Original Article

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING Rosmarinusofficinalis LEAF EXTRACT AND STUDY OF ANTICANCER EFFECT AND APOPTOSIS INDUCTION ON PROSTATE CANCER CELL LINE (PC-3)

T. Gokul Vimal[1], Gayatri Devi.R[2*], J. Selvaraj[3]

[1]Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Science (SIMATS), Saveetha University, Chennai, India

[*2]Associate Professor, Department of Physiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Science (SIMATS), Saveetha University, Chennai - 600077, India

[3]Associate Professor, CoMManD, Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Science (SIMATS), Saveetha University,

Chennai - 600077, India

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Abstract

Introduction: Rosmarinusofficinalis consist of many medicinal values like for the treatment of disorders associated with nervous, cardiovascular, gastrointestinal, menstrual, hepatic and reproductive systems. The main aim is to synthesize silver nanoparticles using Rosmarinusofficinalisleaf extract and to study the anti-cancer and apoptosis induction on prostate cancer cell line

Materials and method: The plant was collected, pressed and was made into powder. The prostate cancer cell line was purchased and was subcultured in the laboratory. The cell line and different concentrations of drugs were subjected to MTT assay and an IC-50 value was found. The prostate cancer cells RNA were isolated and were under gene expression. The RNA was made into cDNA using reverse transcriptase which was then subjected to real time polymerase chain reaction for specific genes which promotes anti-cancer and apoptosis induction.

Results: The analysis of MTT assay showed that the IC-50 where only 50 percent of the cell was alive to certain concentrations of dosage of the drug used. In this study, it was found out that 200 microgram of concentration was enough to reduce 50 percent of cells in a cell culture. The genes caspase 3 and caspase 9 were analyzed and showed significant increase at 200 microgram of the drug Rosmarinusofficinalis.

Conclusion: Rosmarinusofficinalis has a significant role in controlling prostate cancer cell line proliferation by upregulating the gene expression. The results showed that R.officinalis exert cytotoxic activity on prostate cancer cell line

Keywords: Anti-cancer, Apoptosis, caspase genes,MTT assay, prostate cancer,Rosmarinusofficinalis, RNA proliferation.

INTRODUCTION

Nanoparticles are defined as molecules with a size less than 100 nm. The main key to develop metal nanoparticles with the appropriate properties, such as size and form, is through a variety of physical and chemical procedures, which have some significant drawbacks such as being expensive, commodity, and potentially harmful to the environment and living things (1). Consequently, an alternative, affordable, safe, and biocompatible technology is unquestionably required for the synthesis of nanoparticles (2). Plant extracts can function as both reducing and fixing agents in the green manufacture of metal nanoparticles using any secondary metabolic process of therapeutic plants. The green synthesis of nanoparticles involves reduction of size of certain elements like the silver, selenium, gold, zinc oxide and so on with the help of parts of plants such as stem, leaf, flower (3). The green synthesis is found to be cost effective and also increase the efficiency and efficacy of working to certain targeted cells only. The plant mediated green synthesis of nanoparticles was found to be useful in the field of cosmetics and in derma pharmaceutical applications (4).Green synthesized silver nanoparticles were proved to have shown antimicrobial and antifungal activities.

Rosmarinusofficinaliscommonly known as rosemary is best known for its fragrance. The plant are usually found in the Mediterranean regions and is known to have high medicinal values and are also used as food preservatives. The plant, Rosmarinusofficinalis has several pharmacological activities such as antimicrobial, anti-inflammatory, antioxidant, anti-proliferation, anti-tumor and protective, inhibitory and attenuating properties (4,5). Rosmarinusofficinalis consist of many medicinal values like for the treatment of disorders associated with nervous, cardiovascular, gastrointestinal, menstrual, hepatic and reproductive systems (6). The plant has so many of these properties because of the presence of phenolic groups present in the plant. This plant is also considered as one among the biologically important plants.

