (25 µM) as standard drug and untreated cells as control. The plates were then incubated in 5 % CO_2 incubator for 24 h at 37 °C. Post incubation, floating and adherent cells were trypsinized using 500 µL trypsin – EDTA solution (0.25 % w/v). Further, the cells were harvested and washed with 1x PBS by centrifuging at 1800 rpm for 5 min. 5 µL of Annexin V – FITC and 10 µL of propidium iodide along with 400 µL binding buffer was added to obtained cell pellet and incubated in dark at room temperature for 15 min. Samples analysis were carried out using BD FACSCalibur flow cytometer by counting the effect on 10,000 cells. The analyzed cells were expressed as percentage viable (Annexin V–/PI–), necrotic (Annexin V–/PI+), late apoptotic (Annexin V+/PI–) and early apoptotic cells (Annexin V+/PI–) using FlowJo software.

Analysis of cell cycle by flow cytometry: Distribution of cells in different phases of cell cycle was analyzed through flow cytometer using propidium iodide (PI), a DNA staining method. In this method, using 6-well plates, A549 and L929 cells (2×10^5 cell per well) were seeded and treated with IC_{50} values of synthesized nano silver (AgNP-C and AgNP-B), cisplatin (25 µM) as standard drug and untreated cells as control. The plates were incubated in 5 % CO_2 for 24 h at 37 °C. After 24 h, the cells were trypsinized, harvested, washed and fixed with 1x PBS, 70 % cold ethanol respectively. Fixative agent was removed by washing with 1x PBS. 400 µL PI stain, 50 µL RNase A solution was then added and incubated for 10 to 15 min in dark at room temperature and 10,000 cells were analyzed for cell cycle progression using BD FACSCalibur flow cytometer. Using FlowJo software, cells arrested in different phases (G_0/G_1, S and G_2/M) were calculated.

Analysis of capase-3 activity: Caspase-3, a downstream executioner enzyme was analyzed using caspase-3 assay kit obtained from BD Biosciences India Pvt. Ltd, Bangalore for determination of caspase activity. In this method, using 6-well plates, A549 and L929 cells (3×10^5 cells per well) were seeded and treated with IC_{50} values of synthesized nano silver (AgNP-C and AgNP-B), cisplatin (25 µM) as standard drug and untreated cells as control and incubated in 5 % CO_2 incubator for 24 h at 37 °C. Post incubation, trypsinization was carried out using 500 µL of trypsin-EDTA solution (0.25 % w/v). Cells were harvested and fixed using prechilled 70 % ethanol, washed and treated with 1xPBS and 5 µL of FITC Caspase-3 antibody respectively. The plates were incubated at room temperature for 0.5 h in dark and washed with 1xPBS containing 0.1 % sodium azide. Further, analysis was carried out using flow cytometer for sample set of 10,000 cells.

RESULTS AND DISCUSSION

Synthesis of chemogenic and biogenic nano silver: In the present study, chemical reduction method was employed to synthesize chemogenic (AgNP-C) and biogenic nano silver (AgNP-B).

Powder X-ray diffraction (PXRD) analysis: The crystalline structure of synthesized nano silver (AgNP-C and AgNP-B) was evaluated by powder X-ray diffraction (PXRD) (Fig. 1). The results showed five major diffraction peaks at (1 1 1), (2 0 0), (2 2 0), (3 1 1) and (2 2 2) corresponding to 5 diffraction facets of silver confirming face centred cubic structure ((JCPDS file No. 04 - 0783). Further, crystallite size was calculated using Debye-Scherrer formula and was found to be 10.8 nm and 17.35 nm for AgNP-C and AgNP-B respectively [29,35].

