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RESEARCH ARTICLE

Cytotoxic Effect of Ginger Oleoresin Against Lung Cancer Cell Line

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ABSTRACT

Introduction: Indian spice contributes to food as both aroma and taste. Indian spices have been shown to treat diseases that range from colds, cough, and cancer. Ginger is a member of the Zingiberaceae genus, scientifically known as Zingiber officinale. It is the plant with medicinal and nutritional qualities that is the most essential.

Aim: The present study was conducted to evaluate the cytotoxic effect of ginger oleoresin against A549 lung cancer cell line.

Materials and Method: The Ginger oleoresin extract is collected from Synthetic Industries, with a product code - 4010000370. MTT assay was performed on the A549 cell line to assess the cytotoxic effect. The A549 cells are treated with different concentrations of ginger oleoresin (10, 20, 30, 50, 100 and 200 µg/ml) for 24 hrs and the cell viability was analysed using MTT assay.

Results: The results of MTT assay showed a dose dependent increase in cytotoxic effect of ginger oleoresin against A549 lung cancer cell line. The IC_{50} value was 20 µg/ml for the extract.

Conclusion: The number of viable cells decreases as the drug concentration increases. Therefore, the present study concludes that ginger oleoresin possesses a strong cytotoxic effect. Hence, it is a promising candidate for anticancer therapy.

KEYWORDS:
Cytotoxic effect; ginger; oleoresin; MTT assay; A549 cells,natural, innovation

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INTRODUCTION

Lung cancer, with an estimated 1.2 million new cases per year, is the most prevalent type of cancer in the world. Tobacco smoking is the leading source of lung cancer, with tobacco smokers 80% to 90% (1). Incidence disparities are significant in geography, race and gender, and some studies indicate that women may become at greater risk of lung cancer because of exposure to cigarette smoke cancer. A life-long smoker is 20-30 times more likely than a non-smoker to develop lung cancer (2). While in the USA, China and Eastern Europe, the rate of smoking is declining, there is a smoking epidemic that in the course of this century will produce ten million new cases (3). Lung disease is the most preventable cancer of all and the reduction of smoking results in a lower incidence following 7 years of lagging. This lower risk however never exceeds the baseline rate and former smokers are becoming ill with lung cancer in India (4). Despite therapy advances, 90 percent of patients with lung cancer will die. Lung cancer is expected to lead to millions of deaths globally in the coming future. But only certain heavy tobacco smokers eventually grow lung cancer, which suggests that genetic causes are likely to be susceptible to lung cancer (5). Chemotherapy, radiation and cancer-dependent surgery are included in cancer treatment

(6). Many plants are exploring the cytotoxic influence recently in various models (7). In the evaluation of cytotoxic influence, cell lines are widely used (8). A useful model for the investigation of characteristics such as cellular regulation, uncontrollable cell proliferation, limited inhibitor concentration and cell death evasion is lung cancer cell (9).

Plants are being explored for antidiabetic, antimicrobial and anti-inflammatory properties. Indian spice is too explored (10). Indian spice contributes to food as both aroma and taste. Indian spices such as turmeric, ginger, mint, etc. have been shown to treat diseases that range from colds, cough, and cancer. Ginger is a member of the Zingiberaceae genus, scientifically known as Zingiber officinale. It is the plant with medicinal and nutritional qualities that is the most essential (11). It is traditionally used to treat various illnesses such as nausea, vomiting, asthma, inflammation, etc., in Ayurveda, siddha, etc and various other medical systems (12). Ginger, a member of the plant family which includes cardamom and turmeric. The ginger plant has a long tradition of growing, known to have originated from Asia and then to India, Southeast Asia, West Africa and Caribbean. Ginger products include mineral oil and Oleoresin and are marketed worldwide for use in processing food and medicines (13). The intense flavour of ginger is the product of potent ketones, including gingerol, which are used mostly for research purposes (14). It has many biological characteristics, including antimicrobial, antioxidant and anticancer and has an immune system stimulant impact (15).

There is no exact mechanism of action on the antiemetic properties of ginger, but serotonin receptors seem to be inhibited and to exert an antiemetic effect at the level of gastrointestinal and central nervous systems (16). Ginger extract inhibits tumour necrosis factor α and cyclo oxygenase 2 activation in vitro experiments with its possible anti-inflammatory effects. Ginger oil is widely used as the flavouring agent in food products, such as confectionery and perfumes as a natural essential oil prepared by dried and unpeeled zingiber officinale rhizome. Ginger oleoresins include compounds like 6-gingerly, Zingerone, Resin, Shogaol, Phenol, Vitamin B and other minerals which are pharmaceutically active ingredients. The involvement of several ingredients in the oleoresin extract, shows the inhibitory action of plant Zingiber officinale(17) (18) (19) (20).