Prostate cancer, a cancer occurring most commonly in men's prostate. Based on GLOBOCAN (Global Cancer Observatory) 2018, estimates had shown that approximately 1,276,106 new prostate cancer cases were reported across the globe in 2018 with higher prevalence in developed countries. African American men relatively have a higher incidence of prostate cancer which was found to be more aggressive compared to normal white men (7). More than a million cases were diagnosed per year. Symptoms include trouble in urination, blood in semun and urine, losing weight without trying, bone pain and erectile dysfunction. Treatments like conventional surgical methods or modern radiation or even stem cell mediated therapy can be a curative treatment for localized disease but have adverse symptoms such as urinary problems (8). However for metastatic cancer, immunotherapies and harmony mediated therapies were found to be effective with moderate side effects (9).

Apoptosis is a programmed cell death which is initiated by cell blebbing or by shrinkage of cells. Sometimes due to mutation in genes, there can be an increase in the apoptosis which eventually causes cell death. Many studies were done on apoptosis induction with the help of medicinal plants. However, it was found out that further research is necessary in view of its therapeutic use (10). Dysfunctions in the regulation or execution of cell suicide are implicated in a wide range of developmental abnormalities and diseases (11). Anticancer drugs are drugs used to control the abnormally dividing cells. Despite vast investment in oncology R&D, the translation of research advances into medicines that substantially improve the treatment of many cancers remains frustratingly slow (4,5,12). Our team has extensive knowledge and research experience that has translated into high quality of publications (13)(14–27).

The main purpose of this study is to green synthesize silver nanoparticles by Rosmarinusofficinalisand are used to control the prostate cancer cell line.

MATERIALS AND METHODS:

Chemicals

Trypsin-EDTA, fetal bovine serum (FBS), antibiotics-antimycotics, Dulbecco's modified Eagle's medium (DMEM) and phosphate buffered saline (PBS) were purchased from Gibco, Canada. JC-1 (5,5 ,6,6 - tetrachloro-1,1 ,3,3 - tetraethylbenzimidazolocarbocyanine iodide) and real time PCR kit (MESA Green) were purchased from Invitrogen, USA. All the chemicals used were extra pure of analytical grade.

Extract preparation:

Rosmarinusofficinalis L leaves were soxhlet extracted with 70% ethanol. The extract was then filtered with Whatman no. 1 filter paper and the solvent evaporated at reduced pressure by using a Rotary evaporator apparatus to get a viscous mass, which was then stored at 4°C until used.

Procurement and culture of PC 3 cells:

The prostate cancer cell line (PC 3), was obtained from The National Centre for Cell Science (NCCS), Pune, India and cultured according to the cell culture instructions provided. Briefly, PC – 3 cells were grown in MEM containing 10% FBS at 37˚C in an atmosphere containing 5% CO2.

Cell viability assay:

PC – 3 cells were seeded at a density of 5x105 cells/well in 96-well plates and allowed to attach to the well overnight. After incubation, cultured cells were stimulated with various concentrations of Rosmarinusofficinalis L leaf extracts in triplicate and incubated at 37˚C in a 5% humidified CO2 incubator for 24h. Subsequently, MTT was added to each well, and incubation was continued for a further 4 h at 37℃. To dissolve the formazan formed from MTT, the cells were resuspended in 200 µl dimethyl sulfoxide (DMSO), and the optical density (OD) of the solution was determined using a spectrometer at a wavelength of 570 nm. The experiments were repeated 3 times, independently. The mean optical density (OD) ± SD for each group of replicates was calculated. The entire procedure was repeated 3 times. The inhibitory rate of cell growth was calculated using the equation:

% Growth inhibition = (1 - ODextract treated)/OD negative control x 100.

Gene expression analysis by Real Time PCR

mRNA expression levels were examined using real-time PCR. The total RNA was isolated by using Tri Reagent (Sigma). Total RNA (2 µg) from each sample was reverse transcribed using a commercial SuperscriptIII first strand cDNAsynthesis kit (Invitrogen, USA) according to the manufacturer's protocol. Real time-PCR was carried out in a MX3000p PCR system (Stratagene, Europe). Reaction was performed using MESA Green PCR master mix (It contains all the PCR components along with SYBR green dye.) Eurogentec, USA. The specificity of the amplification product was determined by melting curve analysis for each primer pair. The data were analyzed by comparative CT method and the fold change is calculated by 2−CT method described by Schmittgen and Livak (2008) using CFX Manager Version 2.1 (Bio Rad, USA).

