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RESEARCH ARTICLE

Anticancer Activity of Cassia Oleoresin Mediated Selenium Nanoparticles Against Lung Cancer Cell Line

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ABSTRACT

Introduction: Selenium nanoparticles (SeNPs) have various advantages over different nanomaterials due to the promising role of selenium within the stabilization of the system and activation of the defense response. The utilization of SeNPs and their supplements solely have pharmacological significance. Cassia oleoresin helps to prevent nausea, prediabetes, diarrhoea, infections, loss of appetite, chest pain and kidney disorders.

Aim: Aim of the present study was to determine the anti-cancer activity of cassia oleoresin mediated selenium nanoparticles against lung cancer cell lines.

Materials and Methods: Plant extract, cassia oleoresin was obtained from Synthite industries pvt limited, Kerala. The cytotoxic effects of cassia oleoresin were assessed on A549 cells. Cells were treated with different concentrations of cassia oleoresin (10, 20, 40, 60, 80, 100µg/ml) for 24 hours and cell viability was evaluated by MTT assay.

Results: The cassia oleoresin caused a dose dependent increase in cytotoxicity in the A549 cell line. The maximum cytotoxic effect was noticed with the maximum dose used in the study (ie) 100µg/ml. Thus cassia oleoresin demonstrated good anticancer activity against lung cancer cell lines.

Conclusion: The study results depicted that the IC50 value was 15µg/ml almost half of the viable cells were destroyed. Thus it was evident that cassia oleoresin possesses good anticancer activity. However more research is needed to understand the mechanism of cytotoxicity of the extract .

KEYWORDS:
selenium nanoparticles ; cassia oleoresin ; A549 cells ; MTT assay ; cytotoxicity ,eco friendly, green synthesis

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INTRODUCTION

Nanoparticles play a major role in refining the compatibility and bio availability of natural products for the treatment of various chronic diseases and also cancer. Selenium nanoparticles (SeNPs) have various advantages over different nanomaterials due to the promising role of selenium within the stabilization of the system and activation of the defense response (1) . The utilization of SeNPs and their supplements solely have pharmacological significance. Also additionally boost and prepare the body's system to fight the pathogens. Selenium nanoparticles with distinctive physio chemical features have emerged as a novel nano carrier and therapeutic agent with broad-spectrum medicinal properties (1,2). In a study, cassia auriculata mediated selenium nanoparticles were synthesised and its anti-cancer potential was explored by its

anti-leukaemia activity using in vitro studies (3). Plants extracts are broadly utilized in the fabrication of the nanoparticles as compared to the microorganisms, as it may be correlated to the exclusion of the tedious cell culture and isolation method. Cassia oil is obtained by steam distillation of the leaves and twigs of Cinnamomum cassia Blume. Cassia oleoresin helps to prevent nausea, prediabetes, diarrhoea, infections, loss of appetite, chest pain, kidney disorders et cetera (4).

Cancer is one of the most common health problems responsible for a large number of deaths. The incidence of cancer in India among males was noted to be 679,421 (94.1 per 100,000) and among females 712,758 (103.6 per 100,000) for the year 2020. One in 68 males (lung cancer), 1 in 29 females (breast cancer) and 1 in 9 Indians will develop cancer during their lifetime (5). Thus nanomedicine plays a very crucial role in the treatment of cancer therapies (4,6) . Previous studies reported non-toxicity and biocompatibility of some nanoparticles in the normal cell line but high toxicity in cancer is a line. A study revealed that silver selenium bimetallic nanoparticles synthesized using gallic acid displayed potential anti-tumour activity against Dalton's lymphoma cells and also exhibited significant cytotoxic effects against MCF -7 breast cancer cells (7).

The biosynthesis of nanoparticles is an approach of synthesizing nanoparticles using microorganisms and plants having biomedical applications. The anticancer activity of nanoparticles is size dependent, the smaller the particle the greater the inhibition of cancer cell proliferation (8). The current therapeutics for cancer and other chronic illnesses have limitations that are associated with side-effects, drug resistance, solubility of nature etc. Our team has extensive knowledge and research experience that has translate into high quality publications (9–13), (14), (15), (16), (17), (18), (19),((11,20,21),(22–26),(27),(28). Thus it is important to conduct research on medicinal plant extracts and metallic nanoparticles for the advancement of anti-cancer drugs(25) (29) (30) (31) (32) (33) (34) (35) (36) (37) (38) (39) (40) (41) (42). Thus the aim of this study was to determine the anti-cancer activity of cassia oleoresin mediated selenium nanoparticles against lung cancer cell lines.

