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RESEARCH ARTICLE

Biosynthesis Of Selenium Nanoparticles Using Cassia Oleoresin and Its Anticancer Activity Against Liver Cancer Cell Lines

SRIVATCHAVA. S[1], ANITHA ROY[2*], S. Rajesh kumar[3], S. RAGHUNANDHAKUMAR[4], LAKSHMI THANGAVELU[5]

[1]*Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai 77, Tamil Nadu ,India, Email:151901028.sdc@Saveetha.com*

[2]*Professor, Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences , Saveetha University,Chennai77,Tamil Nadu ,India, Email : anitharoy@saveetha.com*

[3]*Associate Professor,Nanomedicine lab, Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Science, Saveetha University, Chennai, Tamil Nadu, India, Email: rajeshkumars.sdc@saveetha.com.*

[4]*Associate Professor,Cancer and Stem Cell Research Lab, Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai77,Tamil Nadu ,India, Email: raghunandhakumar.sdc@saveetha.com*

[5]*Professor, Department of Pharmacology, Saveetha Dental College & Hospitals, Saveetha Institute of Medical & Technical Sciences, Chennai-77,Tamil Nadu, India, Email: lakshmi@saveetha.com*

**Corresponding author*

ABSTRACT

Introduction: Cassia is scientifically known as Cinnamomum Cassia, and it is a member of the Lauraceae family. Cassia is the botanical name for the Cassia bark. Cassia cinnamon is also used for chest pain, high blood pressure, kidney disorders, cramps, and cancer.

Aim: The present study was designed to analyse the anticancer activity of cassia oleoresin mediated selenium nanoparticles in HepG2 liver cancer cell line.

Materials And Method: Cassia oleoresin was obtained from synthite industries Ltd,Kerala. MTT assay was performed using HEPG2 cell lines to asses the anticancer activity.The HEPG2 cell were treated with different concentration of cassia oleoresin mediated selenium nanoparticle (20,40,60,80,100,200µg/ml) for 24 hours and the cell viability was analysed .

Results: The results of MTT assay showed a dose dependent decrease in cell viability of cassia oleoresin mediated selenium nanoparticle against the HEPG2 liver cancer cell line.In the HepG2 cell line, the oleoresin induced selenium increased cytotoxicity in a dose-dependent manner. The maximum cytotoxic effect was observed at a dose of 200µg/ml, which was used in the study.The IC50 value was 15µg/ml.

Conclusion: The present study within the limitations conclude that cassia oleoresin mediated selenium nanoparticles possess a good level of cytotoxic activity against HEPG2 cell lines. Hence the extract may be further explored in animal models to establish its activity

KEYWORDS:
Anti-cancer ; oleoresin ; MTT assay ; HEPG2 cells ; Selenium nanoparticle, green technique, eco-friendly

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INTRODUCTION

Hepatic cancer, also known as primary hepatic cancer or primary hepatic malignancy, is a form of cancer that begins in the liver. Liver cancer may be primary which begins in the liver or secondary which begins elsewhere in the body . Liver metastasis is more common than liver metastasis. (1) Globally, liver cancer is on the rise. (2),(3).Liver cancer is a common and growing problem around the world. (3) According to the most recent epidemiological statistics, liver cancer (4) is among the top ten cancers in terms of both prevalence and mortality ;noted to be the 6th leading cause of cancer and 4th most

common cause of death. (3,5) According to the Global Burden of Disease Liver Cancer Collaboration, the number of new cases of liver cancer each year rose by 75% between 1990 and 2015.Cancer prevention can be divided into three categories: primary, secondary, and tertiary prevention. Preventing exposure to a risk factor for liver cancer is known as primary prevention. Hepatitis B vaccine is one of the most successful primary liver cancer prevention methods. (5) Currently, there is no vaccine available to protect against the hepatitis C virus.(6)

Cassia is scientifically known as Cinnamomum cassia, and it is a member of the Lauraceae family. Cassia is the botanical name for the Cassia bark. India, Bangladesh, Vietnam, and South China are all home to this evergreen oak. Guangdong, Guangxi, and Yunnan are the major growing regions in China. It is now commonly grown in Indonesia, Malaysia, Thailand, and Laos.Cassia oleoresin's health benefits include preventing fatigue, pre-diabetes, and treating diabetes, as well as treating gas , muscle and stomach spasms, diarrhoea, vomiting, infections, the common cold, and a lack of appetite. It can also be used to treat bedwetting, hernias, joint pain, menstrual issues, menopausal symptoms, and abortions. Cassia cinnamon is also used for chest pain, high blood pressure, kidney disorders, cramps, and cancer.