Statistical analysis

Data were expressed as the means ± SD of 3 individual experiments performed in triplicate. Statistical analysis was performed using the one-way ANOVA and $p<0.05$ was considered to indicate a statistically significant result.

RESULTS AND DISCUSSION:

The MTT assay works on the principal by conversion of MTT to formazan crystals by living cells, which helps in determining the mitochondrial activity. Since for most cells the total mitochondrial activity is related to the number of viable cells (28). In MTT assay, the untreated cell line without theRosmarinusofficinalis drugs, the viability is 100 percent. The cell viability decreases as the amount and concentration of drugs increase. At 50 microgram of dosage of drug, cell viability is decreased to 90 percent. At 100 microgram of Rosmarinusofficinalis drug extract, 60 percent of the cells were alive in the wells. But at 200 microgram of Rosmarinusofficinalisextracts 50 percent of the cells show cell death (Figure 1). Therefore the IC-50 Point was obtained which was used for further research. The main reason to take IC-50 point is because with viability greater than 50 percent, it is very difficult to identify if the drug is actually working as the cell death would be very feeble and with drug dosage less than 50 percent tend to kill most of the cells and is again very difficult to identify if the drug has actually worked. The IC-50 (200 microgram of Rosmarinusofficinalis) is used for further characterisation of genes like caspase 3 and caspase 9 genes.

The caspase 9 mRNA is one of the molecules involved in the apoptosis pathway whose down regulation usually signifies the presence of cancer cells (29). In the graph, as the concentration of dosage of Rosmarinusofficinalis increases, fold changes increase which signifies that there is inhibition of apoptosis in the prostate cancer cell line.

An inability to undergo apoptosis is widely thought to contribute to both tumorigenesis and tumor progression. One of the key mediators of apoptosis is the thiol protease caspase3 (30). The caspase 3 mRNA deficiency may exhibit specific defects in apoptotic pathways including delayed kinetics and lack of DNA fragmentation during brain development (31). The graph shows slight increase in the fold change compared to that of control which provides the evidence that the apoptosis inhibition is occurring at 200 microgram of Rosmarinusofficianlisat greater rate (Figure 2). Nanoparticles can be hazardous at greater doses but are harmless at lower doses. Commonly, cells exposed to different nanoparticle concentrations exhibit an amount of the drug rise in cell inhibitory activity. Different silver nanoparticle formulations have dosage effects that might change cytotoxicity or enhance anticancer efficacy.

The presence of high concentrations of flavonoid components including carnosic acid, rosmarinic acid, and ursolic acid, which are present in maximum concentration, is thought to contribute to R.officinalis anticancer activities. In several different tumor cell lines, rosemary extracts showed anti-proliferative activity. This characteristic has been linked to the presence of certain chemicals, including carnosol, carnosic acid, and rosmarinic acid, which have been reported to have antioxidant action (32).

The study was limited to in vitro study. The time duration taken was longer than expected. In cases of treatment based on nanomedicine, the AgNPs demonstrated remarkable potential. However, in order to influence the future direction of their utilization, clinical trials of AgNPs-based nanomedicine are essential. Research into biodegradability, dosage, and delivery methods are currently the main challenges that need to be overcome in clinical studies. Additionally, AgNPs are a crucial tool for early cancer diagnosis and cancer cell imaging and detection. The potentiality of Rosmarinusofficinalis can also be further exploited and further research can be done in combination with anti-cancer effects and other important pharmaceutical and pharmacological applications.

