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# Green Synthesized Silver Nanoparticles: A Promising Anticancer Agent

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Silver nanoparticles (AgNPs) have attracted a great deal of attention in the recent years. It is mostly due to their availability, chemical stability, catalytic activity, conductivity, biocompatibility and anticancer activity. There are three major approaches for AgNPs synthesis; i.e., chemical, physical, and biological methods. Today, many chemical and physical methods have become less popular due to usage of hazardous chemicals or their high costs, respectively. The green method has introduced an appropriate substitute synthesis strategy for the conventional physical and chemical approaches. The utilization of the plant extracts as reducing, stabilizing and coating agent of AgNPs is an interesting eco-friendly approach leading to high efficiency. The anticancer synergistic effects among the AgNPs and phytochemicals will enhance their therapeutic potentials. Surprisingly, although many studies have demonstrated the significant enhancement in cytotoxic activities of plant-mediated AgNPs toward cancerous cells, these nanoparticles (NPs) have been found nontoxic to normal human cells in their therapeutic concentrations. This paper provides a specific insight into the mechanism of plant-mediated AgNPs synthesis, their anticancer and cytotoxic activities *in vitro* cancer cells, *in vivo* model animals and clinical trials.

*Keywords*: Silver nanoparticle; green synthesis; anticancer; apoptosis.

## 1. Introduction

Cancer, a condition of unrestrained cell differentiation, has usually been treated by chemotherapy, radiation and surgery during the past several decades.[1–3] These therapies are certainly efficacious in the destruction of cancer cells, but, alongside that, they come up with the cost of an increasing rate of adverse consequences due to unselective effects directed towards normal cells as well.[3] These therapies are now gradually becoming outdated in cancer treatment due to the development of nanomedicine, targeted drug delivery and multi-target inhibitors.[1,4–7] Nanomedicine is the field of biomedical application of nanotechnology in which engineered

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nanoparticles (NPs) are used to treat disease. Nanomedicine, with its advanced imaging and therapeutic capabilities, has the potential for early detection of cancer and cancer treatment.[8,9] It has the additional benefits of active/passive targeting, high solubility/bioavailability, biocompatibility and multifunctionality over conventional cancer therapies.[10]

### 1.1. *Nanoparticles properties*

Nanomaterials show the following unique properties, due to which they have recently become extensively conferred research topic and a preferable substitute to conventional cancer treatment strategy: (1) Biomolecules, whose size is comparable with NPs, play an important role in regulating various cellular cycles of the body and maintaining crucial cellular homoeostasis. With proper engineering, NPs can be localized in any system of the body and almost lookalike to perform the activity of biomolecules, thus pony-trekking the system biology of the body according to the need for human benefit. (2) NPs are highly soluble due to their small size and their solubility can be further increased by proper surface modification. (3) Due to NPs' high surface area to volume ratio, they have sufficient surface area to encapsulate drugs and other materials, thus providing higher therapeutic payload. (4) Due to their selective targeting nature, NPs can specifically release a therapeutic onto the target, reducing the side effects on normal cells.[9,10] Besides cancer, nanomedicine is now showing increasing application in personalized medicine[11,12] and diagnosis and therapy of cardiovascular diseases.[13] The major aspect of nanomedicine comprises inorganic NPs. Many inorganic NPs conjugated with anticancerous drugs or bio-active molecules (peptides, proteins, DNA, etc.) have already been approved by the U.S. Food and Drug Administration (FDA) and European markets, such as Feridex, Resovist, Doxil, Abraxane, etc.[8,9] Furthermore, inorganic NPs themselves show selective cytotoxicity towards cancer cells.[14–20] Inorganic NPs such as iron oxide NPs, titanium dioxide NPs, cerium oxide NPs, zinc oxide NPs, copper oxide NPs, silica NPs, etc., are being widely researched and used for anticancer therapy.[21] Each of these NPs has its own unique features, which makes them a novel and efficient tool for anticancer therapy. Iron oxide NPs conjugated with anticancer drugs are used to make magneto-sensitive NPs for selective targeting using magnetic fields in cancer treatment.[12,22,23] Likewise, titanium dioxide NPs are used in photodynamic therapy used for cancer therapy. They are used as a replacement for photosensitizer, which is excited by radiation to induce reactive oxygen species (ROS) generation and thus apoptosis.[24,25] Cerium oxide NPs are used in radiation therapy for cancer treatment, in which they selectively kill, irradiated cancer cells while posing no effects on the surrounding normal cells.[26] Zinc oxide NPs are also used for selective cytotoxicity towards cancer cells, where they show cytotoxicity by zinc-dependent protein activity disequilibrium and ROS induction.[27] The controllable pores of silica NPs make them a good carrier for drugs in anticancer therapy.[27,28] In addition, gold, silver and platinum NPs, known as precious metal or noble metal NPs, are also being used for cancer therapy as drug delivery and therapeutic agents.[19,29] The low reactive nature of these noble elements is advantageous for drug delivery purposes. This paper aims to explore these unique properties of Ag NPs, their role *in vitro*, *in vivo* and in human body and their mechanism of cytotoxicity towards cancer cells.

## 2. Silver Nanoparticles

Silver NPs have gained considerable interest because of their unique properties, and proven applicability in diverse areas such as medicine, catalysis, textile engineering, biotechnology, nanobiotechnology, bioengineering sciences, electronics, optics, and water treatment.[30–32]

Silver nanoparticles (AgNPs), because of their wide spectrum of activities and physical and chemical properties, are nowadays studied extensively. However, careful studies on living organisms should be performed, with strong attention to biocompatibility. Multiple effects displayed after AgNP treatment show an interesting potential of metal-based NPs, not only in bio-nanotechnology but also in molecular medicine and anticancer therapy. AgNPs are promising anticancer agents: they influence the cell cycle, inhibit cancer cell proliferation, induce oxidative stress, and propagate programmed cellular death (apoptosis).[22,33] The toxicity was evaluated using cell viability, metabolic activity and oxidative stress. MDA-MB-231 breast cancer cells were treated with various concentrations of AgNPs. AgNPs inhibited the growth in a dose-dependent manner using MTT assay. AgNPs showed dose-dependent cytotoxicity against

MDA-MB-231 cells through activation of the lactate dehydrogenase (LDH), caspase-3, ROS generation, eventually leading to induction of apoptosis which was further confirmed through resulting nuclear fragmentation.[1,7,34] AgNPs have now been recognized as promising therapeutic molecules and are extending their application such as, medical imaging, filters, drug delivery, nanocomposites, cell electrodes, antimicrobial products, and use in cancer diagnosis and therapy.[4,33,35,36] AgNPs due to their superior physicochemical and biological properties are intensively dealt with. The proper knowledge of these characteristics is essential to maximize their potential applications in many areas while minimizing their hazards to humans and the environment.[37] AgNPs are one of the most commonly used nanomaterials both in everyday life, and in research laboratories.[15] These NPs are a solution to many technological and environmental challenges in the area of medicine,[38] water treatment,[39] as catalysts[40] and in energy conservation.[41] So, the synthesis of metal nanoparticles for these fields is an area of interest.[42] Recently AgNPs have found a wide range of applications in cancer treatment,[43] antimicrobial[44] and anti-inflammatory agents.[45] Therefore, it is necessary to develop clean, nontoxic and an eco-friendly method for its synthesis. There are many methods to synthesize AgNPs such as a chemical reaction, co-precipitation, sol–gel method, etc. the problem with most of these methods is that they are very expensive and involve the use of toxic and hazardous chemicals, which may pose potential environmental and biological risks. In recent years, green synthesis of NPs has had several advantages as this technique eliminates the use of energy, high pressure, temperature, and toxic chemicals. There are many reports on green synthesis of AgNPs using plants.[28,46–50] Cancer continues to be a worldwide deadly disease, even though much research and rapid developments have been made during the last decade.[51,52]