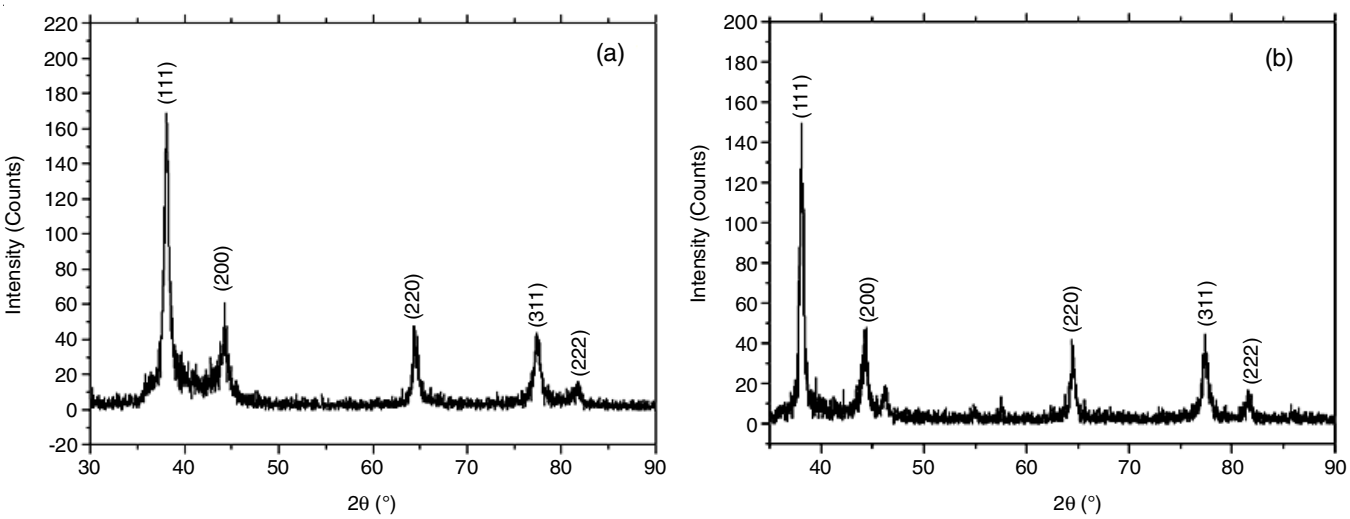

Fig. 1. XRD pattern of (a) AgNP-C and (b) AgNP-B

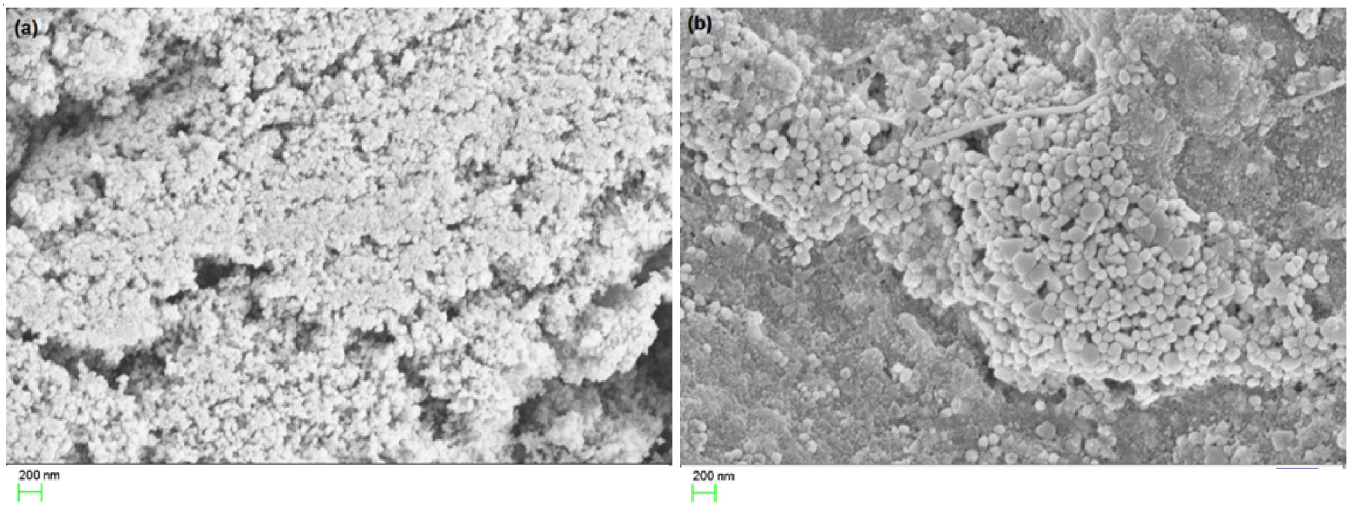

Fig. 2. SEM micrograph of (a) AgNP-C and (b) AgNP-B

Scanning electron microscopic analysis (SEM): The surface morphology of synthesized nano silver (AgNP-C and AgNP-B) was studied by SEM (Fig. 2). The scanning electron micrograph of AgNP-C revealed that the particles were spherical in morphology with smooth surface and aggregation. While in case of AgNP-B, the particles were agglomerated with spherical, triangular and hexagonal morphology and uneven surface.