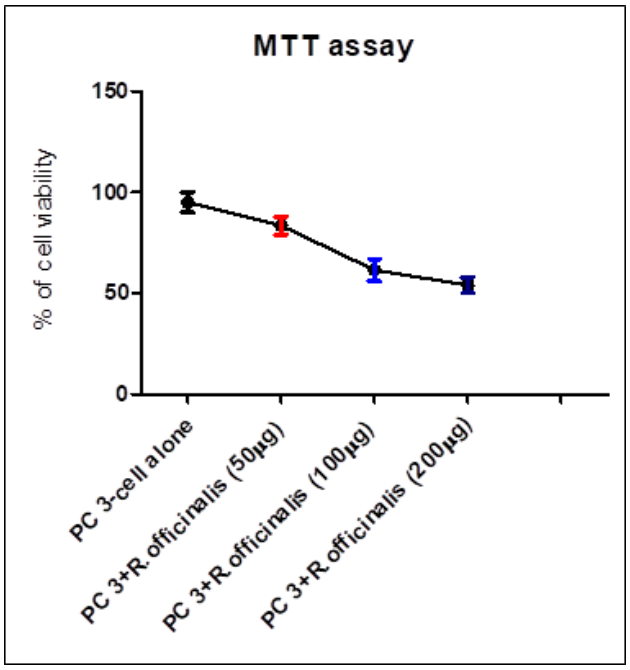

Figure 1: MTT assay

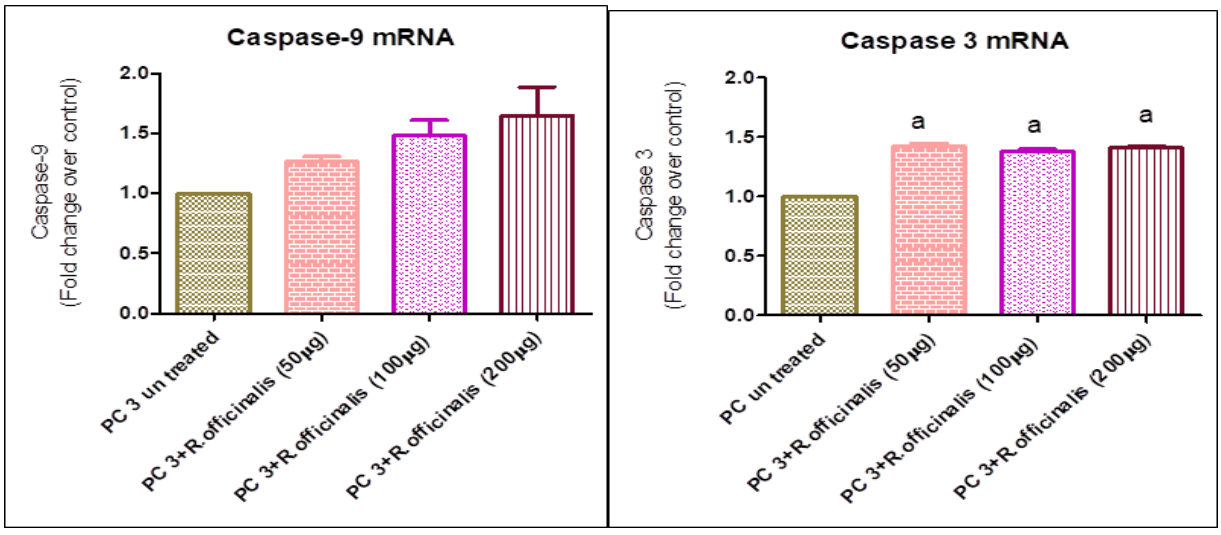

Figure 2: Caspase 3 and Caspase 9 mRNA expression

CONCLUSION:

Using the R.officinalis extract, silver nanoparticles were biosynthesised in a cost-effective, non-toxic, and ecologically sustainable manner. A possible anticancer agent for cancer therapy, green synthesis nanosilver exhibits a good cytotoxic effect against prostate cancer cells. The Rosmarinusofficinalishas a significant role in controlling prostate cancer cell proliferation via activation of caspase 3 and caspase 9 mRNA.

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Author Contributions:

GokulVimalT :Literature search, Data collection analysis, Manuscript drafting

Dr.R. GayatriDevi :Data Verification, Manuscript draft

Dr.J.Selvaraj: Data collection analysis, Data Verification, Manuscript draft

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