### 2.1. *General protocol for the synthesis of silver nanoparticles from plant extracts*

In green methods as indicated in Fig. 1, biomolecules replace the reducing agents and validate environmentally friendly synthesis by avoiding the need to use toxic chemicals. These methods encompass the green chemistry approach aimed at the design, development and implementation of chemical products and processes to reduce or eliminate the use and generation of harmful substances.[53] Three major sources of biological AgNPs production are bacteria, fungi and plant. Due to the extensive secretory components in fungi, they are able to produce larger amounts of extracellular AgNPs in comparison with bacteria. Currently, the use of plants as the production assembly of AgNPs has drawn attention, because of its rapid, eco-friendly, nonpathogenic, economical protocol and providing a single step technique for the biosynthetic processes. The reduction and stabilization of silver ions by a combination of biomolecules such as proteins, amino acids, enzymes, polysaccharides, alkaloids, tannins, phenolics, saponins, terpenoids and vitamins which are already established in the plant extracts having medicinal values and are environmental benign, yet chemically complex structures.[54–56] The protocol for the nanoparticle syntheses involves: the collection of the part of plant of interest from the available sites can be done and then washed thoroughly twice/thrice with tap water to remove both epiphytes and necrotic plants; followed with sterile distilled water to remove associated debris if any. These clean and fresh sources are shade-dried for 10–15 days and then powdered using domestic blender. For the plant broth preparation, around 10 g of the dried powder is boiled with 100 mL of deionized distilled water.[57] The resulting infusion is then filtered thoroughly until no insoluble material appeared in the broth. To 90 mL of 1 mM $AgNO_3$ solution, on addition of few

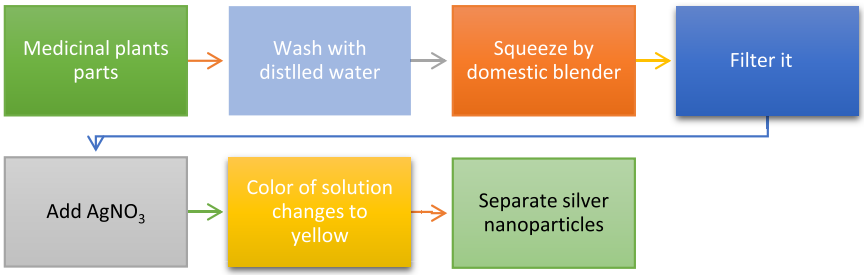


Fig. 1. Protocol for synthesis of AgNPs using plant extract.

mL of plant extract follow the reduction of pure Ag $(^{+})$ ions to Ag (0) which can be monitored by measuring the UV-Visible spectra of the solution at regular intervals.[9,26,58] In order to obtain AgNPs from plants, two types of extracts can be used: aqueous or alcoholic and, in the majority of cases, aqueous plant extracts are preferred because of the further use of the synthesized AgNPs in medical or biological applications.[59]

As[60] highlighted that the lists below by the steps involved for obtaining aqueous plant extract are:

- collecting the plant's parts that are of interest;
- washing thoroughly with tap and distilled water;
- shade-drying 7–10 days (or more if necessary);
- grinding the dried part of the plant to a fine powder;
- boiling a determined quantity of the dried powder with distilled water;
- filtering the resulted infusion until no debris is present.[60]

## 2.2. *Silver nanoparticles as anti-cancer agent*

Several physical and chemical properties of AgNPs implicate possible applications in the human environment: in agriculture, food industry, and cosmetology and finally in human health protection and medicine.[29,33,35,43,46,58] AgNPs, as cellular sensitizers with pro-oxidative and pro-apoptotic potential, also serve as therapeutic agents in photodynamic therapy against cancer cells.[61]

When AgNP enters cancer cells, a cascade of processes starts with loss of inner homeostasis and redox state destabilization. A series of free radical waves damages mitochondrial and nuclear membranes and propagates oxidative stress. Additionally, in S-phase (DNA replication) of the cell cycle, damaged DNA is not repaired effectively because repair enzymes are blocked by $Ag^{+}$ ions and replication stop.[62,63] Because of uncoupling in Mitochondria and effects on mitochondrial membrane potential, the ROS level increases to propagate the canonical apoptotic pathway. The mitochondria-dependent apoptosis pathway was studied in SCC-25 cells at the transcriptional level, where expression of the genes Bax and Bcl-2 was assayed.[62,63] The pro-apoptotic Bcl-2 gene was upregulated after 24 h of treatment with AgNPs.[62] ROS production in Caco-2 cells was manifested also by an inflammatory state that resulted in cellular death due to release of the pro-inflammatory cytokine interleukin (IL)-8 after 24 h of AgNP exposure.[64,65] This state was also propagated between cells by external pro-apoptotic signals. Use of AgNPs as good pro-apoptotic agents in cancer therapy seems to be reasonable. Toxicity of AgNPs is shown through the intrinsic ROS-mediated mitochondrial apoptotic pathway.[66] AgNPs could propagate a free radical wave, with further lysosomal rupture and free radical accumulation. Lysosomal damage leads to cathepsin release into the cytoplasm, which is a signal for lysosome-mediated apoptosis.[67] Any of these disruptions have been described as cytotoxic effects of AgNPs of different origins; however, the most desirable one is the lethal apoptotic effect on cancer cells. AgNPs are promising anticancer agents: they influence the cell cycle, inhibit cancer cell proliferation, induce oxidative stress, and propagate programmed cellular death (apoptosis). In future applications, some possible controversies must be resolved: dosage for different tissues, because of tissue-specific biocompatibility and side effects during therapy.

The role of ROS in tumor cell proliferation has been reported in a number of experimental systems. In one study, exogenous $H_2O_2$ at a low concentration promoted cell proliferation by increasing intracellular ROS levels[68] as shown in Fig. 1.

AgNPs markedly inhibited the proliferation of HePG-2 cells through induction of apoptosis with caspase-3 activation and PARP cleavage. AgNPs with dose-dependent manner significantly increased the apoptotic cell population (sub-G1). Furthermore, AgNP-induced apoptosis was found dependent on the overproduction of ROS and affecting of MAPKs and AKT signaling and DNA damage-mediated p53 phosphorylation to advance HePG-2 cells apoptosis.[69]

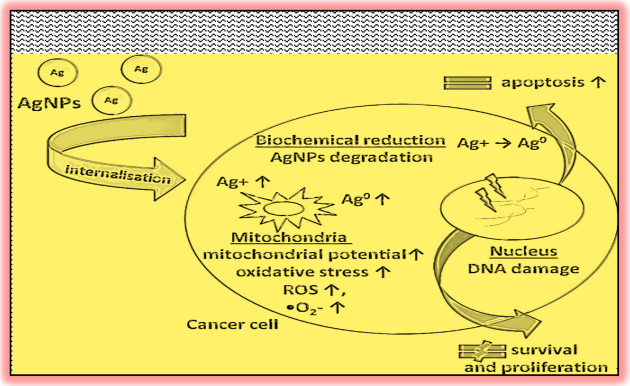


Fig. 2. Pro-oxidative activities of AgNPs in cancer cells.