Transmission electron microscopy (TEM): The size and shape of the synthesized nano silver (AgNP-C and AgNP-B) were analyzed by high resolution transmission electron microscope (HR-TEM) (Fig. 3). The particles were found to be spherical in shape with average particle size of ~ 60 nm and ~ 80 nm for AgNP-C and AgNP-B respectively.

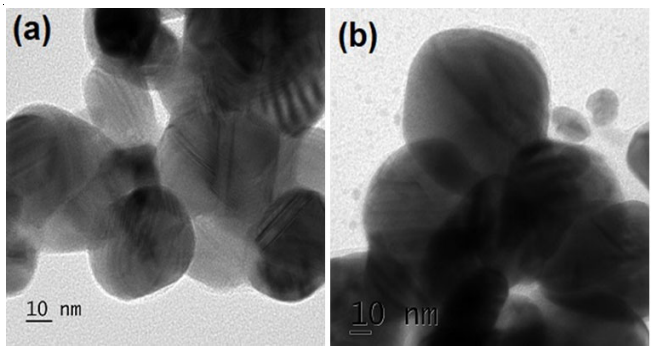

Fig. 3. TEM micrograph of (a) AgNP-C and (b) AgNP-B

Cell viability assay: Cytotoxicity assay was carried out to assess the effect of synthesized nano silver (AgNP-C and AgNP-B) at different concentrations (12.5, 25, 50, 100, 200 µg/mL) against A549 and L929 cell lines. Results unveiled decrease in viability of treated cells in a concentration dependent manner. The half maximal inhibitory concentration (IC_{50}) of AgNP-C and AgNP-B against A549 cell lines were found to be 173.5 ± 2.51 and 29.2 ± 0.22 µg/mL, respectively. In case of L929 cell lines, the IC_{50} values were 317.2 ± 3.43 and 622.3 ± 1.6 µg/mL for AgNP-C and AgNP-B, respectively. It is evident from the results that AgNP-B showed higher cytotoxic activity as compared to AgNP-C against A549 cell lines. Further, higher IC_{50} values were observed against L929 cell lines compared to A549 cell lines, indicating that synthesized nano silver (AgNP-C and AgNP-B) are less toxic to normal cells. This selectivity of nano silver towards cancer cells have been reported against several types of cancer cells such as breast cancer cells (MCF-7) [36], colon cancer cells (HT29) [37], cervical cancer cells (HeLa) [38], lung cancer cells (A549) [39,40] and Dalton's lymphoma ascites tumor [41] and proves it to be potential anticancer agent.

Rapid internalization of nano silver by lung cancer cells because of higher metabolic rate and cell division might be the cause for inhibition and cell death. De Matteis *et al.* [42] reported that internalization of nanoparticles cause the release of silver ions in A549 and HeLa cell lines leading to acidic lysosomal environment and cell death by common-ion effect. In addition to above, large number of researchers have reported that the possible mechanism by which nano silver induces cell death of cancer cells is by oxidative stress, ROS generation and DNA damage [9].

Analysis of apoptosis by flow cytometry: Apoptotic process plays a prominent role in maintaining homeostasis, biochemical and morphological changes in cells. Thus, Annexin V/PI staining method was used to determine the percentage apoptotic effect of IC_{50} concentrations of synthesized nano silver (AgNP-C and AgNP-B) against A549 and L929 cells using flow cytometer. Results depicted that the cell lines treated with nano silver exhibited considerable increase in cell population percentage of early and late apoptotic cells as well as decrease in percentage of viable cells (Table-1). Percentage of early and late apoptotic cells in untreated A549 cells (1.25 ± 0.20 % and 0.10 ± 0.02 %) and L929 cells (1.01 ± 0.10 % and 1.46 ± 0.38 %) were less compared to nano silver treated cells, indicating that apoptosis was induced. AgNP-C and AgNP-B treated A549 cells showed increased percentage of late apoptotic cells (14.01 ± 0.54 % and 19.12 ± 60 %) and early apoptotic cells (32.73 ± 0.23 % and 4.20 ± 0.49 %). However, L929 cells treated with AgNP-C and AgNP-B showed very low percentage of late apoptotic (2.39 ± 0.26 % and 1.74 ± 0.31 %) and early apoptotic (3.30 ± 0.34 % and 1.61 ± 0.23 %) cells. The results indicate that nano silver induced higher apoptotic cell death in A549 cells compared to L929 cells.