A nanoparticle, also known as an ultrafine particle, is a small particle of matter with a diameter of 1 to 100 nm. (7) The term is often applicable to larger particles with diameters of up to 500 nm, as well as fibres and tubes with diameters of less than 100 nm in just two directions. (8) Metal particles smaller than 1 nm are commonly referred to as atom clusters instead. Nanoscience is the analysis of extremely small structures and materials. One billionth of a metre is a nanometre. At the nanoscale, matter's physical and chemical properties alter. Nanotechnology has the ability to change a wide variety of sectors, including health care and manufacturing .Nanoparticles have the ability to cause medical and environmental damage. (9)-(10) The majority of these are caused by the particles' high surface-to-volume ratio, which can make them highly reactive or catalytic.They can also move across cell membranes in animals, but little is known about their interactions with biological systems. Owing to particle size and intercellular agglomeration, the particles are unlikely to reach the cell nucleus, Golgi complex, endoplasmic reticulum, or other internal cellular components(11,12). In previous years many nanoparticles were synthesized using natural products and evaluated and confirmed their pharmacological activities (13–23)

The chemical element selenium has the symbol Se and the atomic number 34. It is a nonmetal with properties that are intermediate between sulphur and tellurium in the periodic table, as well as having similarities to arsenic. Selenium is an important micronutrient for animals, despite the fact that it is harmful in large quantities. It occurs as a bystander mineral in plants, often in harmful concentrations in forage . Selenium levels of blood, plasma, serum, and urine may be tested to detect severe environmental or occupational exposure, confirm a poisoning diagnosis in hospitalised patients, or investigate a possible case of fatal overdose. Some analytical techniques can tell the difference between organic and inorganic forms of the element.Prior to elimination in the urine, both organic and inorganic forms of selenium are largely converted to monosaccharide conjugates (selenosugars) in the body. Cancer patients who take selenomethionine orally every day can have very high selenium levels in their blood and urine.(24) Our team has extensive knowledge and research experience that has translated into high quality publications (25–29)
(30)(31)(32)(33)(34)(35)(36)(37)(38)(39)(40)(41)(42)(42,43)(44)(44,45)(46)((47).Aim of the present study was designed to analyse the anticancer activity of cassia oleoresin mediated selenium nanoparticles in HepG2 liver cancer cell line.

MATERIALS AND METHOD

Study setting

The study was conducted in the Cancer and Stem cell Research Lab, Saveetha Dental College after obtaining approval from the Scientific Review Board

Chemicals

DMEM medium, 0.25% Trypsin-EDTA solution, sodium bicarbonate solution, bovine serum albumin (BSA), low melting agarose, MTT from Sigma Chemicals Co., St. Louis, USA. fetal bovine serum (FBS) 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) India.

Plant Material

Cassia oleoresin was obtained from Synthite Industries Pvt Limited , Kerala with product code 4010000195. It was isolated from Cinnamomum cassia Blume (Laureraceae) by solvent extraction of the dried bark. The volatile oil content was 30-35ml/ 100g.

Preparation of nanoparticle

Cassia oleoresin mediated selenium nanoparticles were 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 and stored in an airtight eppendorf tube.

Cell Culture Reagents

Dmem

Commercially available DMEM contains 7.5% sodium bicarbonate solution. To 500ml of DMEM, 5ml of penicillin/streptomycin solution and 0.5ml of amphotericin B

solution was added. Then the medium was sterile filtered (0.22µ) inside the hood. The medium was then dispensed into a sterile container and stored at 4°C.

Growth Medium (DMEM with 10% FBS)

10ml of FBS was made up to 100ml using sterile DMEM. It was stored in a sterile container in cool and aseptic condition.

Phosphate Buffered Saline (PBS; pH 7.4)

0.63 g of sodium phosphate monobasic (NaH2PO4), 0.17 g of sodium phosphate dibasic (Na2HPO4) and 4.5 g of sodium chloride (NaCl) were dissolved in 500 ml of double autoclaved milliQ water. The pH was then adjusted to 7.4 using 1 N HCl and 1 N NaOH, sterile filtered (0.22 µ) and then stored in a sterile container.

Trypsin-EDTA versus Glucose Solution

Trypsin was purchased as 1 x with EDTA (0.5% trypsin, 5.3 mM EDTA sodium salt). (Note: Freeze-thaw process does not affect the enzyme activity. Thawing is done at room temperature).

Physiological Saline

890 mg of sodium chloride was dissolved in 100 ml of double autoclaved milliQ water to get 0.89% .

Cell Line

Human liver adenocarcinoma-HepG2 cell line was procured from National Centre for Cell Science (NCCS, Pune), India. The cells were grown in T255 culture flasks containing DMEM medium supplemented with 10% FBS. Upon reaching confluence, the cells were detached using Trypsin-EDTA solution.