MATERIALS AND METHODS

Study setting

The study was carried out in the Cancer and Stem Cell Research Lab, Dept of Pharmacology, Saveetha Dental College after getting approval from the institutional review board . Ethical clearance was obtained from the ethical committee of the research department in Saveetha Dental College with an ethical number of IHEC/SDC/UG-1959/21/121.

Extract

Plant extract cassia oleoresin was obtained from Synthite industries Pvt limited, Kerala . The initial stock solution was 1 mg/ml. Cassia oleoresin was a dark brown fluid.

Chemicals

DMEM medium, 0.25% trypsin EDTA solution, sodium bicarbonate solution, bovine serum albumin, low melting agarose, MTT from Sigma chemicals Co., St. Louis, USA, Fetal bovine serum and antibiotic/antimycotic solution, DMSO were from Himedia, sodium phosphate monobasic and dibasic, sodium chloride, sodium hydroxide, sodium carbonate, hydrochloric acid and methanol were purchased from Sisco Research Laboratories (SRL), Pvt Ltd, Chennai, India.

Cell culture

The cells were grown in a T25 culture flask containing DMEM medium supplemented with 10% FBS. Upon reaching confluence, the cells were detached using Trypsin EDTA solution.

Preparation of Nanoparticle

Cassia oleoresin mediated selenium nanoparticles was prepared by adding 0.861g of selenium in 70 ml distilled water to 30 ml of Cassia oleoresin solution. After the formation of the nanoparticle, it was centrifuged at 8000 rpm for 15 minutes by a lark refrigerated centrifuge and the pellets were collected and washed with distilled water. The final purified pellets were collected and dried at 60° C for three hours. The powder is collected and stored in an airtight Eppendorf tube.

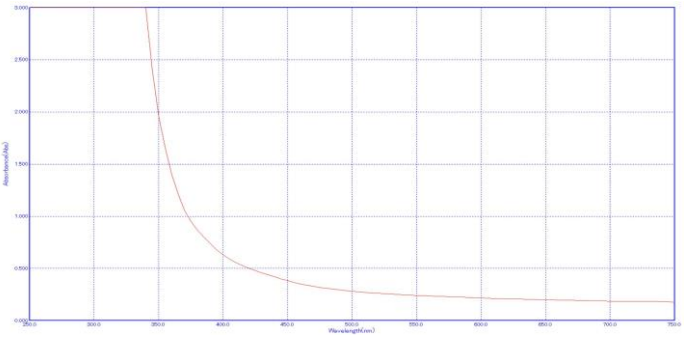


Fig.1: The graph represents the UV spectroscopy of cassia oleoresin. The peak value was found to be 340nm.

MTT Assay Analysis

In vitro cytotoxicity assay: The effect of cassia oleoresin on cell viability was measured by MTT assay following the method by Mosmann(43). Briefly, the cells (1 × 105 cells per ml) were seeded in a 96 well microtiter plate (100 µl per well) with

replications. Treatment was conducted for 24 and 48 h with different concentrations (20,40,60,80,100,200µg/ml) of cassia oleoresin mediated selenium nanoparticles. After incubation, 20 µl of 5 mg/ml MTT stock solution was added to each well and incubated for 4 h at 37 °C. The obtained formazan crystals were solubilised with DMSO and the absorbance was measured at 570 nm using a microplate reader (SpectraMax M5, Molecular Devices, USA). Cell viability (%) has been shown as a ratio of absorbance (A549) in treated cells to absorbance in control cells (0.1 % DMSO) (A549). The IC50 was calculated as the concentration of sample needed to reduce 50 % of the absorbance in comparison to the DMSO-treated control.

Cell viability estimation

Cell viability is calculated using the formula

Cell viability (%) = {A549 od of (sample)/A549 od of (control)} x 100

Statistical analysis

All data obtained were analysed and the results were computed statistically using the SPSS 10 software package using one way ANOVA. In all tests the level of statistical significance was set at $P < 0.05$.

RESULTS

In the present study, we evaluated the cytotoxic potential of cassia oleoresin in A549 cells by MTT assay. MTT assay was employed to assess the count of viable cells to measure the growth modulation of cells in vitro. The A549 cells were treated with different concentrations of cassia oleoresin for 24 hours. The cassia oleoresin caused a dose-dependent increase in cytotoxicity in the A549 cell line (Figure 2). The maximum cytotoxic effect was noticed with the maximum dose used in the study (ie) 100µg/ml (Figure3). Thus cassia oleoresin demonstrated good anticancer activity against lung cancer cell lines 50% of inhibition was observed in concentration of 15µg/ml, which has been taken as IC50 value and fixed for further experiments.