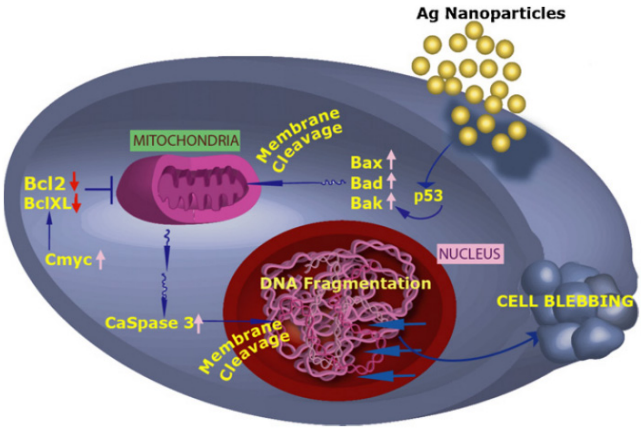


Fig. 3. Schematic representation of AgNP induced apoptotic pathway.

The ROS production and damages resulting from oxidative stress are AgNP size-dependent; smaller NPs cause greater ROS overproduction. Those observations result from the ability of AgNPs to interact with cellular components and to penetrate to organelles (mitochondria, nuclei, liposomes, endoplasmic reticulum, etc.) and to release free $Ag^+$ ions there (Fig. 2).[36] AgNPs, because of their wide spectrum of activities and physical and chemical properties, are nowadays extensively researched. However, careful studies on living organism should be performed, with strong attention to biocompatibility. Multiple cellular effects, displayed after AgNP treatments, show interesting potential of metal-based NPs, not only in bio-nanotechnology but also in molecular medicine and anticancer therapy.[62] AgNPs are promising anticancer agents, influencing the cell cycle, inhibiting cancer proliferation, and inducing oxidative stress and propagation of programmed cellular death (apoptosis). Additionally, they protect against bacterial, fungal and viral infections. During chemo and radiotherapies, such antimicrobial protection will be desirable because of the decreased immunological resistance of cancer patients. In conclusion, AgNPs often present in the human environment should be studied for novel findings and better characteristic.[36]

### 2.2.1. *In vitro cancer therapy of silver nanoparticles*

Silver nanoparticles (AgNPs) have now been recognized as promising therapeutic molecules and are extending their use in cancer diagnosis and therapy.

The findings from Refs. 4 and 70 revealed that the antitumor activity of green-synthesized AgNPs against lung cancer *in vitro*. Cytotoxicity effect was explored on human lung cancer H1299 cells *in vitro* by MTT and trypan blue assays. Apoptosis was measured by morphological assessment, and nuclear factor-$\kappa$B (NF-$\kappa$B) transcriptional activity was determined by a luciferase reporter gene assay. The expressions of phosphorylated stat3, bcl-2, survivin, and caspase-3 were examined by Western blot analysis. AgNPs showed dose-dependent cytotoxicity and stimulation of apoptosis in H1299 cells. The effects on H1299 cells correlated well with the inhibition of NF-$\kappa$B activity, a decrease in bcl-2, and an increase in caspase-3 and survivin expression. AgNPs significantly suppressed the H1299 tumor growth in a xenograft severe combined immunodeficient (SCID) mouse model. The results demonstrate the anticancer activities of AgNPs, suggesting that they may act as potential beneficial molecules.

Evaluation has been done on the influence of low doses of nanosize scale silver particles on the proliferation and viability of malignant oral epithelial keratinocytes *in vitro*, alone and in conjunction with the plant alkaloid berberine. Cells of human tongue squamous carcinoma SCC-25 (ATCC CRL-1628), cultivated with the mixture of Dulbecco's modified Eagle's medium, were exposed to AgNPs alone (AgNPs, concentrations from 0.31 to 10 $\mu$g/mL) and to a combination of AgNPs with berberine chloride (BER, 1/2 IC50 concentration) for 24 h and 48 h. The cytotoxic activity of AgNPs with diameters of 10 nm $\pm$ 4 nm was measured by 3-(4,5-dimethyl-2-thiazyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay. Cell cycle analysis was performed by treating cells with propidium iodide followed by flow-activated cell sorting. RT-QPCR reaction was used to assess expression of anti-apoptotic proteins Bcl-2 and pro-apoptotic protein Bcl-2-associated X protein Bax genes expression. Monodisperse AgNPs at a concentration of 10 $\mu$g/mL arrested SCC-25 cells cycle after 48 h at the G0/G1 phase in a dose- and time-dependent manner through disruption G0/G1 checkpoint, with increase of Bax/Bcl-2 ratio gene expression. AgNPs exhibit cytotoxic effects on SCC-25 malignant oral epithelial keratinocytes, which is diminished when combined with BER. The AgNPs concentration required to inhibit the growth of carcinoma cells by 50% (IC50) after 48 h was estimated at 5.19 $\mu$g/mL. AgNPs combined with BER

increased the expression of Bcl-2 while decreasing the ratio of Bax/Bcl-2 in SCC-25 cells. Silver particles at low doses therefore reduce the proliferation and viability of oral squamous cell carcinoma cells. SCC-25 cells are susceptible to damage from AgNPs-induced stress, which can be regulated by the natural alkaloid berberine, suggesting that NPs may be potentially used in a chemoprevention/chemotherapy by augmentation of action of standard anti-cancer drugs.[62] The performance of newly synthesized chitosan-coated silver nanotriangles (Chit-AgNTs) with strong resonances in near-infrared (NIR) to operate as photothermal agents against a line of human nonsmall lung cancer cells (NCI-H460). Found that the rate of cell mortality in the presence of Chit-AgNTs was higher than in the presence of thiolated poly(ethylene) glycol capped gold nanorods (PEG-AuNRs) — a common hyperthermia agent used as reference, while no destructive effects were noticed on the control sample (cells without NPs) under identical irradiation conditions.[71]

Green synthesized AgNPs from *Melia azedarach* was studied about its cytotoxicity against *in vitro* HeLa cells and *in vivo* Dalton's ascites lymphoma (DAL) mice model. Cytotoxicity of biosynthesized AgNPs against *in vitro* Human epithelial carcinoma cell line (HeLa) showed a dose-dependent activity. Lethal dose ($LD_{50}$) value was found to be 300 $\mu$g/mL of AgNPs against HeLa cell line. Cytotoxicity against normal continuous cell line human breast lactating, donor 100 (HBL 100) was found only in increased concentration of both AgNPs and 5-FU.[72]

A study demonstrated that efficacy of biologically-synthesized silver AgNPs as an antitumor agent using Dalton's lymphoma ascites (DLA) cell lines *in vitro*. The AgNPs showed dose-dependent cytotoxicity against DLA cells through activation of the caspase 3 enzyme, leading to induction of apoptosis which was further confirmed through resulting nuclear fragmentation. Acute toxicity, i.e., convulsions, hyperactivity and chronic toxicity such as increased body weight and abnormal hematologic parameters had not occurred.[7]

Green synthesized AgNPs using Pandanus odorifer leaf extract had shown activity of their anti-cancer potentials. Anti-cancer potential of these NPs was evaluated by scratch assay on the monolayer of rat basophilic leukemia (RBL) cells, indicating that the synthesized AgNPs inhibit the migration of RBL cells and they assessed the cytotoxicity of AgNPs, using a colorimetric method based on MTT [3-(4,5-dimethylthiazol2-yl)-2,5-diphenyl tetrazolium bromide]. The assay indirectly measures the mitochondrial activity of viable cells as a function of cell growth and proliferation. MTT salt gets reduced by reducing enzymes such as mitochondrial dehydrogenases of biologically active cells into water-insoluble formazan.[73]

Results reported by Inbathamizh[5] showed that AgNPs from *M. pubescens* can be potent natural antioxidants and can be essential for health preservation against oxidative stress-related degenerative diseases, such as cancer.

