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Cytotoxic Potential of Biosynthesized Silver Nanoparticles Using *Cucumis melo* (L*).* Fruit

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Abstract

Biosynthesized nanoparticles are gaining attention because of biologically active plant secondary metabolites that help in green synthesis and also due to their unique biological applications. The present study was designed to investigate the cytotoxic activity of the Cucumis melo (L) fruit against HepG2 cell lines. Biosynthesis of silver nanoparticles using the aqueous extract of Cucumis melo fruit (SNPs-AECMF) was prepared and it was confirmed and characterized through various techniques like UV-visible spectroscopy, Scanning Electron Microscopy (SEM), Energy dispersive x-ray spectroscopy (EDAX) and X-ray diffraction (XRD) analysis. The cytotoxic activity for cancer cell lines was evaluated by MTT assay. The in vitro cytotoxicity of different concentrations (3.25 - 50µg/mL) of the silver nanoparticles of Cucumis melo fruit was evaluated by the MTT assay. The IC50 value is measured by the concentration of extract causing 50% growth inhibition of cancer cells. The results indicated that the cytotoxic effect of the silver nanoparticles of Cucumis melo fruit against HepG2 cells is dose dependent. At low concentrations the extract was found to be less toxic towards the HepG2 cells, whereas at higher concentrations the toxicity was increased. The concentration at 42.59 µg / ml was found to be an effective dose because at this concentration, it exhibited 50 % cytotoxicity against HepG2 cells. This work revealed the potentials of silver nanoparticles of Cucumis melo fruit as a cytotoxic agent against liver cancer cell lines. The plant can be further screened against various diseases using toxicity models in order to find out its unexplored efficacy.

Introduction

Cytotoxicity is the quality of being toxic to cells and *in vitro* cytotoxicity testing procedures reduces the use of laboratory animals and hence the use of cultured tissues and cells have been increased[1]. In order to study the anticancer activity of a new drug, it is important to determine the cytotoxic concentration of the drug. Among the various possible experimental models of cancer, human cancer cell lines have been the most widely used. They have retained the hallmarks of cancer cells, namely uncontrolled growth, insensitivity to antigrowth

signals, escape from checkpoints (apoptosis), genetic instability and invasiveness. *In vitro* cytotoxicity test is mainly performed to screen upper limit of the extract concentration that affect basic cellular functions[2].

Green synthesis is an alternative harmless and environment-friendly method for producing nanoparticles[3].Silver is the metal of choice in the field of biological system, living organisms and medicine. The role of silver nanoparticles as an anti-cancer agent should open new doors in the field of medicine[4]. The biomedical applications of silver nanoparticles are promising with their tremendous effects in the fields of medicine, drug delivery and anti-angiogenic property of cancer[5]. Human hepatoma-derived cell line HepG2 was employed as an *in vitro* model, as the liver is a major target organ of AgNPs and HepG2 is the cell line that is most widely used in evaluating the *in vitro* toxicity of AgNPs among all the liver cell lines[6]. Silver nanoparticles serve as antitumor agents by decreasing progressive development of tumor cells. AgNPs can induce cytotoxic effects on cancer cells, inhibiting tumor progression and thereby effectively controlling disease progression without toxicity to normal cells[7].

Traditionally, *Cucumis melo* fruit is used for treatment of painful discharges, dysuria, cooling agent, moisturizer for the skin. Three components found in melons are cucurbitacin-β, lithium and zinc, which exhibit promise in cancer prevention, fighting depression, dandruff, and ulcers and stimulates the immune system[8]. Pharmacological studies conducted on *Cucumis melo* indicate its immense potential in the treatment of conditions such as pain, inflammation, cardiovascular disorders, liver diseases, cancer, coughs, and dysuria[9]. Being a nutritional fruit and having many health benefits in traditional medicine, since documental evidence is not available on biosynthesis of silver nanoparticles of *Cucumis melo* fruit and its activities. Based on the preliminary studies conducted in our laboratory, the method of synthesis of silver nanoparticles was standardized using the aqueous extract of *Cucumis melo* fruit. The synthesized silver nanoparticles were also confirmed by the various characterization techniques. Therefore, the present study deals with the cytotoxic effect of silver nanoparticles synthesis from *Cucumis melo* fruit.