Passaging Of Cells

Upon reaching confluence liver cell lines HepG2 were passaged as follows. The medium from the culture flask was aspirated. The flask was rinsed with 2ml of PBS and aspirated again quickly. 1.5-ml of trypsin-EDTA solution was added and incubated at 37ºC for about 2 minutes until cells started detaching. As soon as the cells were detached, transfer the trypsinized medium containing cells using a transfer pipette into a 15 ml falcon tube and it was centrifuged at 1000 rpm for 5 min. The medium was carefully aspirated off and care was taken not to put the pipette tip in the bottom of the tube, where the cells were pelleted. The cells were gently resuspended in the DMEM medium with 10% FBS by pipetting up and down 5-8 times gently. From the cell suspension, a drop was placed on the edge of the coverslip of neubauer hemocytometer. The drop was led to run under the cover slip by capillary action. Care was taken not to force the liquid and the entry of the air bubble was avoided. Then the cells from the E1, E2, E3, E4 and E5 squares were counted under a microscope. The cells were then gently resuspended and transferred to sterile T 75 culture flasks and the volume of medium was made upto 10 ml with growth medium per flask.

Testing Viability Of Cells

The viability of HepG2 cells was assessed by trypan blue exclusion test Perry et al., (1997).

Reagents

1. Trypan blue solution: (0.5% trypan blue (w/v) in physiological saline).

100 ml of trypan blue solution was mixed with 100 l of cells contained in the medium and incubated for 5 min at 37ºC. The cells were then washed thrice with saline and 10 l of this suspension was placed in a haemocytometer and viewed under a microscope. The unstained cells represented the viable cells whereas the damaged cells were stained. The number of stained and unstained cells was counted and the percentage of viable cells was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{No. of unstained cell}}{\text{Total no. of cells}} \times 100$$

The viability of the cells was found to be between 90-95%.

Cell Proliferation Assay

The proliferation of HepG2 cells was assessed by MTT assay Safadi et al., (2003). The assay is based on the reduction of soluble yellow tetrazolium salt to insoluble purple formazan crystals by metabolically active cells. Only live cells are able to take up the tetrazolium salt. The enzyme (mitochondrial dehydrogenase) present in the mitochondria of the live cells is able to convert internalized tetrazolium salt to formazan crystals, which are purple in colour. Then the cells are lysed using 20% SDS solution, which releases the formazan crystal. These crystals are solubilized by DMF present in the solubilizer. The colour developed is then determined in an ELISA reader at 620 nm.

Reagents

1. MTT [3-(4,5-dimethythiazol-2-yl) 2,5-diphenyl tetrazolium bromide]: 0.5 mg MTT/ml of serum-free DMEM.

2. Solubilization solution: 20% w/v SDS in 50% of Dimethyl formamide.

3. Phosphate Buffered Saline (PBS; pH 7.4).

Procedure

HepG2 cells were plated in 24 well plates at a concentration of 5 x 104 cells/well 24 hours after plating, cells were washed twice with 500 µl of serum-free medium and starved by incubating the cells in serum-free medium for an hour at 37ºC. After starvation, cells were treated with ION of different concentrations for 24 hours. At the end of treatment, the medium from control and ION treated cells were discarded and 500 µl of MTT containing DMEM (0.5 mg/ml) was added to each well. The cells were then incubated for 4 h at 37ºC in the CO2 incubator. The MTT containing medium was then discarded and

the cells were washed with 1x PBS (1 ml). The crystals were then dissolved by adding 500 µl of solubilization solution and this was mixed properly by pipetting up and down. Spectrophotometric absorbance of the purple blue formazan dye was measured in a microplate reader at 620 nm. The OD of each sample was then compared with the control OD and the graph was plotted.

Based on MTT assay we selected the low and high doses for further studies. The characterisation of morphological changes in cells treated with (compound name with low and high doses) compared to their respective controls were observed under phase contrast microscope.

Statistical Analysis

All data obtained were analyzed and represented as mean ± SE. The results were computed statistically (SPSS/10 Software Package; SPSS Inc., Chicago, IL, USA) using one-way ANOVA. Post-hoc testing was performed for inter comparisons using the LSD. In all tests, the level of statistical significance was set at $p<0.05$.

RESULT AND DISCUSSION

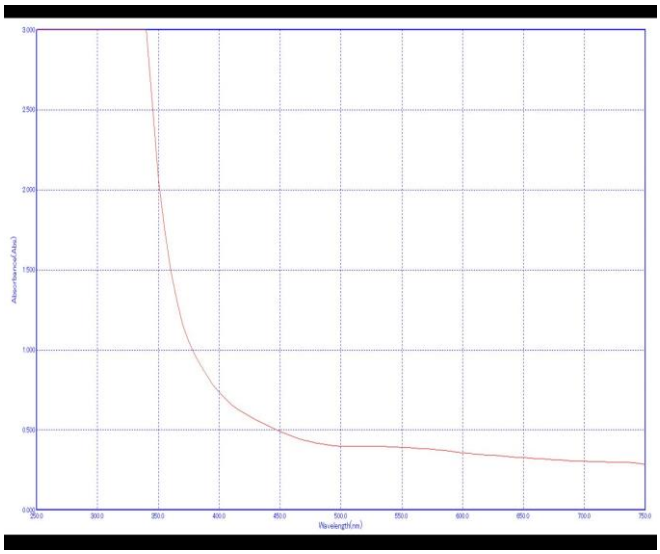

Fig. 1: The graph represents the UV spectroscopy of cassia oleoresin. The Y-axis represents the absorbance. The X-axis represents the wavelength (nm). The peak value was found to be 340nm.