AgNPs perform well as cancer therapeutics because they can disrupt the mitochondrial respiratory chain, which induces the generation of ROS and ATP synthesis, which can induce DNA damage.[32]

AgNPs synthesized with Sesbania grandiflora leaf extracts were demonstrated to be cytotoxic to MCF-7 cancer cells. Morphological characteristics, including the disruption of membrane integrity, decreased cell growth, cytoplasmic condensation and cell clumping, were observed in MCF-7 cells treated with AgNPs, whereas control cells remained active. In addition, apoptotic features, such as cell shrinkage and nuclear condensation and fragmentation, were also observed in MCF-7 tumour cells 48 h after treatment with 20 $\mu$g/mL of AgNPs. AgNPs synthesized with *S. grandiflora* extracts induced the generation of free radicals, which resulted in oxidative damage and caspase mediated apoptosis.

As the study was conducted by Khorrami *et al.* indicated that the cell line cytotoxicity which has dose-dependent manner treatment of MCF-7 cells at different concentrations of AgNPs 3.90, 7.81, 15.62, 31.25, 62.5, 125, 250, 500 and 1000 $\mu$g/mL was measured by the cytotoxicity MTT assay within 24 h. After increasing the concentration of AgNPs, the cell viability was decreased. The produced AgNPs at 1000 $\mu$g/mL concentration exhibited 85% inhibition of MCF-7 cell proliferation, which was significantly different with the untreated cells as control group ($P < 0.001$). However, the lowest suppressions in cell growth at 3.90, 7.81, 15.62, 31.25 and 62.5 $\mu$g/mL concentrations indicated no significant differences in comparison with the control group ($P < 0.05$).[74]

Treatment of Human Chang liver (HeLa) cells with AgNPs induces cell growth and morphological changes, oxidative cell damage via mitochondria-mediated apoptosis and modulation of the level of

reduced glutathione in the cells.[75] When lung epithelial cells exposed to AgNPs cell viability were reduced, leakage of LDH was increased, cell cycle distribution was altered, apoptotic gene expression was upregulated and anti-apoptotic genes were downregulated.[76] When A549 cells were exposed to AgNPs at 10, 50 and 200 $\mu$g/mL for 24 h, the cell morphology was significantly altered, which is a characteristic feature of cell death, including cell shrinkage, few cellular extensions, restricted spreading pattern and increased floating cells.[76] During exposure of a normal human lung bronchial epithelial cell line (BEAS-2B) to AgNPs at different concentrations between 0.01 and 10 $\mu$g/mL for 24 h, internalization of AgNPs into the cells as aggregates encased in endocytic vesicles was seen, ultimately causing genotoxic effects with increased ROS generation, formation of micronucleus and enhanced DNA damage.[32,77] Subsequently, Zhang *et al.*[32] elucidated the endocytic mechanism of entry of AgNPs into epithelial cells and the localization of AgNPs in intracellular vacuoles. In addition, AgNPs inhibited epidermal growth factor (EGF)-dependent signal transduction through production of high ROS levels and reduced Akt and ERK signaling. AgNPs, which were green synthesized with various methods, have been tested in different cancer cell lines *in vivo* wise method. Table 1 summarizes the recent studies. AgNPs synthesized with from *Sesbania grandiflora* leaf extracts were demonstrated to be cytotoxic to MCF-7 cancer cells. Morphological characteristics, including the disruption of membrane integrity, decreased cell growth cytoplasmic condensation and cell clumping, were observed in MCF-7 cells treated with AgNPs, whereas controlled cells remained active. In addition, apoptotic features, such as cell shrinkage and nuclear condensation and fragmentation, were also observed in MCF-7 tumor cells 48h after treatment with 20 $\mu$g/mL of AgNPs. AgNP synthesized with *S. grandiflora* extracts induced the generation of free radicals which resulted in oxidative damage and caspase-mediated apoptosis.

This study reports a simple and eco-friendly synthesis of AgNPs using leaf extract of *Rhynchosia suaveolens*. UV-Vis analysis of *R. suaveolens*-synthesized AgNPs (RS-AgNPs) showed surface plasmon resonance (SPR) peak at 426 nm. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) analysis revealed that RS-AgNPs were 10–30 nm in size with spherical shape. X-ray diffraction (XRD) analysis of RS-AgNPs confirmed the crystalline nature with face-centered cubic (FCC) lattice. Fourier transform infrared (FTIR) interprets that polyphenols and proteins take part in bioreduction and capping of RS-AgNPs. RS-AgNPs exhibited dose-dependent inhibition of proliferation of different cancer cells including DU145 and PC-3(human prostate carcinoma cell lines), SKOV3 (human ovarian carcinoma) and A549 (human lung adenocarcinoma) with IC50 values of 4.35 lg/mL, 7.72 lg/mL, 4.2 lg/mL and 24.7 lg/mL, respectively. The plausible reasons behind anticancer activity of RS-AgNPs were explained using different assays on the most susceptible SKOV3 cells. RS-AgNPs induced oxidative stress in SKOV3 cells by generating ROS, enhancing lipid peroxidation (LPO) levels and decreasing glutathione (GSH) levels. RS-AgNPs induced the apoptosis of SKOV3 cells by up regulating the caspase-3, caspase-8, caspase-9, p53 and BAX and down regulating the antiapoptotic protein Bcl-2. Further, RS-AgNPs showed elevation of caspase 3/7 activity and also exhibited antimigratory effect by inhibiting the migration of SKOV3 cells into the wounded area. The findings suggested that biogenic RS-AgNPs provide an alternative approach to overcome several limitations of chemotherapy.[78]

The silver nanoparticles (AgNPs) synthesized by physical and chemical methods are not much desirable due to the use of high energy consumption, environmentally toxic and biological hazards chemicals. Therefore, green synthesis of AgNPs using natural plant extract is more appropriate method. Hence in this work, they have employed an anticancer plant, which is used in the Indian system of medicine *Flacourtia indica* to develop the AgNPs and revealed their anticancer potential in Dalton Lymphoma Ascites (DLA) cell line model. Among the solvent extracts of *F. indica*, methanol extract was found to contain higher level of yield (5.14 g/100 g) and total phenolic compounds (9104 mg gallic acid equivalents/L) with good antioxidant power. Hence, the preparation of methanolic extract from *F. indica* was optimized using Response Surface Methodology (RSM), which indicated that 88% of methanol concentration, 50°C of temperature and 88 min of extraction time results in higher phenolic yield. The optimized *F. indica* extract strongly reduced the silver into AgNPs. The synthesized AgNPs were characterized using Ultraviolet-Visible spectroscopy scanning (major peak at 455 nm), TEM (particle size of 14–24 nm) and zeta potential (−15 mV). The higher level of

Table 1. Effect of green synthesized AgNPs used in different cancer cell lines.