TABLE-1
APOPTOTIC STUDY (*i.e.*, ANNEXIN V-PI EXPRESSION STUDY) OF AgNP-C AND AgNP-B AGAINST A549 AND L929 CELL LINES

Quadrant	A549 cell lines				L929 cell lines			
	Necrotic cells (%)	Late apoptotic cells (%)	Viable cells (%)	Early apoptotic cells (%)	Necrotic cells (%)	Late apoptotic cells (%)	Viable cells (%)	Early apoptotic cells (%)
Label	UL	UR	LL	LR	UL	UR	LL	LR
Untreated	3.27±0.49	0.10±0.02	95.39±0.71	1.25±0.20	0.06±0.01	1.46±0.38	97.47±0.45	1.01±0.10
Standard drug	0.73±0.15***	16.17±0.61***	60.75±1.31***	22.35±0.59***	1.97±0.33***	34.57±0.71***	42.63±0.93***	20.83±0.36***
AgNP-C	0.10±0.03***	14.01±0.54***	53.15±0.67***	32.73±0.23***	0.23±0.06	2.39±0.26	94.18±0.74*	3.30±0.34**
AgNP-B	3.44±0.18	19.12±0.60***	73.24±0.16***	4.20±0.49**	0.05±0.02	1.74±0.31	96.47±0.52	1.61±0.23

Values are expressed as the mean ± SEM (n = 3); Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's test - $^{***}P< 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ were considered as statistically significant and by comparing treated group with control group.

TABLE-2
CELL CYCLE ANALYSIS OF AgNP-C AND AgNP-B AGAINST A549 AND L929 CELL LINES

Cell cycle stage	A549 cell lines				L929 cell lines			
	Untreated	Standard drug	AgNP-C	AgNP-B	Untreated	Standard drug	AgNP-C	AgNP-B
Sub G0/G1	1.30±0.21	3.71±0.37**	39.20±0.44***	7.29±0.33***	1.84±0.19	2.04±0.33	3.13±0.15*	2.94±0.48
G0/G1	79.02±0.81	35.95±0.93***	42.54±0.84***	39.42±0.40***	50.41±0.53	15.27±0.52***	48.15±0.53*	48.75±0.56
S	11.07±0.44	12.40±0.46	9.18±0.45	22.02±0.75***	17.41±0.36	41.39±0.49***	18.33±0.46	16.54±0.55
G2/M	8.61±0.19	31.73±1.26***	9.41±0.62	24.15±0.13***	30.33±0.80	25.61±1.24**	30.65±0.38	31.42±0.49

Values are expressed as the mean ± SEM (n = 3); Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's test - $^{***}P< 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ were considered as statistically significant and by comparing treated group with control group.

