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Anticancer activity of plant mediated silver nanoparticles on selected cancer cell lines

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ABSTRACT

Cancer is a lethal disease or a destructive phenomenon arises in human beings because of genetic factors or exposure to toxic chemicals or viral infections etc. Radio therapy is very promising in the treatment of cancer compared to other treatments. However normal cells are also destroyed along with tumor cells in this treatment. Application of nanomedicine in the treatment of cancer is a novel approach. From research findings it can be known that MTT assay is generally used and widely accepted method in screening invitro anticancer activity of metal naoparticles. In addition biosynthesized silver nano particles(AgNPs) have exhibited remarkable activity against cancer cell lines in MTT assay. Green synthesized silver nanoparticles inhibit the proliferation of cancer cells by activating the apoptosis process or changing the cellular chemistry. Biosynthesized AgNPs may also block the tumor growth by damaging the function of mitochondria or generaration of ROS. Plants are the natural treasures of secondary metabolites. In the green synthesis these phytochemicals acts as reducing and stabilizing agents of silver nanoparticles. The plant mediated AgNPs have shown dose and time dependant antitumor activity on cancer cells. The present study deals with the nature of different plants used in the synthesis of silver nanoparticles. The review mainly focussed on invitro cytotoxic activity of plant mediated AgNPs on selected cancer cell lines.

Key words: AgNPs, Green synthesis, MTT assay, Cell line, Cytotoxicity.

INTRODUCTION

Cancer is a complicated health issue in the present world and a complete cure from this deadly disease is an expensive and much time taking issue. Scientists and budding researchers from biotechnology and clinical field are working hard to restrict or suppress the growth of cancer in humans. The disease mainly arises in human beings because of genetic factors, life style factors such as chewing and smoking tobacco, certain types of infections and exposure to different types of chemicals and radiation [1]. Whatever may be the reason the cancer patients develop malignant tumours from uncontrolled division and growth of cells and invade to near parts of the body. Cancers are named based on their origin, such as leukaemia, breast cancer, thyroid cancer and colorectal cancer etc [2]. There are different types of cancer treatments available such as surgery, chemotherapy, radiation therapy and immune therapy etc. Among all, the treatment of cancer by radiation therapy is widely accepted. Radiation therapy is given alone to a patient after the surgery or in combination with chemotherapy and other treatments. The main drawback of radiation therapy is normal cell death also occurs in addition to cancer cell death [3]. In 2012 the disease caused most number of deaths worldwide accounting for 8.2millions and in lower-income-countries 5.3 million deaths occurred a year. According to the national cancer registry program of the Indian council of medical research (ICMR), Cancer emerged as the biggest cause of death in India i.e. 5 lakhs every year [4].

Natural forests are treasure houses of phytochemicals and never loss their identity. According to the World Health Organization about 80% of the world's population relies on traditional medicinal system for some aspect of primary health care [5]. There were no synthetic medicines three hundred years ago and plants were the only the main source of medicine for the world [6]. Based on the folk remedies and subsequent ethnopharmacological studies today many drugs are available in the market. About 100 drugs of known structure used in allopathic medicine were extracted from higher plants [7]. For example notable and 100 years old drugs like morphine, digitalis and atropine are most wanted in the market till now. In the present scenario sceince has developed and the nutrients and chemicals present in the plant extracts are easily treasure troved in scientific testing. Plant extracts are used in the manufacture of some cosmotics, shampoops, soaps, food flavoring agents and medicines [8]. The secondary metabolites like alkaloids, glycosides, phenols, terpenoids and volatiles oils present in the plant extracts are used in active drugs [9]. In the present decade there is a considerable scientific and commercial interest in the discovery of new anticancer agents from natural resources. Over 50% of all drugs in clinical trials for anticancer activity were from natural sources [10]. Taking in to consideration that plants are the natural factories for the biologically active metabolites, the National Cancer Institute collected about 35,000 plant samples from different countries and screened for anticancer activity. Different extracts of 3000 plants has shown anticancer activity. Vincristine and Vinblastine from *Catharanthus roseus*, Taxol and Docetaxel from *Taxus brevifolia* and Camptothecins from *Camptotheca acuminate* are the best potential anticancerous agents derived from plants [11].