Previously, our team had conducted numerous studies with plant extract, essential oils and oleoresins (21)- (22). Our team has extensive knowledge and research experience that has translated into high quality publications(23–27)(28–32) . The present study was done to evaluate the cytotoxic effect of ginger oleoresin against lung cancer cell lines.

MATERIALS AND METHOD

Study Setting

The study was conducted in the Cancer and Stem Cell Laboratory, Saveetha Dental College, Chennai, India. The Ethical Clearance number is IHEC/SDC/UG-1973/21/125.

Preparation of Plant extract

The Ginger oleoresin extract was collected from Synthite Industries Pvt Limited, Kerala, with a product code - 4010000370. The preparation was done by solvent extraction of the dried rhizomes. It contains a volatile oil in a composition of about 30 -35 ml/100g. The initial stock solution was 1mg/ml.

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.

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 sterile containers 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 (NaH_2PO_4), 0.17 g of sodium phosphate dibasic (Na_2HPO_4) 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: Enzyme production is not impaired by the freeze-thaw technique. Thawing is done at room temperature). 0.89% Physiological Saline, 890 mg of sodium chloride was dissolved in 100 ml of double autoclaved milliQ water.

Cell line

Human lung adenocarcinoma-A549 cell line was procured from the National Centre for Cell Science (NCCS, Pune), India. The cells were grown in T255 culture flasks containing DMEM medium supplemented with 10% FBS. The cells were isolated by Trypsin-EDTA solution when the confluence was attained.

MTT assay

In vitro cytotoxicity assay, The effect of ginger oleoresin on cell viability was measured by MTT assay following the method by Mosmann. Briefly, the cells (1 × 10^5 cells per ml) were seeded in a 96 well microtitre plate (100 µl per well) with replications. Treatment was conducted for 24 hrs with different concentrations (10, 20, 30, 50, 100 and 200 µg/ml) of ginger oleoresin. The stock solution MTT was applied to each well for 20 µl 5 mg/ml after incubation and incubated at 37 ° C for 4 hours. after incubation. DMSO solubilized the collected formazan crystals and the absorption was measured by a microplate reader at 570 nm (SpectraMax M5, Molecular Devices, USA). Cell viability (%) has been shown as a ratio of absorbance (A570) in treated cells to absorbance in control cells (0.1 % DMSO) (A570). In comparison with DMSO-treated power, the IC50 was determined as the sample concentration needed to minimise absorbance by 50%.

Cell viability estimation

Cell viability was calculated following the equation:

Cell viability (%) = {A570 od of (sample)/A570 od of (control)} x 100.

Morphology Study

Based on MTT assay we selected the low and high doses of Ginger oleoresin for further studies. The characterisation of morphological changes in A549 lung cancer cells treated with (Ginger oleoresin with low and high doses) compared to their respective controls were observed under phase contrast microscope.

Statistical analysis

The analysis of all the data collected was performed and the findings have been statistically calculated by means of ANOVA one-way (SPSS/10 Software Package; SPSS Inc., Chicago, IL, USA). The statistical value standard was calculated at $p<0.05$ for all testing.

RESULTS

From the present study, we evaluated the cytotoxic effect of Ginger oleoresin in A549 cells by MTT assay. MTT assay is used to assess the number of viable cells to measure the growth modulation of cells in vitro. This assay is an economical method as well as a reliable method. The A549 lung cancer cells were treated with different concentrations of Ginger oleoresin extract (10, 20, 30, 50, 100 and 200 µg/ml) for 24 hrs (Figure 1). The oleoresin caused a dose dependent increase in cytotoxicity in A549 lung cancer cell line. The morphological representation of lung cancer cells upon treatment with Ginger oleoresin extract for 24 hrs compared to control cells is seen in Figure 2. The results clearly indicate that the findings were clearly cytotoxic to lung cancer cells at a specific concentration and incubation time. It shows that nearly half of the viable cells were killed at the concentration (IC_{50}) of 20 µg/ml. At 20 µg/ml, only 50% of the cells are viable (Table 1). Thus, it can be said that as the drug concentration increases, the viable cells decrease. It proves that ginger oleoresin possesses a strong cytotoxic effect against A549 lung cancer cells.