Materials and Methods

Collection and identification of plant material: *Cucumis melo* (L.) (Family - Cucurbitaceae) fruits were collected from the local markets of Coimbatore district, Tamilnadu, India. The specimen sample was identified and authenticated by Dr. M. Palanisamy, Scientist-C, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamilnadu, India. The identification No. BSI/SRC/5/23/2014-15/Tech/482.

Synthesis of silver nanoparticles using *Cucumis melo* fruit: The 10 g of the *Cucumis melo* fruit powder was weighed and mixed in 100 ml of distilled water and the mixture was boiled in heating mantle at 60ºC for 10 to 20 minutes. The extract was cooled to room temperature, filtered through Whatman No.1 and used for further analysis. Aqueous solution of 1mM $AgNO_3$ was prepared and used for the synthesis of silver nanoparticles. 5 ml of aqueous extract of *Cucumis melo* was mixed to 95 ml of $AgNO_3$ solution and incubated at room temperature in dark condition for 24 hours for the synthesis of silver nanoparticles. The yellow coloured solution was changed into reddish brown after 3 hours under room temperature. The appearance of reddish brown colour after 3 hours indicates the formation of silver nanoparticles. The synthesis of silver nanoparticles was characterized and confirmed by UV-Visible Spectrophotometer, SEM, EDAX, PSA and XRD analysis.

Cytotoxic effect of silver naoparticles of aqueous extract of *Cucumis melo* fruit (SNPs-AECMF) on HepG2 cell lines[10,11]

Cell lines and culture: The human liver cancer

cell line (HepG2) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10 % fetal bovine serum (FBS). All cells were maintained at 370 C, 5 % CO2, 95 % air and 100 % relative humidity.

Cell treatment procedure: The monolayer cells (HepG2 cells) were detached with trypsin-ethylene diaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted by tryphan blue exclusion assay using a hemocytometer. The cell suspension was diluted with medium containing 5 % FBS to give a final density of 1x105 cells / ml. 100 micro litres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells / well and incubated to allow for cell attachment at 370C, 5 % CO2, 95 % air and 100 % relative humidity. After 24 hours the cells were treated with serial concentrations of the test samples. They were initially dispersed in phosphate buffered saline (PBS) and diluted to twice the desired final maximum test concentration with serum free medium. Additional four, 2fold serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulted the needed final sample concentrations.

Following drug addition, the plates were incubated for an additional 48 hours at 370 C, 5 % CO2, 95 % air and 100 % relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations. After 48 hours of incubation, 15 µl of MTT (5mg / ml) in phosphate buffered saline (PBS) was added to each well and incubated at 370C for 4hours. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100 µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.

$$\% \text{ Cell Inhibition} = 100 - [\text{Abs (sample)} / \text{Abs (control)} \times 100].$$

Nonlinear regression graph was plotted between % Cell inhibition and Log concentration and IC50 was calculated using Graph Pad Prism software.

Results and Discussion

The HepG2 cell line used in the present study, is a reliable model that is well characterized and is widely used for biochemical and nutritional studies of many antioxidants and conditions[12]. MTT assay is an established method of determining viable cell number in proliferation and cytotoxicity studies for a variety of chemical compounds[13].

Cytotoxic activity by MTT assay: In the present study, the cytotoxic effect of silver nanoparticles of *Cucumis melo* fruit was tested against HepG2 cell lines by using MTT assay. The viability of the HepG2 cell lines was observed after 48 hours of treatment with silver nanoparticles of *Cucumis melo* fruit. Cells underwent concentration dependent cytotoxicity by AgNPs and a significant decrease in the cell viability was observed as inhibitory concentration (IC50 value). It shows reduced cell viability of HepG2 cell lines at different concentration (3.25, 6.25, 12.5, 25, 50 µg/ml) of silver nanoparticles of *Cucumis melo* fruit extract (Fig. 1). The results obtained from MTT assay after 48 hours of incubation showed that silver nanoparticles showed significant effect on Hep-G2 cells with IC50 values of 42.59 µg/ml. The reduced cell viability of HepG2 cells observed in this study is suggestive of the anticancer effects of SNPs-AECMF.