The present review mainly focused on the anticancer activity of silver nanoparticles(AgNPs) synthesized by using different plant varieties in the lab condition i.e. invitro. Nanobiotechnology deals with the synthesis of nanoparticles and their application in various feilds. Biological synthesis of metal nanoparticles is one of the area of nanotechnology. However the biological synthesis of metal nanoparticles by using plant extracts is called green synthesis which is ecofriendly in nature. It reqiures less time, less technology, cost effective and causes no health hazards when compared to physical and chemical synthesis of metal nanoparticles [12]. Green synthesis with plants is a favorable techniques than other biological methods i.e. synthesis of nanoparticles with microbial extracts requires complicated procedures in bacterial and fungal culture maintinance [13]. Among green synthesized metal nanoparticles AgNPs are the most widely used in science because of its antimicrobial activity. In pure form silver is highly toxic to microbes and when converted in to nanolevel its activity increases several times [14]. Another reason of accepting the AgNPs through out the world is that silver is not toxic to humans and when tested for curing of various diseases it lefts no allergic reactions [15]. The articles contains the nature of different plants used in the synthesis of AgNPs. The main aim of this review is to bring awareness about green synthesized AgNPs and their cytotoxic activity on selected cancer cells invitro.

***In Vitro* Anticancer Activity of AgNPs**

The essential goal of research scientists working on cancer treatment is to discover and identify new anticancer drugs with low side effects on the immune system of the patients. Taking above point into consideration natural compounds with anticancer activity have been explored from plants, bacteria, fungi and algae etc. Invitro cytotoxicity testing is a cost effective process reduces the use of laboratory animals. The most accepted method of cytotoxicity on cancer cells lines invitro is MTT assay [16]. It is a colorimetric method that measures the reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase of viable cells. The procedure involves the following steps. The cultured tumor cell lines in the laboratory are seeded at a density of 4×10^4 cells/ml in a volume of 0.1ml per well in 96-well plates respectively. After 24hrs, AgNPs to be tested (50-250mg/ml) which were dissolved in a medium are added to each well and incubated for 48hrs at 37^0C in a CO_2 incubator. The inhibition rate of AgNPs on cancer cells depends on the dose, time and also the plant extract used in the AgNPs synthesis. MTT solution (100 µl/well, 1 mg/ml) is added to each well after incubation and again incubated for 4 hours. After removing culture media 100 µl of DMSO is added to each well and incubated for 1hour. Here the viable cells reduces the yellow MTT in to purple formazan crystals by using mitochondrial succinate dehydrogenase. After that the absorbance was detected by microplate ELISA reader at 570nm. The inhibition ratio of cancer cell proliferation by AgNPs was determined as follows:

$$\text{Inhibition rate (\%) of AgNPs on Cancer cells} = \frac{\text{1-Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Application of nanomaterials in the control of cancer is a novel approach and previous reports have demonstrated that nanoparticles eradicate cancer cells by flow and penetration to different regions of tumors through blood vessels and then to interstitial space to arrive at the target cells. The environmental and physiological characteristics vary from one tumor tissue to another. Hence nanoparticles should be designed in such a manner, taking in to account the target site and route of administration to generate optimal therapeutic effects [17]. Silver nanoparticles inhibit the proliferation of the cancer cell lines mainly by different mode of actions. Apoptosis is a highly regulated and efficient cell death programme consists the interplay of many factors. Silver nanoparticles mediates and amplify the

death signal by triggering the activation of Caspase-3 molecule. The IC_{50} values would determine the level of toxicity to cells. The DNA is broken in to fragments by the action of Caspase-3 [18]. AgNPs may interfere with the proper functioning of cellular proteins and induce subsequent changes in cellular chemistry [19]. Some times AgNPs alter the function of mitochondria by inhibiting the catalytic activity of Lactate dehydrogenase [20]. AgNPs may also encounter the cancer cell proliferation by generating reactive oxygen species (ROS) which ultimately leads to DNA damage [21]. Moreover the green synthesized metal nanoparticles lefts less or no side effects on the patients. In 2013 a research group synthesized silver nanoparticles using the aqueous extract of *Origanum vulgare* by reducing 1mM silver nitrate solution. It is a perennial herb and belongs to the mint family Lamiaceae. The AgNPs of the plant were characterized by different techniques i.e. UV-Vis spectroscopy, Fourier infrared spectroscopy (FTIR), Scanning electron microscopy, X-ray diffraction (XRD) and Dynamic light scattering. The synthesized nanoparticles were spherical in shape with an average particle size distribution of 136 ± 10.09 nm. In the MTT assay the plant mediated AgNPs have shown dose dependant antiproliferative activity on human lung cancer A549 cell line [22]. *Heliotropium indicum* is an annual, branched and erect plant belongs to the family Boraginaceae. In english language the plant commonly called Indian heliotrope and grows in waste lands. The silver nanoparticles synthesized by using the powder of the plant *H. indicum* were studied for anticancer activity invitro against HeLa cancerous cells. The average size of synthesized AgNPs was 80 to 90 nm. The cervical cancer cells taken from Henrietta Lacks are called HeLa cells and cultured invitro. In the MTT assay the cultured HeLa cells obtained from National Centre for cell science, Pune were used and the antitumor activity was screened for 48hrs. The plant mediated silver nanoparticles inhibited the growth of cancer cells and cytotoxic activity was dose and time dependant with an IC_{50} of 20μg/ml and 40μg/ml for 24hrs and 48hrs respectively [23].