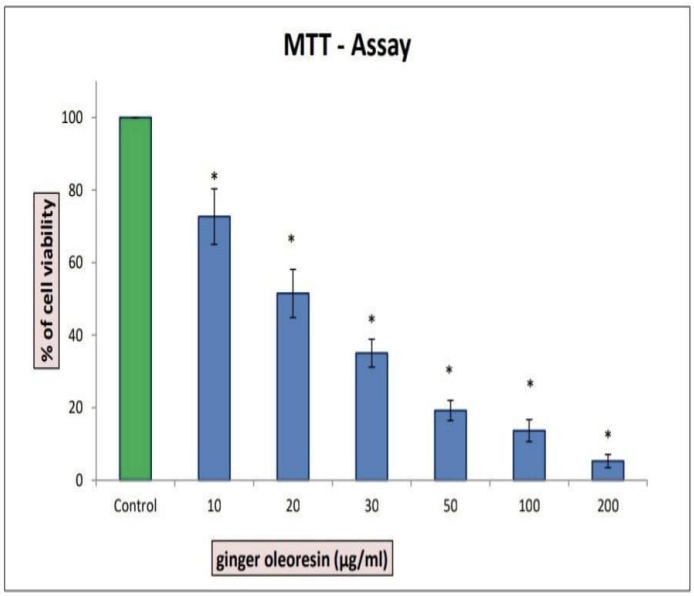

Fig.1: The bar chart represents the cytotoxic effect of ginger oleoresin with control via MTT assay for 24 hours. X - axis- blue colour represents the different concentration of Ginger oleoresin extract in 10-200 µg/ml and green colour the control used and Y - axis represents the percentage of cell viability. The cell viability was showing a decrease with increase in concentration of ginger oleoresin. The p value was 0.006 at 20µg/ml and was considered as significant as it was $p< 0.05$

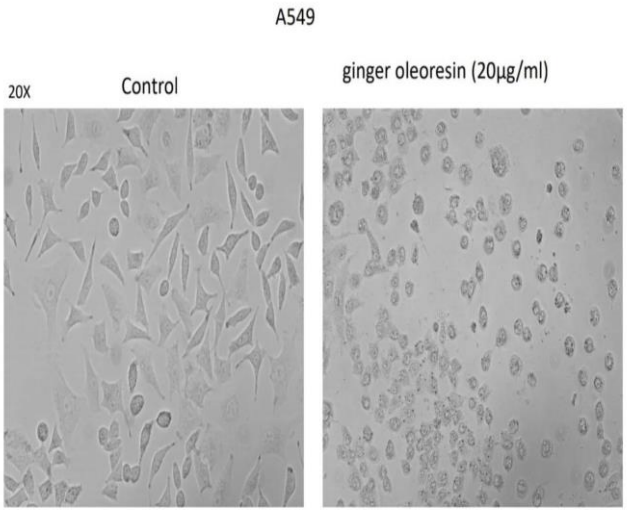

Fig.2: Picture depicts the different morphological representation of lung cancer cells upon treatment with Ginger oleoresin extract for 24 hrs compared to control cells. Images were captured with an inverted phase contrast microscope in 20x magnification.

DISCUSSION

The Cytotoxic activity of the Ginger oleoresin in A549 cells was assessed in the present study using the MTT test. In order to quantify in vitro cell growth modulation, the MTT trial evaluates the number of viable cells. This test is both a cost-effective and a reliable test. In A549 lung cancer line, oleoresin induced an increase in dose-dependent cytotoxicity. The data clearly shows, at certain times of concentration and incubation, that lung cancer cells have been cytotoxic. It demonstrates that about half the viable cells are killed at 20 µg/ml (IC50). Only 50% of cells are viable at 20 µg/ml (Table 1).

Ginger is a spice famous for its distinctive floral scent and magnificent taste with documented curing properties. In field studies in many countries in Asia and Africa, the common use of ginger in the treatment of cancer has been discovered. In-vitro and in-vivo trials have tested the biological validation of its extracts and extracted substances, including gingerols, shogaols, zingiberene and zingerone (33). Hessien, et al, reports the cytotoxic effects of green tea and ginger polyphenolic extracts on human cell line H460, which are mediated by antiproliferation and apoptosis, signalling their promising chemical prevention effect on lung cancer (34). Dwivedi, et al, findings indicate that eugenol could be a possible antitumor agent for a variety of cancer cells, based on their sensitivity (35). Curcumin, ginger oleoresin and rutin solid lipid nanoparticles are a novel mix of Vietlife Antican (VLA), which all exhibit low toxicity and enhanced bioavailability. Thao, et al, concluded that VLA may be a very promising and effective pathogens that help in the prevention or treatment of cancer (36). Previously, our team has conducted various studies based on nanoparticles (37), (38), (39), (40), (41), (42), (43), (44), (45), (46), (47), (48), (48,49), (50), (50,51), (52), (53).

Limitations of this study is that, since it is an in vitro analysis, only the laboratory and therapeutic use of the herb was analysed but not about its effects and interaction inside the body. Further studies should concentrate more on the assessment of the interaction of the Ginger Oleoresin in vivo models .

CONCLUSION

The number of viable cells decreases as the drug concentration increases(54)-(55). Therefore, the present study concludes that ginger oleoresin possesses a strong cytotoxic effect. Hence, it is a promising candidate for anticancer therapy.

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CONFLICT OF INTEREST

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

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