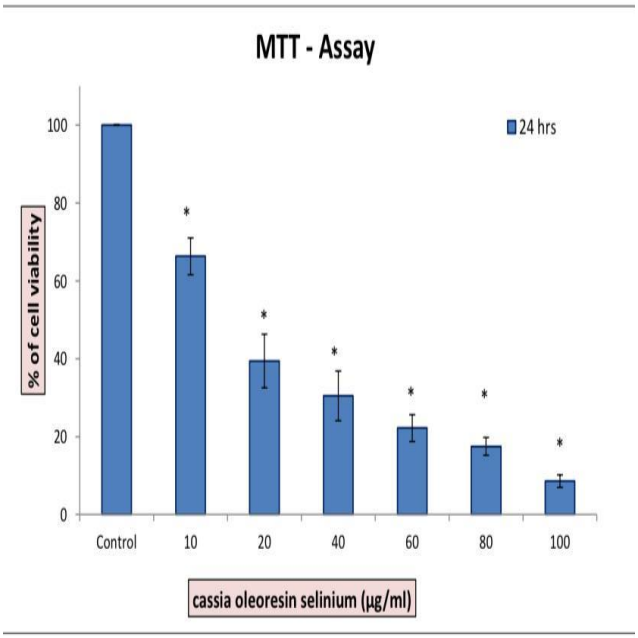

Fig.2: The cytotoxic effects of cassia oleoresin on HEPG2 cells. The x -axis represents the different concentration of the Cassia oleoresin mediated selenium nanoparticles and the control in blue color.. The y -axis represents percentage cell viability. Data presented as means ± SE (n = 3). * compared with the control , at 50µg the p value was 0.005 which was highly significant since $p < 0.05$ was considered as significant.

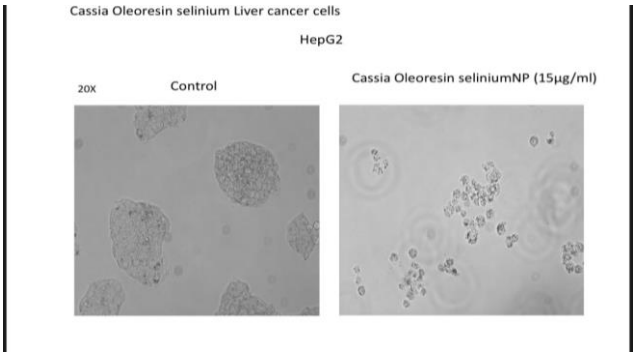

Fig.3: Assessment of cell morphology of HepG2 treated with cassia oleoresin and control . Cells were treated with cassia oleoresin (100 µM) for 24 h along with the control group. Images were obtained using an inverted Phase contrast microscope in 20X magnification. IC50 value was 15 µg/ml.

The graph represents the UV spectroscopy of cassia oleoresin. The peak value was found to be 340nm. (figure 1).The MTT assay was used to assess the cytotoxic potential of cassia oleoresin in HEPG2 cells in this study. The MTT assay was used to evaluate the number of viable cells in order to assess the growth modulation of cells in vitro. The in vitro cytotoxicity assay is a cost-effective and dependable tool.(48). For 24 hours, HepG2 cells were treated with varying concentrations of cassia oleoresin (20 to 200µg/ml). In the HepG2 cell line, the oleoresin induced selenium increased cytotoxicity in a dose-dependent manner. The maximum cytotoxic effect was observed at a dose of 200µg/ml,(figure 2) which was used in the study. The anticancer properties of cassia oleoresin were found to be effective against a liver cancer cell line.

When compared to the previous in vitro research performed (49) to investigate the anticancer activity of cassia against cholangiocarcinoma, the present study findings clearly show that cassia oleoresin has a strong degree of anticancer activity against liver cancer cell line.(figure 3) .The limitation of the current study was that it was done in inviro . To make the complete use in human , further studies in animal and human volunteers are required in future.,

CONCLUSION

The current study could show that cassia oleoresin mediated selenium nanoparticles have a good cytotoxic effect on HEPG2 cell lines. (50-59).Hence, it may be used for the management of liver cancer after further invivo studies.

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CONFLICT OF INTEREST

All the authors declare no conflict of interest in the study.

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