Silver nanoparticles gained much popularity and have a significant role in the pharmaceutical sceince. The AgNPs synthesized by some selected plants has shown potential biological activities especially anticancer activity. *Cassia fistula* belongs to the family Fabaceae and commonly called as golden shower tree. The south asian native plant is national tree to Thailand. The AgNPs of the plant were characterized by UV-Visible, FTIR, SEM, EDX and AFM. The AgNPs were irregular in shape and the size is in the range of 30-50nm. Invitro cytotoxic activity of AgNPs was studied on MCF 7 human breast cancer cell line. The plant mediated AgNPs has shown dose dependant cytotoxicity on MCF 7 cell line. The anticancer activity of the AgNPs is mainly due to the physicochemical interaction of silver atoms with the intracellular proteins and also with the nitrogen and phosphate groups in the DNA [24]. Silver nanoparticles synthesized by plants has shown potential anticancer activity againt KB cell line also. It is a high level multi drug resistant subline of the ubiquitous Keratin-forming tumor cell line HeLa. *Melia dubia* is a fast growing tree of the family Meliaceae and the wood of the plant is widely used in plywood industry provides excellent income. The AgNPs of *M. dubia* were test against human breast cancer KB cell line. The biological assay provided remarkable results with high therapeutic index value [25]. It is necessary for humans to search for a cost effective drug which can save the life of the cancer patients. Nanovesicles as novel drugs can solve this problem in near future. Here there is another finding of AgNPs against cancer cell lines with promising results in addition to enhanced antimicrobial activity. *Olax scandens* is a plant belongs to the family Olacaceae. The leaf extract mediated AgNPs of the plant were tested against human lung cancer cell line (A549), mouse melanoma cell line (B16) and human breast cancer cell line (MCF7). The green synthesized AgNPs has shown potential anticancer activity against all the cancer cells tested. Another advantage in this assay is the bright red flouresence emitted by AgNPs of *Olax scandens* is used to detect localization of them inside the cancer cells [26].

Nature is a good friend of human beings and has given wonderful gifts in the form of plants and their products to cure any healthy issue. A continous and dedicated research attemps on plants against pathogens or cancer cells invitro could explore the secrets in the nature. *Jatropa curcas* is a plant native to american tropics and belongs to the family Euphorbiaceae. This flowering and poisonous plant is cultivated in tropical and subtropical regions all over the world. The seeds of the plant is used in the production of high quality biodiesel. *Jatropa gossypifolia* is a flowering plant of Euphorbiaceae family and commonly called cotton leaf Physicnut in english. In a recent investigation the silver nanoaprticles synthesized by using the stem extract of *J. curcas* and *J. gossypifolia* were studied against human lung cancer cells(A549). The synthesized AgNPs were characterized by UV-Visible spectroscopy, Scanning electron microscope and Fourier transform infrared spectroscopy. The proliferation of cancer cell line was significantly inhibited by AgNPs of *J. curcas* and *J. gossypifolia* in a dose dependant manner with an IC_{50} value of 19.5*μg/ml* and 13.5*μg/ml* respectively [27]. Another research team worked on the anticancer activity of *Rosa damascena* silver nanoparticles. It is a decidous shrub of family Rosaceae and commonly called Damask rose. The size of the AgNPs were analysed in dynamic light scattering and was in the range of 74 to 94 nm. The AgNPs of *R. damascena* in MTT assay invitro on human lung adeno carcinoma have shown potential antiproliferative activity with an IC_{50} value of 80 μg/mL [28]. In the search of novel anticancer drug, some researchers worked on the cytotoxicity of *Tragia involucrata* leaf extract mediated AgNPs against MOLT-4 cancer cell line. The plant *T. involucrate* is a slender and twining herb and belongs to the family Euphorbiaceae. The assay revealed the potentiality of biosynthesized silver nanoparticles as novel cancer agents [29]. Research on silver

nanoparticles increasing day by day and especially biotechnologists working hard in support of cancer patients to find a cost effective anticer agents. *Ocimum kilimandscharicum* is commonly called african blue basil and belongs to the family Lamiaceae. Recent research reveals that the AgNPs synthesized by ethanolic stem extract of *O. kilimandscharicum* were highly stable and has shown remarkable cytotoxicity on Hep G2, liver cancer cell line with an IC50 value of 49 µg/ml [30].

