

CYTOTOXIC EFFECT OF CORIANDER OLEORESIN AGAINST LUNG CANCER CELL LINE A549

ABSTRACT

Aim: The main aim of present study was to assess the cytotoxic effect of coriander oleoresin against lung cancer cell line A549.

Introduction: *Coriandrum sativum* (Coriander), family *Umbelliferae*. Coriander contains mainly essential oil and has antioxidant, diuretic, anti-diabetic anticonvulsant, hypnotic sedative, anti-mutagenic, antimicrobial, anthelmintic activity.

Materials and methods: Coriander oleoresin (product number: 4010000243) was obtained from Synthite Industries Private Limited, Kerala. In the present study, lung cancer cell line A549 was treated with coriander oleoresin at different concentrations and later evaluated for its cytotoxic activity using MTT assay.

Results: The cytotoxic effect of coriander oleoresin on lung cancer cell line was proved; the drug concentration increased, the percentage of cell viability decreased proving its cytotoxic effect. The coriander oleoresin has shown a dose dependent cytotoxic effect on lung cancer cell lines. As the drug concentration increased, the percentage of cell viability decreased proving its cytotoxic effect. The IC_{50} value was 80 $\mu\text{g/ml}$.

Conclusion: In the present study, coriander oleoresin showed a good cytotoxic effect on lung cancer cell lines which may be helpful in treatment of lung cancer. However more research is needed to understand the underlying mechanisms of the cytotoxicity property of the plants.

Keywords: coriander oleoresin, lung cancer, therapeutic properties, cytotoxic activity, new approach

Running title: Cytotoxic effect of coriander oleoresin against lung cancer cell line A549.

INTRODUCTION

Coriandrum sativum (Coriander) part of the *Umbelliferae* family, is a herbaceous vine of Mediterranean and Middle Eastern origin. Coriander contains mainly essential oil and has antioxidant, diuretic, anti-diabetic anticonvulsant, hypnotic sedative, anti-mutagenic, antimicrobial, anthelmintic activity. The effect of the commercial value of coriander depends on

its physical properties, chemical composition, and bioactivity. Linalool (78.45 percent), alpha-pinene (5.03 percent), camphor (3.90 percent), gamma-terpinene (3.80 percent), D-limonene(2.58 percent) and geranyl acetate (2.13 percent) are the key components of Coriander oleoresin (1). Coriander oleoresin is a natural food additive. It is obtained by solvent extraction of fruits of *Coriandrum sativum*. It is a dark brown volatile liquid used in the pharmaceutical industry. Fresh coriander's distinctive smell is due to the presence of aldehyde in volatile oil.(2).

Coriander seed is used by people for treatment. Coriander is used for problems of digestion, such as stomach pain, lack of appetite, hernia, nausea, diarrhoea, bowel spasms, and intestinal gas. It is often used to treat diseases caused by bacteria and fungi, such as measles, haemorrhoids, toothaches, worms, and joint pain. Coriander is used by some breast-feeding women to improve milk supply. In manufacturing, coriander is used in medications (3). Uncontrolled growth of cells and apoptosis resistance characterise cancer cells via mutations in key signaling molecules, which regulate pathways that are directly involved in regulating cell proliferation and apoptosis, these two main features are initiated in cancer cells (4).

Cell lines have been helpful in elucidating essential signaling pathways in lung cancers, as well as in developing therapeutic applications based on these studies. Lung cancer cell lines have been spread widely among the research community, which have used them to great effect, and this trend is expected to continue (5). In an article written by Bu- Yeo Kim et al, they stated that the seeds of *D. sophia* had potent cytotoxic effect on lung cancer cells A549. Seeds of *D. sophia* treatment induced dose dependent response in lung cancer cells A549 that showed down regulation of genes linked with metabolic functions and up- regulation of a large group of genes linked with cell- growth related signaling pathways. It was found that the reciprocal regulatory mechanism may inhibit growth in human lung cancer A549 when treated with seeds of *D. sophia*. But the underlying mechanism must be understood for further future studies (6). Understanding the ability of herbal extracts and formulations to modulate metabolising enzymes will aid the health care system in providing adequate medication for patients and avoiding much of the side effects that come with it. Our team has extensive knowledge and research experience that has translated into high quality publications (7–12). The main aim of this study was to assess the cytotoxic effect of coriander oleoresin against lung cancer cell line A549.

MATERIALS AND METHODS

Study setting: Cell line Research was conducted in Blue Lab, Saveetha Dental College. The study was approved by the Scientific Review Board, Saveetha Dental College, Chennai. [IHEC/SDC/UG-1926/21/91].

Reagents and extract:

Coriander oleoresin (product number: 4010000243) was obtained from Synthite Industries Private Limited, Kerala. 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

The A549 cells were cultured in DMEM supplemented with 10% of phosphate buffer and 1% physiological saline. Cell cultures were maintained at 37° C in a fully humidified atmosphere containing 5% CO₂

Cell treatment

Coriander oleoresin was dissolved in 0.1% DMSO (v/v). A549 cells were plated at 1.2 X 10⁴ cells/cm². Twenty-four hours later, cells were fed with fresh expansion culture medium supplemented with different final concentrations of extract (20, 40, 80, 100, 120 and 140 µg/ml) or the corresponding volumes of the vehicle. After 24 h of treatment, cells were collected after 0.05% trypsin application. Cell viability was evaluated using the MTT assay.