Analysis of cell cycle progression: To assess the effect of cell growth inhibition by nano silver exposure, analysis of cell cycle progression was carried out using flow cytometer. Cell cycle progression analysis was evaluated using propidium iodide staining method in A549 and L929 cells (Table-2). AgNP-C and AgNP-B (IC_{50} concentrations) treated A549 cells showed percentage increase in cells being arrested in G2/M phase of the cell cycle as well as decrease in percentage of cells being arrested in G0/G1 phase compared to control. Percentage increase of cells being arrested in G2/M phase of A549 cells treated with AgNP-C was 9.41 ± 0.62 % and AgNP-B was 24.15 ± 0.13 %, compared to untreated cells (8.61 ± 0.19 %). In case of L929 cells treated with AgNP-C and AgNP-B, the percentage cells arrested in G2/M phase was found to be 30.65 ± 0.38 % and 31.42 ± 0.49 % respectively. In addition to this, considerable accumulation of the cells in G0/G1 phase was observed in A549 cells treated with AgNP-C (42.54 ± 0.84 %) and AgNP-B (39.42 ± 0.40 %), as well as in L929 cells treated with AgNP-C (48.15 ± 0.53 %) and AgNP-B (48.75 ± 0.56 %) compared to standard (A549 cells – 35.95 ± 0.93 % and L929 cells – 15.27 ± 0.52 %). The obtained results are in agreement with previous reports indicating apoptosis is induced [41,43,44]. Further, the upregulation p53 and p21 expression by nano silver causes G2/M phase arrest and apoptosis. Zhu *et al.* [45] have reported that nano silver induces apoptosis in HepG2 cells by triggering the p53, MAPKs and AKT pathways *via* intercellular ROS generation. In addition to this, upregulation of Bax, Bid proapoptotic proteins and downregulation of Bcl-2, Bcl-w anti-apoptotic proteins expression level *via* p53 multifunctional tumor suppressor through treatment of nano silver induced apoptotic cell death in A549 cells by causing outer mitochondrial membrane permeabilization [6,41]. De Martino *et al.* [46] have reported that garlic extract treated HepG2 cells showed G2/M phase arrest and apoptosis by activation of p53/p21 system and JNK signaling cascade. However, the major reason for cell cycle arrest by nano silver in A549 cells might be due to the oxidative stress induced DNA damage and chromosomal abnormalities [6,47].

Analysis of caspase-3 expression: Caspase-3 is one of the key effector and major regulator of apoptotic process in cysteine protease family. In order to evaluate the effect of nano silver in inducing apoptotic pathway, caspase-3 expression analysis was carried out using caspase-3 antibody by flow cytometer. It was found that caspase-3 expression levels in A549 cells treated with AgNP-C and AgNP-B (IC_{50} concentrations) showed significant increase in mean fluorescence intensity (MFI) of 51.37 ± 0.50 and 67.57 ± 0.55 respectively compared to untreated cells (12.86 ± 0.70) (Table-3). However, the expression of caspase-3 levels depicted in the form of mean fluorescence intensity were 13.60 ± 0.44 and 12.37 ± 0.41 for AgNP-C and AgNP-B treated L929 cells respectively, which is significantly less compared to nano silver treated A549 cells. Higher apoptotic activity is due to p53 mediated membrane translocation and activation of Bax leading to release of cytochrome C from cytosol causing upregulation of downstream caspases such as caspase-3, -7, -9 and cell death [48]. Cytochrome C release from mitochondrial membrane by series of cascade events results in active caspase-3 that cleaves diverse

TABLE-3
CASPASE-3 EXPRESSION STUDY OF AgNP-C AND AgNP-B AGAINST A549 AND L929 CELL LINES

Caspase 3	Relative mean fluorescence intensity	
	A549 cell line	L929 cell line
Untreated	12.86 ± 0.70	11.78 ± 0.48
Standard drug	95.3 ± 0.52***	56.37 ± 1.04***
AgNP-C	51.37 ± 0.50***	13.60 ± 0.44
AgNP-B	67.57 ± 0.55***	12.37 ± 0.41

Values are expressed as the mean ± SEM (n = 3); Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's test - $^{***}P< 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ were considered as statistically significant and by comparing treated group with control group.

substrates leading to DNA fragmentation, morphological alterations of nucleus and phosphatidylserine, a phagocytic marker appearance on the cell surface [49]. From the results, it is confirmed that silver nanoparticles induce cell death by mitochondrial-mediated intrinsic apoptotic pathway in A549 cells [26].

Conclusion

Chemogenic (AgNP-C) and biogenic (AgNP-B) nano silver was synthesized and characterized by various techniques. PXRD studies revealed that the synthesized nano silver possess face centered cubic structure. TEM results confirmed spherical shape with size of about ~60 nm for AgNP-C and ~80 nm for AgNP-B. Concentration dependent cytotoxic activity of nano silver was observed against A549 cells with minimal effect on normal cells (L929). Present results indicated significant increase in early and late apoptotic cell percentage, expression of caspase-3 and arrest of cell cycle in G_2/M phase in A549 cells compared to L929 cells. Overall, higher apoptotic activity due to significant expression of caspase-3 was observed in A549 cells compared to L929 cells. Thus, this study indicates selectivity of nano silver towards cancer cells and proves its potential as an effective anticancer drug.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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