| Medicinal plant | Cancer type | Cell lines | AgNP doses | Toxicity effects | Reference |
|---|---|---|---|---|---|
| *Moringa olifera* | Human cervical carcinoma | HeLa | IC50 25, 50, 100 $\mu$g/mL | key morphological alterations of apoptotic cells | 70 |
| *Plantago major* | breast cancer | MCF-7 | $IC_{50}$ 0.5–2.5 $\mu$g/mL | potential cytotoxicity of | 71 |
| *Ganoderma neo-japonicum Imazeki* | Breast Cancer | MDAMB- 231 | IC50: 8,7 $\mu$g/mL | Dose-dependent inhibition of cell growth | 42 |
| *Taraxacum offinale* | Liver hepatocellular carcinoma | HepG2 | 10–200 $\mu$g/mL dose-dependent | A high cytotoxic effect was observed in HepG2 | 72 |
| *Commelina nudiflora L.* | Colon Cancer | HCT-116 | IC50:100 $\mu$g/mL | Diminished cell viability and increased cytotoxic effect in HCT-116 | 73 |
| *Melia dubia* | Human breast cancer | MCF-7 | IC50:31.2 $\mu$l/mL | AgNps showed remarkable cytotoxicity activity with evidence of high therapeutic index value against MCF-7 | 74 |
| Inonotus *obliquus* | Human lung cancer human breast cancer | A549 and MCF7 | IC50:100 $\mu$l/mL | Significant cytotoxic effect in A549 and MCF-7 cell lines | 75 |
| *Erythrina indica lam* | Breast and lung cancer | MCF-7 and HEP G2 | 23.89 $\pm$ 0.39 $\mu$l/mL for MCF-7 13.86 $\pm$ 0.95 $\mu$l/mL for HEP G2 | Significant cytotoxic effect in MCF-7 and HEP G2 cell lines | 76 |
| *Acalypha indica Linn* | Human breast cancer | MDA- MB-231 | IC50:100 $\mu$g/mL | Important cytotoxic effects and apoptotic properties | 77 |
| *Dendrophthoe falcata (L.f) ttingsh* | Human breast cancer | MCF-7 | IC50:5 $\mu$g/mL | Significant cytotoxic effect in MCF-7 cell line | 78 |
| *Datura inoxia* | Human breast cancer | MCF7 | IC50:20 $\mu$g/mL | Antiproliferative effect, cell cycle arrest, decreased DNA synthesis and apoptosis | 79 |
| *Piper longum* | Epidermoid Larynx Carcinoma | HEp-2 | IC50:500 lg/mL | Exhibit a prominent cytotoxic effect (94.02%) on HEp-2 cell lines | 80 |
| *Sargassum vulgare* | Human myeloblastic leukemic and cervical cancer | HL60 and HeLa | EC50: 93.57 $\mu$g/mL | Preventing the irradiation- related carcinogenesis with DNA damage and apoptosis | 81 |
| *Saccharomyces boulardii* | Human breast cancer | MCF-7 | (10–100 $\mu$g/mL), | Very low concentration of AgNps showed very high activity in MCF-7 cells compared to silver ions | 82 |
| *Pimpinella anisum* seeds | Human neonatal skin stromal and colon cancer | hSSCs and HT115 | $>$10 $\mu$g (20, 30, 40, 50 $\mu$g/mL) | AgNps doses showed few effects on cell proliferation below 10 $\mu$g, while doses above 10 $\mu$g resulted in increased cytotoxicity | 83 |
| *Indigofera tinctoria* leaf extract | Lung cancer | A549 | IC50:56.62 $\pm$ 0.86 $\mu$g/mL | Nanoparticles has higher antioxidant activities and more cytotoxic effect on cancer cell than the pure leaf extract | 84 |
| *Chaenomeles sinensis* | Human breast cancer | MCF7 | IC50:725.93 $\mu$g/mL | Cytotoxic against breast cancer cells | 85 |
| *Clinacanthus Nutans* | Oral squamous cell carcinoma | HSC-4 | IC50:1.61 $\mu$g/mL | Apoptotic effects observed at G1 phase and IC50 was low compared to few studies | 86 |

Table 2. Studies on green synthesis of silver nanoparticles.

| S.no | Plant name | Plant part | Nanoparticle | Size (nm) | Shape | Characterization | Cells induced | Reference |
|---|---|---|---|---|---|---|---|---|
| 1 | *Coptis chinensis* | Whole part | Silver | 6–45 | Spherical | UV-Vis, DLS, TEM and XRD | A549 lung alveolar carcinoma cell line | 87 |
| 2 | Nepeta *deflersiana* | Whole plant | Silver | 33 | cubic | UV-Vis, DLS, TEM, XRD and FTIR | (HeLa) human cervical cancer cells | 88 |
| 3 | Eucalyptus *chapmaniana* | Whole plant | Silver | — | Cubic | UV-Vis and XRD | HL-60 | 89 |
| 4 | *Dodonaea viscosa* | Leaf | Silver | 15–20 | Dendritic | UV-Vis, FT-IR, XRD, HR-SEM with EDX and HR-TEM with SAED patterns. | A549 NSCLC cells | 41 |
| 1 | *Abelmoschus esculentus* | Pulp | Silver | 3–11 | Spherical | UV-Vis, DLS, TEM, XRD and FTIR | Jurkat cell line (human T-cell lymphoma) | 90 |
| 2 | *Acacia nilotica* | Gum | Silver | 10–78 | Spherical | UV-Vis, FTIR, hydrodynamic size distribution, FESEM and TEM | HeLa (cervix cancer) and A549 | 91 |
| 3 | *Alternanthera sessilis* | Aerial parts | Silver | 10–30 | Spherical | UV-Vis and TEM | MCF-7 cell line | 92 |
| 4 | *Punica granatum* | Leaf | Silver | 35–60 | Spherical | UV-Vis, FTIR, XRD, XPS, FESEM-EDS and HRTEM. | HepG2 | 93 |
| 5 | *Couroupita guianensis* | Leaf | Silver | 28.4 | Spherical and uniform | UV-Vis spectroscopy, SEM, TEM and FTIR | MCF-7 cell lines | 94 |
| 6 | *Anthemis atropatana* | Leaf | Silver | 38.8 | Spherical | UV-Vis, SEM, TEM and XRD | HT29 | 95 |
| 7 | *Beta vulgaris* | Leaf | Silver | 10–20 | Spherical | UV-Visible spectroscopy and TEM | MCF-7, A549 and Hep2 cells | 96 |
| 8 | *Morinda pubescens* | Leaf | Silver | | | | HEP | 6 |
| 9 | *Sesbania grandiflora* | Leaf | Silver | 22 | Spherical | FESEM, EDAX and spectral studies | MCF-7 | 97 |

anti-proliferative activity (76.97%) was noted for AgNPs when compared to crude extract in DLA cell line model through cytotoxicity assay.[9,79]

In this study, the ability of AgNPs to kill pancreatic cancer cells has been assessed and then made to identify the molecular mechanism underlying this effect. Moreover, cytotoxicity of AgNPs was evaluated against nontumor cell of the same tissue (hTERT-HPNE cells) for comparison. The result indicated that AgNPs with size of 2.6 nm and 18 nm decreased viability, proliferation and caused death of pancreatic cancer cells in a size- and concentration-dependent manner. Ultrastructural analysis identified that cellular uptake of AgNPs resulted in apoptosis, autophagy, necroptosis and mitotic catastrophe. These alterations were associated with increased pro-apoptotic protein Bax and decreased level of anti-apoptotic protein Bcl-2. Moreover, AgNPs significantly elevated the level of tumor suppressor p53 protein as well as necroptosis- and autophagy-related proteins: RIP-1, RIP-3, MLKL and LC3-II, respectively. In addition, PANC-1 cells were found to be more vulnerable to AgNPs-induced cytotoxicity compared to pancreatic nontumor cells.