MTT Assay

The effect of coriander oleoresin on cell viability was assessed using the MTT assay according to Mosmann's method in an in vitro cytotoxicity test.~~*In vitro cytotoxicity assay the effect of coriander oleoresin on cell viability was measured by MTT assay following the method by Mosmann.*~~ Briefly, the cells (1 × 10⁵ cells per ml) were seeded in a 96 well microtiter plate (100 µl per well) with replications. Treatments with varied concentrations of coriander oleoresin~~Treatment was conducted for 24 with different concentrations~~ (20, 40, 80, 100, 120 and 140 µg/ml) were carried out for 24 hours. of coriander oleoresin.~~Following incubation, After incubation,~~ 20 µl of 5 mg/ml MTT stock solution was added to each well and incubated ~~for 4 h~~ at 37 °C for 4 hours.~~The formed formazan crystals were dissolved in DMSO and the absorbance was measured with a microplate reader at 570 nm (SpectraMax M5, Molecular Devices, USA).~~ The ratio of absorbance (A₅₇₀) in treated cells to absorbance in control cells (0.1 percent DMSO) was used to calculate cell viability (percentage) (A₅₇₀). In comparison to the DMSO-treated control, the IC₅₀ was computed as the sample concentration required to diminish 50% of the absorbance.~~The obtained formazan crystals were solubilized 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 (A₅₇₀) in treated cells to absorbance in control cells (0.1 % DMSO) (A₅₇₀).~~The IC₅₀ was calculated as the concentration of sample needed to reduce 50 % of the absorbance in comparison to the DMSO treated control.~~ Percent cell viability was calculated following the equation:

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

Morphological Study:

Based on MTT assay we selected the **IC₅₀** value of Coriander oleoresin for further studies. The characterization of morphological changes in lung cancer cells before and after treatment with Coriander oleoresin were observed under phase contrast microscope.

Statistical Analysis:

All data obtained were analyzed and computed statistically (SPSS/10 Software Package; SPSS Inc., Chicago, IL, USA) using one-way ANOVA. In all tests, the level of statistical significance was set at $p < 0.05$.

RESULTS

In the present study, the cytotoxic effect of coriander oleoresin against lung cancer cell line A549 was evaluated using MTT assay. MTT assay provided a quick, simple and cost effective way for testing the cytotoxic activity of coriander oleoresin. In this study, lung cancer cell lines were treated with different concentrations of the extract of coriander oleoresin for 24 hours. Coriander oleoresin extract caused a dose dependent increase in the cytotoxic activity of lung cancer cell lines. Morphological changes and apoptosis of lung cancer cells were observed using coriander oleoresin under inverted phase contrast microscope at 20x magnification (figure 1). As the concentration of the extract increased, the percentage of cell viability decreased which depicted **significant cytotoxic activity** of coriander oleoresin against lung cancer cell line (figure 2). The mean standard deviation (n=3) was calculated by student t- test using MS- Excel.

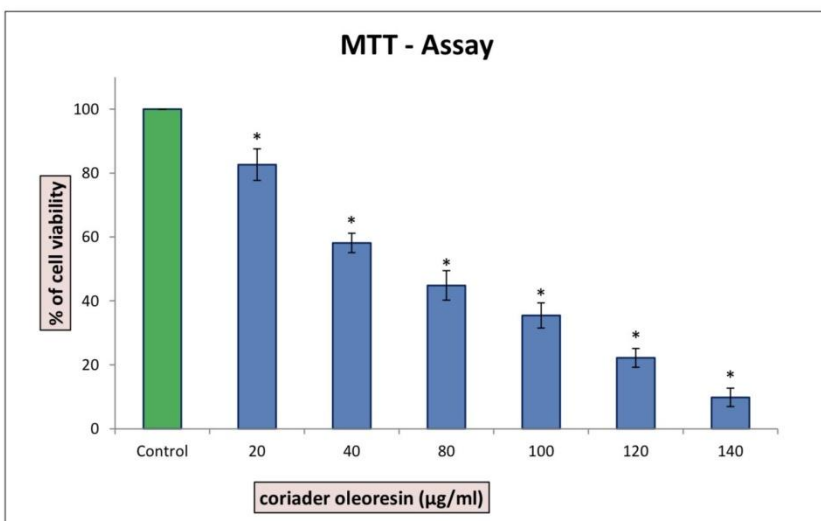


Figure 1: The graph represents the cytotoxic effects of coriander oleoresin on lung cancer cells. Cells were treated with coriander oleoresin (20, 40, 80, 100, 120 and 140 µM) for 24 hours, and cell viability was evaluated by MTT assay. Green denotes the control sample whereas blue denotes the different concentration of coriander oleoresin. X-axis denotes control of the different concentration of coriander oleoresin in. And the Y-axis denotes the percentage of cell viability. Data are shown as means \pm SD (n = 3). * compared with the control group, $p < 0.001$ by student-t test using MS- Excel. The IC_{50} value was found to be 30 µg/ml in 140 µg. The cell viability was also found to be 10%. Thereby the results show that as the drug concentration increases, the percentage of cell viability decreases proving coriander oleoresin's cytotoxic effect.