Anticancer activity of cassia oleoresin mediated selinium nanoparticel against lung cancer cell line

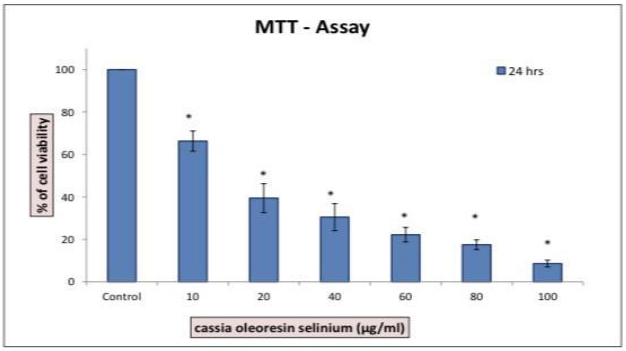
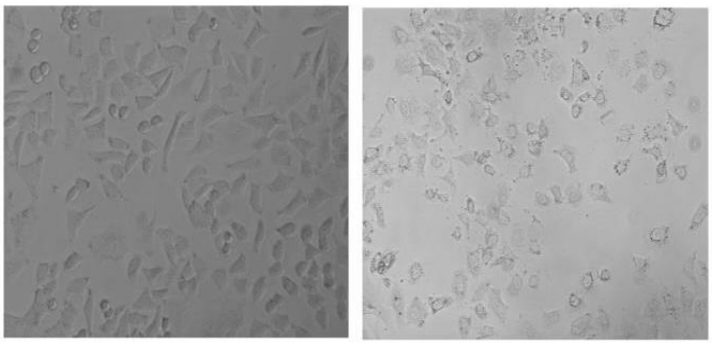


Fig.2 : The graph depicts the cytotoxic effect of cassia oleoresin on A549 cells. Cells were treated with cassia oleoresin (10, 20, 40, 60, 80, 100 µg/ml)) for 24 hours and cell viability was evaluated by MTT assay. X- axis represents the cassia oleoresin mediated selenium nanoparticle andY- axis represents the percentage of cell viability.* Compared with the control, p value was 0.002 at 20µg/ml which was found to be less than $p < 0.05$ and hence considered as significant.



Control **Treated**

Fig.3: Represents the morphological changes of A549 cells in lung cancer cell line without and with treatment of cassia oleoresin by phase contrast microscope at 20x magnification.

DISCUSSION

The present study demonstrated the cytotoxic effect of cassia oleoresin. From this study, it is understood that cassia oleoresin caused a dose-dependent increase in cytotoxicity of the A549 cell line. The maximum cytotoxic effect was noticed with the maximum dose used in the study (ie) 100µg/ml. A similar result was obtained in a study where biogenically synthesised selenium nanoparticles exhibited cytotoxicity at

40 µg/ml, inhibiting cancer cell growth in HeLa cells (44). In vitro anticancer results clearly revealed that the newly synthesized selenium nanoparticles are having promising effects in the medical field. Previous studies proved that the regulation of cell proliferation has been contemplated as one of the anticancer targets and many other anticancer agents have been outlined to kill the cancer cells by blocking cancer cell proliferation via cell cycle arrest (45).

In a previous study, selenium nanoparticles were synthesized with Spirulina polysaccharides and were found to delay the proliferation of cancer cells by causation caspase-mediated cell death (46) . In a study, selenium nanoparticles were found to show hypersensitivity delayed-type response and also stimulated the defensive mechanism by enhancing the interferon production in breast cancer mice model (47). Thus it is worth mentioning that the newly synthesized selenium nanoparticles with organic molecules could induce a cytotoxic effect and damage the cancer cell with minimal toxicity. Cinnamomum has been proved to show cytotoxic effects on various other cancers like basal cell carcinoma, human cancer promyelocytic leukemia, human cervical carcinoma, human colorectal carcinoma, human epithelioid cervix carcinoma, human glioblastoma multiforme tumor, human leukemia and leukemia rat embryonic fibroblast, human liver cancer, human lymphoblast lung, human melanoma cell lines, human NPC, human oral cancer lymphocytic leukemia cells, human oral squamous cell carcinoma, human prostate cancer cell etc. (48). Previously our team has conducted various studies on nanomedicine and other fields of research (49), (50), (51), (52), (53), (54), (55), (56), (57), (58), (59), (60), (60,61), (62), (62,63), (64), (65)

Limitations of this study was that, since it was done only in in-vitro level, further studies should concentrate more on the assessment of the interaction of cassia oleoresin and on the mechanistic elucidation of various active components responsible for its anti tumor activity in- vivo.

CONCLUSION

The study results within the limitation depicted that at the IC50 value of 15µg/ml almost half of the viable cells are destroyed(66)-(67). Thus it is evident that cassia oleoresin possesses a good level of anticancer activity. However more research is needed to understand the mechanism of cytotoxicity of the plants

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CONFLICT OF INTEREST

NIL

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