The cytotoxicity, genotoxicity and biocompatibility of AgNPs depend on many factors such as size, shape, surface charge, surface coating, solubility, concentration, surface functionalization, distribution of particles, mode of entry, mode of action, growth media, exposure time and cell type. Cellular responses to AgNPs are different in each cell type and depend on the physical and chemical nature of AgNPs.[32]

Cytotoxic effect of biosynthesized AgNPs obtained from *Alternanthera sessilis* plant studied by MTT assay against breast cancer cells (MCF-7 cell line) showed significant cytotoxic activity with IC50 value 3.04 lg/mL compared to that of standard cisplatin. The superior activity of the AgNPs may be due to the spherical shape and smaller particle size 10–30 nm as confirmed from transmission electron microscope (TEM) analysis. The data obtained in the study reveal the potent therapeutic value of biogenic AgNPs and the scope for further development of anticancer drugs.[80]

AgNPs, because of their wide spectrum of activities and physical and chemical properties, are nowadays extensively researched. Multiple cellular effects, displayed after AgNP treatments, shows interesting potential of metal-based NPs, not only in bio-nanotechnology but also in molecular medicine and anticancer therapy. Green-synthesized AgNPs (Table 2) are promising anticancer agents, influencing the cell cycle, inhibiting cancer proliferation and inducing oxidative stress and propagation of programmed cellular death (apoptosis)[28,29,46–49,81]

#### 2.2.1.1. Effects of green synthesized silver nanoparticles on proliferation of cell lines

The biological method used for the synthesis of AgNPs and its cytotoxicity against MCF-7 cells are reported. Furthermore, the AgNPs greenly synthesized from *Annona squamosa* leaf extract exhibited a dose-dependent cytotoxicity against human breast cancer cell (MCF-7) and normal breast epithelial cells (HBL-100) and the inhibitory concentration (IC50) were found to be 50 $\mu$g/mL, 30 $\mu$g/mL, 80 $\mu$g/mL and 60 $\mu$g/mL for AgNPs against MCF-7 and normal HBL-100 cells at 24 h and 48 h incubation, respectively. An induction of apoptosis was evidenced by (AO/EtBr) and DAPI staining.[81]

Cytotoxicity of biosynthesized AgNPs from Melia azedarach against *in vitro* Human epithelial carcinoma cell line (HeLa) showed a dose–response activity. $LD_{50}$ value was found to be 300 $\mu$g/mL of AgNPs against HeLa cell line. Cytotoxicity against normal continuous cell line HBL 100 was found only in increased concentration of both AgNPs and 5-FU.[72]

Human lung alveolar carcinoma epithelial cells A549 was continuously treated with AgNPs for 24 or 48 h at concentration range from 2.5 $\mu$g/mL to 20 $\mu$g/mL. AgNPs inhibited the proliferation of A549 cells following dose and time dependent manner. For the total range of concentrations, the most pronounced inhibition was found at 48 h ($p < 0.01$). A549 cells were exposed to AgNPs at 5 $\mu$g/mL, 10 $\mu$g/mL and 20 $\mu$g/mL for 48 h, cell viability of the groups exposed to AgNPs decreased to 87.1%, 75.2% and 68.5%, respectively, compared to the control group.[80,82]

### 2.2.2. *Anti-cancer activity of silver nanoparticles against in vivo cancer cells*

As Sriram *et al.* (2012) reported that AgNPs significantly increased the survival time in the tumor mouse model by about 50% in comparison with tumor controls, AgNPs also decreased the volume of ascitic fluid in tumor-bearing mice by 65%, thereby returning body weight to normal. Elevated white blood cell and platelet counts in ascitic fluid

from the tumor-bearing mice were brought to near-normal range.

Histopathologic analysis of ascitic fluid showed a reduction in DLA cell count in tumor-bearing mice treated with AgNPs. Reference 83 reported Decalepis *hamiltonii* to synthesize the AgNP's.

The apoptosis inducing effect of AgNPs was observed through acridine orange staining (AO and EB) and DNA fragmentation assay. Anti-angiogenic activity was sohiog confirmed by observing vessel development. All these observations indicate that the AgNPs were effective in treatment of DAL.[84]

Investigation was done on antitumor and apoptotic effects of AgNPs on the DAL cells *in vivo*. Thirty Swiss albino male mice were assigned into five groups of six each. Group I was intact animals. Group II animals served as tumor control injected with DAL cells intraperitoneally. Group III induced animals received plant extract (17 mg/kg BW) and Group IV induced animals received AgNPs (35 $\mu$g/kg BW). Group V induced animals received standard anticancer drug 5-Fluorouracil (5-FU, 20 $\mu$g/kg BW). The treatment period was 10 days excluding the day of tumor injection. Tumor cells were collected after euthanizing the animals and real-time PCR was used to analyze p53, caspase-3, 8, 9, 12 and cytochrome C expressions. Results indicate that the AgNPs were efficient in prolongation of life span, reduction of tumor volume and body weight in tumor animals. All the apoptotic genes were upregulated by treatment with AgNPs. To conclude, this study elicits that AgNPs are potent in antitumor activity and the molecular mechanism is by the induction of apoptosis through the mitochondrial dependent and independent pathways.[85]

AgNPs have now been recognized as promising therapeutic molecules and are extending their use in cancer diagnosis and therapy. This study demonstrates for the first time the antitumor activity of green-synthesized AgNPs against lung cancer *in vitro* and *in vivo*. Cytotoxicity effect was explored on human lung cancer H1299 cells *in vitro* by MTT and trypan blue assays. Apoptosis was measured by morphological assessment,and NF-$\kappa$B transcriptional activity was determined by a luciferase reporter gene assay. The expressions of phosphorylated stat3, bcl-2, survivin and caspase-3 were examined by Western blot analysis. AgNPs showed dose-dependent cytotoxicity and stimulation of apoptosis in H1299 cells. The effects on H1299 cells correlated well with the inhibition of NF-$\kappa$B activity, a decrease in bcl-2 and an increase in caspase-3 and survivin expression. AgNPs significantly suppressed the H1299 tumor growth in a xenograft SCID mouse model. The results demonstrate the anticancer activities of AgNPs, suggesting that they may act as potential beneficial molecules in lung cancer chemoprevention and chemotherapy, especially for early-stage intervention.[4]

Nanomedicine concerns the use of precision-engineered nanomaterials to develop novel therapeutic and diagnostic modalities for human use.[7] This study demonstrates the efficacy of biologically synthesized AgNPs as an antitumor agent using DLA cell lines *in vitro* and *in vivo*. The AgNPs showed dose-dependent cytotoxicity against DLA cells through activation of the caspase 3 enzyme, leading to induction of apoptosis which was further confirmed through resulting nuclear fragmentation. Acute toxicity, i.e., convulsions, hyperactivity and chronic toxicity such as increased body weight and abnormal hematologic parameters did not occur. AgNPs significantly increased the survival time in the tumor mouse model by about 50% in comparison with tumor controls. AgNPs also decreased the volume of ascitic fluid in tumor-bearing mice by 65%, thereby returning body weight to normal. Elevated white blood cell and platelet counts in ascitic fluid from the tumor-bearing mice were brought to near-normal range. Histopathologic analysis of ascitic fluid showed a reduction in DLA cell count in tumor-bearing mice treated with AgNPs. These findings confirm the antitumor properties of AgNPs and suggest that they may be a cost-effective alternative in the treatment of cancer and angiogenesis-related disorders.