Till today nanobiotechnology created wonders in the field of medicine and agriculture. However from the research records it can be known that the cytotoxicity of silver nanoparticles on cancer cells is unique and significant among metal nanoparticles. *Abutilon indicum* L. is a small medicinal and ornamental shrub of the Malvaceae family. It grows generally in tropical, subtropical and warmer climates of the world. The leaf extract based nanoparticles were spherical with an average size of 70 to 95nm. In a biological assay the AgNPs of *A. indicum* L. were exposed to human breast cancer cell line MCF-7. The nanoparticles caused cell death and the degree of cell mortality was in proportional to their concentration [31]. The phytochemicals alkaloids and flavanoids etc present in the plant extract were mainly responsible for the stability and cytotoxicity of AgNPs. Highly stable and biologically potent AgNPs were formed from the plants having more amounts of secondary metabolites. *Andrographis paniculata* belongs to the family Acanthaceae and contains flavanoids, chalcones, diterpiniods and flavanone glycocides etc. The plant is generally used in traditional medicine as anti-inflammatory, anti hepatic, anti thrombogenic and anti snake venom agent. Considering its phytochemicals AgNPs were prepared and examined for their anticancer activity against HeLa(Human cervical cancer cells) and Hep-2(Human liver cancer cells) in MTT assay. The silver nanovesicles have shown excellent activity against the selected cell lines but further research is required to use these AgNPs in the invivo conditions [32]. Each plant has its own significant feature and here the extracts of *Chrysophyllum oliviforme* were used in the synthesis of AgNPs and investigated for antitumor activity against human cervical cancer cell line. The tree belongs to the family Sapotaceae and commonly called satin leaf. The highly stable silver nanoparticles have shown anticancer activity on HeLa cells with IC50 value of 40.365µg/ml [33]. According to research literature more findings on anticancer activity of green synthesized AgNPs were screened in the year 2015. Supraja S and Arumugam P (2015) studied the anticancer activity of *Cynodon dactylon*(Apocyanaceae) leaf extract mediated silver nanoparticles on human hepato carcinoma cell line (HEpG-2) and gained positive results [34]. *Rosa indica* is perennial flowering plant and belongs to the family Rosaceae. The AgNPs synthesized from the ethanolic extract of *R. indica* petals were tested for anticancer activity on human colon adenocarcinoma cell line HCT15 and the results were satisfactory [35].

Our earth contains vast number of plants and still there are many plants and their varieties to be explored having pharmaceutical value. For the sustainable development of human race the pharmaceutical value of each plant variety and their parts must be screened. Here Krishnan V.G et al (2015) conducted a bioassay on human larynx carcinoma Hep-2 cell line using the AgNPs of *Elettaria cardamomum*(Zinziberaceae) seed. In another invitro study *Momordica charantia*(Cucurbitaceae) aqueous leaf extract mediated AgNPs were used as anticancer agents on human breast cancer cell line(MCF-7). The AgNPs of the two plants discussed above have shown significant dose and time dependant anticancer activity on respective selected cell lines [36, 37]. *Erythrina indica* is a flowering plant of the family Fabaceae. The biosynthesized AgNPs of *E. indica* were screened for antitumor activity on breast and lung cancer cell lines. The spherical shaped AgNPs with 20-118nm have shown excellent activity against selected cell lines [38]. The application of these AgNPs on human patients in invivo conditions is very complicated issue. It requires extensive research on route and dose of administration. The side effects caused should be studied to each and every plant based AgNPs. *Tylophora indica* Merr is a climbing plant and belongs to the family Apocyanaceae. The perennial vine is a native to tropical and subtropical regions of Asia, Africa and Australia. The silver nanoparticles of aqueous leaf extract of the plant were active against the breast cancer cell line (MCF-7) invitro [39]. In an another bioassay the AgNPs of *Kalanchoe pinnata*(Crassulaceae) and *Synadenium grantii*(Euphorbiaceae) have shown potent anticancer activity on HeLa cell line but the morphology of effected cells, viable cell number and IC50 values are different [40]. Caffiec acid is polyphenol found naturally in the bark of *Eucalyptus globulus*(Myrtaceae). The AgNPs synthesized using caffiec acid were screened anticancer activity against the human hepatoma $HepG_2$ cells invitro. The silver nanoparticles suppressed the proliferation of cancer cell line by inducting apoptosis after entering in to cells and the activity is dose dependant [41]. *Andrographis echioides* is a native flowering plant of asia and belongs to the family Acanthaceae. In an experimental study silver nanoparticles were synthesized using the leaf extract of the plant. The nanoparticles were well characterised and the size was found to be in the range of 68.06 to 91.28nm. The AgNPs of *A. echioides* have shown significant activity against the human breast cancer cell line MCF-7 [42]. Silver nanoparticles were synthesized using extracts of *Cucurbita maxima*(Petals), *Moringa oleifera*(Leaves) and *Acorus calamus*(Rhizome) and screened antiproliferative activity in MTT assay on skin cancer cell line A431. These plants belongs to Cucurbitaceae, Moringaceae and Acoraceae respectively. The AgNPs of the plants listed has shown potential anticancer activity and the IC_{50} values of 82.39, 83.57 and 78.58 were recorded respectively for *C.maxima*, *M.oleifera* and *A.calamus* [43]. *Solanum trilobatum* is a herb and belongs to the family Solanaceae. The biologically synthesized silver nanoparticles from the fruit extract of