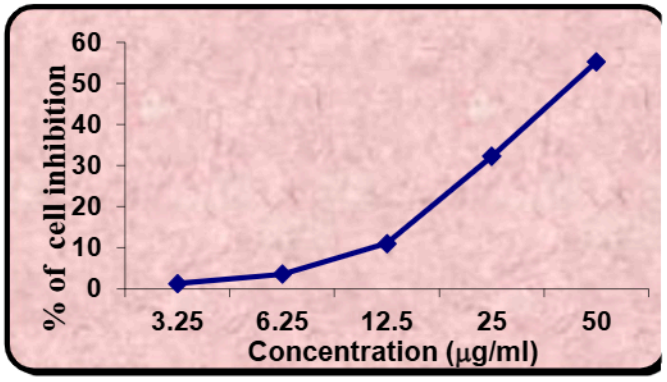

Fig.1: MTT assay of silver nanoparticles of aqueous extract of *Cucumis melo* fruit

The cytotoxic effects of silver are the result of active physicochemical interaction of silver atoms with the functional groups of intracellular proteins, as well as with the nitrogen bases and phosphate groups in DNA[14]. The antioxidant properties of AgNPs generated by the fruit extract, possibly due to the flavonoids and other phytochemicals capping on the AgNPs[15].

Fig.2 showed the decreased cell proliferation and increased cytotoxic activity of silver nanoparticles of *Cucumis melo* fruit extract. The nano size, surface area and surface functionalization are the chief factors that control bio - kinetics and thus cause toxicity leading to inhibition of cell proliferation. Cytotoxic effects on cancer cells can be induced by silver nanoparticles from fruit extract, which can inhibit the progression of cell proliferation[16].

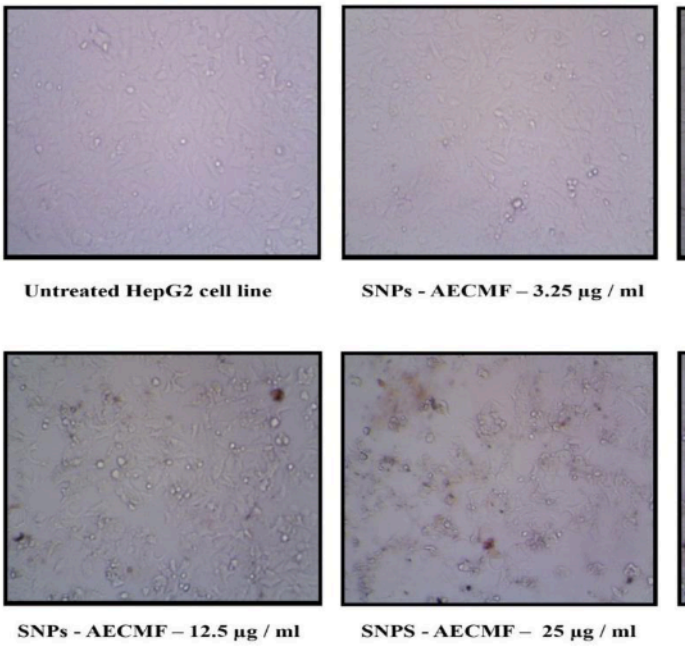

Fig.2: Cytotoxic activity of silver nanoparticles of aqueous extract of *Cucumis melo* fruit

on HepG2 cell lines

Similar results are obtained by Lokina *et al*., (2004) demonstrating that biologically synthesized silver nanoparticles from apple extract showed significant cytotoxic effects exerted against MCF-7 breast cancer cells[17]. Silver nanoparticles from aqueous extracts of red seaweed *Pterocladiella capillacea* exhibited effective cytotoxic activity against the human hepatocellular carcinoma (HepG2) cell line at higher concentrations[18].

Conclusion

The findings of the present study, thus, validate and strengthen the method of synthesis of silver nanoparticles from *Cucumis melo* fruit using an inexpensive and eco-friendly method. *In vitro* cytotoxicity study shows that silver nanoparticles from aqueous extract of *Cucumis melo* fruit is more cytotoxic for HepG2 cell lines, the reason might be the phytochemicals present in the *Cucumis melo* fruit extract are the capping materials of silver nanoparticles. The cytotoxic effect of AgNPs on HepG2 cells

suggests that AgNPs can play a major role in developing potential and useful nanomedicine against several cancer types. Hence silver nanoparticles of aqueous extract of *Cucumis melo* fruit (SNPs-AECMF) should be considered as an anticancer drug and it can be used for further *in vivo* studies.

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