Cascades of systemic and intracellular obstacles, including low stability in blood, little tumor accumulation, weak tumor penetration, poor cellular uptake, inefficient endosomal escape and deficient disassembly in the cytoplasm, must be overcome in order to deliver nucleic acid drugs for cancer therapy. Nanocarriers that are sensitive to a variety of physiological stimuli, such as pH, redox status and cell enzymes, are substantially changing the landscape of nucleic acid drug delivery by helping to overcome cascaded systemic and intracellular barriers. This paper discusses nucleic acid-based therapeutics, systemic and intracellular barriers to efficient nucleic acid delivery and nanocarriers responsive to extracellular and intracellular biological stimuli to overcome individual barriers. In particular, responsive nanocarriers for the cascaded

delivery of nucleic acids *in vivo* are highlighted. Developing novel cascaded nanocarriers that transform their physicochemical properties in response to various stimuli in a timely and spatially controlled manner for nucleic acid drug delivery holds great potential for translating the promise of nucleic acid drugs and achieving clinically successful cancer therapy.[86]

AgNPs synthesized from *Ficus religiosa* plant demonstrated cytotoxic effects in different cancer cell lines. Induction of apoptotic cell death was confirmed by various staining techniques, increased expression of cleaved caspases-8, 9, 3, lamin, PARP and oxidative stress markers in A549 and Hep2 cells. The *in vivo* studies performed in rats revealed that significant increase in serum levels of AST, ALT and LDH, TNF-$\alpha$ and IL-6 on day 29 following oral administration of FRAgNPs. However, these levels reverted back to normal at the end of wash out period on day 89. ICP-OES analysis revealed accumulation of silver in liver, brain and lungs on day 29 with respective concentration of 4.77 $\mu$g/g, 3.94 $\mu$g/g and 3.043 $\mu$g/g tissue. However, complete elimination of silver was observed on day 89. Histological analysis performed in vital organs indicated pathological changes only in liver which was also normalized after 89 days.[87]

AgNPs fabricated from *Pumbago indica* also showed dose-dependent cytotoxicity against DLA cells. AgNPs significantly increased the survival time in the mice model with survival days about 34($\pm$3) days in comparison with controls 17($\pm$1) days. AgNPs also decreased the volume of ascitic fluid in bearing mice, thereby returning bodyweight to normal. Elevated white blood cell and platelet counts in ascitic fluid of the bearing mice were brought to near normal level.[88]

*In vivo* toxicity was done in Wistar rat model by a AgNP derived from aqueous rhizome extract of *Acorus calamus* (ACRE). Further, the apoptotic changes induced by AgNPs in more susceptible Hep2 cells were observed through AO/EB, DCFH-DA, Rhodamine 123, PI/DAPI staining, oxidative stress markers and Western blotting. *In vivo* toxicity study revealed substantial alterations in the levels of serum biochemical markers including AST, ALT, LDH and inflammatory markers such as TNF-$\alpha$ and IL-6 on day 29 when rats treated with AgNPs as compared to control, however, these levels were restored to normal at the end of washout period on day 89. No remarkable changes were observed in liver oxidative stress enzymes. ICP-OES analysis indicated bio-distribution of silver in spleen (5.67 $\mu$g/g) and liver (4.98 $\mu$g/g) in rats treated with 10 mg/kg b.w of AgNPs on day 29 and elimination of silver from all organs was observed at the end of washout period on day 89. Histopathological analysis revealed no significant changes in kidney, spleen, lungs, heart, testis and brain with 5 and 10 mg/kg b.w of AgNP. However, 10 mg/kg b.w of AgNPs showed moderate degree of cell swelling and vacuolar degeneration in liver and these alterations were reverted back to normal at the end of washout period. Findings from this study signify green-synthesized AgNPs at low concentrations might be useful in many ways with eco-friendly nature.[89]

Identification of differential sensitivity of cancer cells as compared to normal cells has the potential to reveal a therapeutic window for the use of AgNPs as a therapeutic agent for cancer therapy. Exposure to AgNPs is known to cause dose-dependent toxicities, including induction of oxidative stress and DNA damage, which can lead to cell death. Triple-negative breast cancer (TNBC) subtypes are more vulnerable to agents that cause oxidative stress and DNA damage than other breast cancer subtypes. They hypothesized that TNBC may be susceptible to AgNP cytotoxicity, a potential vulnerability that could be exploited for the development of new therapeutic agents. They showed that AgNPs are highly cytotoxic toward TNBC cells at doses that have little effect on nontumorigenic breast cells or cells derived from liver, kidney and monocyte lineages. AgNPs induced more DNA and oxidative damage in TNBC cells than in other breast cells. *In vitro* and *in vivo* studies showed that AgNPs reduce TNBC growth and improve radiation therapy. These studies showed that unmodified AgNPs act as a self-therapeutic agent with a combination of selective cytotoxicity and radiation dose-enhancement effects in TNBC at doses that are nontoxic to noncancerous breast and other cells.[90]

The *in vivo* studies revealed that the LD50 was higher than 2000 mg/kg and there was no significant difference ($p > 0.05$) between the treatment groups compared with the control group for mean organ-to-body weight ratio except in the liver and in all hematological parameters except WBC and hematocrit. Similarly, there was no significant difference ($p > 0.05$) for serum electrolytes (Na+, Mg2+ K+, Cl− and Ca2+), total protein, urea, $\delta$-glutamyl transferase (GGT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP),

Alanine aminotransferase (ALT), albumin, total and conjugated bilirubin between the treatment and the control group. However, there were changes in creatinine, urea and cholesterol. In the *in vitro* assays, ECE and ECAgNPs showed IC50 values of $70.87 \pm 2.99\,\mu g/mL$ and $138.8 \pm 3.98\,\mu g/mL$, respectively against MDA-MB-231 cells compared to paclitaxel, which showed an IC50 value of 80 ng/mL.

Conclusion: The results showed that the LD50 of the ECE and ECAgNPs in Wister rats was determined to be greater than 2000 mg/kg body weight. The aqueous extract also showed more cytotoxic than the ECAgNPs suggesting that the toxic compounds in aqueous extract were involved in the capping of the AgNPs.[4,7,73,84–87,89–95]

Metal silver induced up to 100% L5178Y-R cells cytotoxicity, with an LC50 of $1.8 \times 10^{-8}$ M, whereas AgNPs caused up to 78% cytotoxicity, with an LC50 of $14.4 \times 10^{-8}$ M. In addition, intramuscular administration of metal silver and AgNPs administered at the time of tumor injection significantly ($P = 0.05$) increased mice survival, where 70% and 60% of mice survived at day 35, respectively, as compared with such treatments administered 7 days after tumor induction (55% and 25% survival, respectively); vincristine treatment caused 50% mice survival and tumor-bearing control mice had 20% survival. These results open further approaches on treating several types of cancer using free and nanoparticle-encapsulated silver-based therapies.[96]

### 2.2.3. *Anti-cancer activity of silver nanoparticles in clinical trials*

Advance in nanotechnology has enabled us to utilize particles in the size of the nanoscale. This has created new therapeutic horizons and in the case of silver, the currently available data only reveals the surface of the potential benefits and the wide range of applications. We have yet to elucidate the exact cellular pathway of AgNPs. Furthermore, it remains to be seen whether any potential complications for the AgNPs would surface after prolonged clinical use. Nonetheless, a bright future holds for this precious metal.[97]