S. trilobatum were screened for anticancer activity against human breast cancer cell line(MCF-7) using MTT assay. The AgNPs have shown dose dependant antitumor activity on selected cell line [44].

Presently research on synthesis and screening anticancer activity of plant mediated silver nanoparticles is increasing day by day. However scientists and researchers should produce more accurate and promising results which can save the life of human beings in cost effective manner. There are some more recent research findings on AgNPs and their significant anticancer activity on selcted cell lines. The AgNPs of *Pentatropis capensis* (Asclepiadaceae) in the MTT assay on Hep-2 cell line has shown more antitumor activity to lower doses than higher doses. The nanoparticles were spherical in shape and the size was in the range of 76.38-88.13nm [45]. The results discussed above clearly indicates that these AgNPs are suitable nanomedicine in the treatment of cancer. In an another finding the AgNPs synthesized from the aqueous flower extract of *Lonicera hypoglauca*(Caprifoliaceae) have exhibited significant antiproliferative activity on MCF-7 cells [46]. The AgNPs of *Carica papaya*(Caricaceae) latex extract have shown remarkable dose dependant anticancer effect with an IC_{50} value 19.88 µg/ml on human breast carcinoma MCF-7 cell line in MTT assay. High resolution scanning electron microscope reveals that the AgNPs were spherical with an average size of 6-18nm. [47]. The AgNPs synthesized using the aqueous extract of *Clerodendrum phlomidis*(Lamiaceae) exhibited remarkable antitumor effect on Ehrlich ascites carcinoma (EAC) and human colorectal adenocarcinoma (HT29) cells with 91.84% and 84.91% inhibition, respectively in MTT assay [48]. Recently another research team synthesized silver nanoparticles from the bark extract of *Ficus benghalensis*(Moraceae) and *Azadirachta indica*(Meliaceae) and screened their antiproliferative activity on osteosarcoma cancer cell line. The AgNPs of both the plants have shown remarkable dose dependant activity against the selected cancer cell line [49].

CONCLUSION

Nanobiotechnology is an inter desciplinary science and flourishing rapidly in the field of medicine and agriculture. It deals with the synthesis and application of nanoparticles in various fields. According to earlier records metal nanoparticles especially silver nanoparticles(AgNPs) exhibited significant antimicrobial and pesticidal activity. In addition AgNPs have also shown to exhibit antitumor activity invitro. When screened in MTT assay the AgNPs synthesized from plants like *Andrographis paniculata, Cassia fistula* and *Olax scandens, Pentatropis capensis* have shown remarkable cytotoxicity on selected cancer cell lines. Most interesting feature is that plant mediated AgNPs can also suppress the proliferation of multi drug resistant human breast cancer cell line KB. AgNPs synthesized from *Melia dubia* supressed the growth of KB cell line invitro with high therapeutic value index. From the research records discussed above it could be known that the AgNPs of most of the plants were dose and time dependant potential antiproliferative agents on cancer cell lines. However extensive research is required in application of these AgNPs invivo regarding the dose and route of administration as well as side effects arise in patients.

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