Nanomedicine, defined as the application of nanotechnology in the medical field, has the potential to significantly change the course of diagnostics and treatment of life-threatening diseases, such as cancer. In comparison with traditional cancer diagnostics and therapy, cancer nanomedicine provides sensitive cancer detection and/or enhances treatment efficacy with significantly minimized adverse effects associated with standard therapeutics. Cancer nanomedicine has been increasingly applied in areas including nanodrug delivery systems, nano pharmaceuticals and nanoanalytical contrast reagents in laboratory and animal model research. In recent years, the successful introduction of several novel nanomedicine products into clinical trials and even onto the commercial market has shown successful outcomes of fundamental research into clinics. This paper is intended to examine several nanomedicines for cancer therapeutics and/or diagnostics-related applications, to analyze the trend of nanomedicine development, future opportunities and challenges of this fast-growing area.[9,17]

Over the last few decades, the development of wide range of NPs with the ability to tune size, composition and functionality, has provided an excellent resource for nanomedicine. Inorganic NPs provide a great opportunity as drug carriers, due to the easy modification of targeting molecules, the control of drug release by different stimuli and the effective delivery to target sites, thus resulting in having an improved therapeutic efficacy and in reducing side effects. Inorganic NPs are investigated in preclinical and clinical studies for the detection, diagnosis and treatment of many diseases. The stability of inorganic NPs offers a potential advantage over the traditional delivery methods. Inorganic NPs could enhance and improve current imaging and diagnostic techniques, such as MRI or PET. Even though, they have not yet been approved for drug delivery applications, their ability to respond to external stimuli is now widely investigated in clinic. Conclusion: The successful translation of inorganic NPs to the clinic requires the development of a simple, safe, cost-effective, eco-friendly mode of synthesis and a better understanding of the safety mechanisms, biodistribution and the pharmacokinetics of NPs. However, more attention should be given to concerns on long-term toxicity, carcinogenesis, immunogenicity, inflammation and tissue damage. Although, some inorganic NPs, which were apparently promising in the preclinical phase were found not to be successful when translated to the clinic, several encouraging NPs are currently being developed for treatment and cancer care and for a wide variety of other diseases.[17]

### 2.3. *Toxicological profile of Ag nanoparticle*

In recent years, there has been escalating interest in the biomedical applications of NPs. In particular, AgNPs are increasingly being investigated as tools for novel cancer therapeutics, capitalizing on their unique properties to enhance potential therapeutic efficacy. However, questions such as whether we are able to contain or control the toxicity effects of AgNPs and how much do we know about the toxicological profile of AgNPs which are commonly used in emerging nanotechnology-based applications, still remain. Hence, serious considerations have to be given to the hazards and risks of toxicity associated with the use of AgNPs.[98]

Studies also illustrated that NPs can enter organisms during ingestion or inhalation and can translocate within the body to various organs and tissues where the NPs have the possibility to exert the reactivity being toxicology effects.

The uses of AgNPs in numerous consumer products lead them to their release to the aquatic environment and become a source of dissolved Ag and thus exert toxic effects on aquatic organisms including bacteria, algae, fish and daphnia.[99,100] The respiratory system represents a unique target for the potential toxicity of NPs due to the fact that in addition to being the portal of entry for inhaled particles, it also receives the entire cardiac output.[101] NPs are used in bio-applications widely but despite the rapid progress and early acceptance of nanobiotechnology, the potential for adverse health effects due to prolong exposure at various concentrations levels in human in the environment has not yet been established.

One of the NPs toxicity is the ability to organize around the protein concentration that depends on particles size, curvature, shape and surface characteristics charge, functionalized groups and free energy. Due to this binding, some particles generate adverse biological outcomes through protein unfolding, fibrillation, thiol crosslinking and loss of enzymatic activity. Another paradigm is the release of toxic ions when the thermodynamic properties of materials favor particles dissolution in a suspending medium or biological environment. NPs tend to aggregate in hard water and seawater and are greatly influenced by the specific type of organic matter or other natural particles (colloids) present in fresh water. The state of dispersion will alter the ecotoxicity, but many abiotic factors that influence this, such as pH, salinity and the presence of organic matters remain to be systematically investigated as part of ecotoxicological studies.[102]

There are several possible ways in which patients can be exposed to AgNPs, such as dermal contact, oral administration, inhalation and blood circulation. Macrophages are the first cells that AgNPs will encounter in the human body.[103] It is known that the size of the AgNP dictates its mode of cytotoxicity to murine macrophages (Ag+ ion-specific and/or particle-specific). The toxicity of AgNPs ($< 10$ nm) is mostly mediated by the released $Ag^+$ ions, with liver being the major target organ, followed by spleen, lungs and kidney. One study showed that the effect of both 20 nm and 100 nm AgNPs on Wistar-derived WU rats treated at 6 mg/kg body weight doses was an increase in spleen weight; moreover, the clinical chemistry parameters also indicated liver damage.[104] A separate study on the inhalation toxicity of AgNPs showed that AgNPs had an influence on the neutral mucins in the respiratory mucosa of Sprague–Dawley (SD) rats exposed to AgNPs at concentrations of 0.5–61 $\mu g/m^3$, yet without toxicological significance.[103]

### 2.4. *Mechanism of action of AgNPs in cancer cells*

Although the mechanism of antitumor action of AgNPs are not properly understood, three proposed mechanisms has been reported. The first mechanism reports that the AgNP sinduces loss of survival of cancerous cell, may be due to ROS generation which leads to apoptotic morphological changes, DNA fragmentation and oxidative stress resulting in apoptosis.[88,105] The second mechanism depends on interference, proper functioning of proteins which results in changes in cellular chemistry.[106] Silver nanoparticles are likely to interact with thiol rich enzymes,[107,108] which may result in partial unfolding of proteins. AgNPs provide a relative hydrophobicity inside bovine hemoglobin, which results in a transition to the alpha helix to beta sheets which also leads to partial unfolding and aggregation of proteins.[109] These changes in protein may lead to cytoxicity. In the third proposed mechanism, AgNPs treatment makes changes in cell permeability which leads to entry of Ca ions there by activation of enzymes like protease and endonuclease which results in mitochondrial membrane dysfunction and ROS generation, subsequent oxidative

stress, DNA damage, errors in chromosome segregation and production of micronuclei leads to cell death.[110]

The working principles and mechanism of action of AgNPs are vital and different in each and every cell type. Induction of cytotoxicity and genotoxicity by AgNPs depends on many factors such as size, shape, surface charge, surface coating, solubility, concentration, media, surface functionalization, distribution into the cells, mode of entry and cell type. Recently, Duran and co-workers extensively reviewed the toxicity of AgNPs based on protein coronas.[111] The biological activities of AgNPs depend on proteins present in the cell culture media: the presence of a protein corona contributes to toxicity and facilitates interactions between AgNPs and cells to induce or mitigate toxicity.[111] After introduction of AgNPs to cells, the initial events are entry into and distribution within the cells, a critical event for determining toxicity. AgNPs penetrated into the cells through several cellular compartments including endosomes, lysosomes and mitochondria. The probable mechanisms of NPs uptake by cells include pinocytosis-, caveolae- and clathrin-dependent mediated endocytosis and phagocytosis.[32]

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