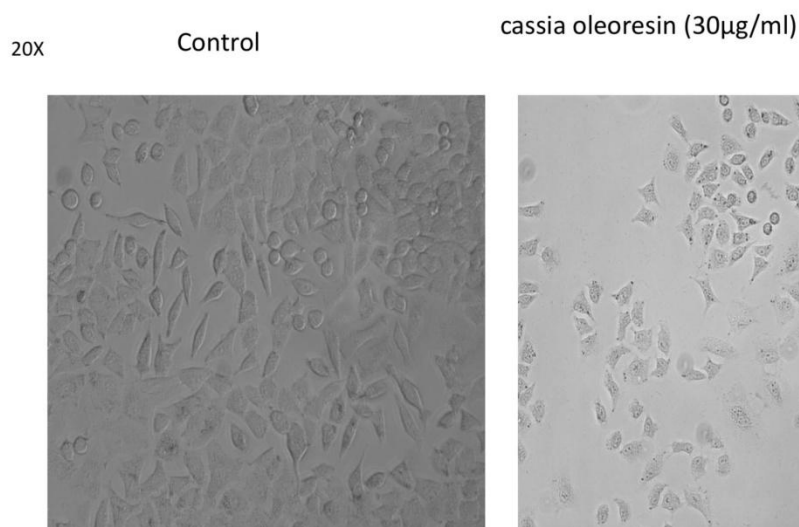


Figure 2: Assessment of cell morphology of lung cancer cells treated **without or with** coriander oleoresin. Cells were treated with coriander oleoresin (30 µg/ml) for 24 hours along with the control group. Images were obtained using an inverted Phase contrast microscope at 20x magnification.

DISCUSSION

In the present study, lung cancer cell line A549 was treated with coriander oleoresin at different concentrations and later evaluated by MTT assay (Figure 1, Figure 2). In another research done by Filomena Silva et al, coriander oil had antimicrobial activity against all bacteria that have been tested in the study. With the exception of *Bacillus cereus* and *Enterococcus faecalis*, coriander oil was found to have bactericidal activity against nearly all bacteria tested. Coriander oil's main mechanism of action was found as membrane disruption, which contributes to cell death. The findings of the study support the use of coriander oil in antibacterial formulations since it efficiently destroys pathogenic bacteria linked to foodborne diseases and hospital infections (13). P. Kowshihan et al has stated that after preparing coriander oleoresin mediated silver nanoparticles and confirmed by using UV- Visible spectroscopy, it was evaluated for its antifungal and antibacterial properties against *S. mutans*, *Lactobacillus*, *S. aureus* and *C. albicans*. It was seen that coriander oleoresin mediated silver nanoparticles had a good antimicrobial effect and can be used against the above mentioned infections (14).

The MTT assay, first defined by Tim Mosmann in 1983, is the most widely used viability assay in the world. The assay is based on metabolically active cell transferring of soluble yellow tetrazolium salt to insoluble purple formazan crystals. The tetrazolium salt can only be taken up

by living cells. Internalized tetrazolium salt was converted to purple formazan crystals by an enzyme (mitochondrial dehydrogenase) located in the mitochondria of living cells (15, 16).

Prathapan et al used the same method for studying the effect of lutein on differentiation and the cells were incubated at different concentrations later quantified using multiple plate readers at 490 nm. This preparation was similar to the preparation used in the present study (17,18). The leaves of coriander show stronger antioxidant activity than seeds, but in both the parts of coriander, ethyl acetate extract showed the strongest ability. Addition of coriander to food increases antioxidant content and may have potential to act as a natural antioxidant to inhibit unwanted oxidation process (19-33). Surgery, radiofrequency, cryosurgery, chemotherapy, radiotherapy, targeted therapy with monoclonal antibodies, and angiogenesis inhibitors are all common therapeutic options. These are successful preventive screening programs in treatment options. Due to a rise in cancer-related mortality and the harmful or toxic side effects of cancer chemotherapy and radiation therapy, new anticancer agents have been extracted from plants and also have been identified as screening of medicinal plants as a source of anticancer molecules (34,35).

Coriander oleoresin exhibits antimicrobial activity against gram- negative and gram- positive bacteria, yeast, dermatophytes and filamentous fungi. Anti cancer effect of coriander oleoresin, source of dietary fibres and manganese, iron and magnesium helps increase levels of good cholesterol (HDL) (36). Selenium nanoparticles mediated by coriander oleoresin had strong anti-inflammatory efficacy, suggesting that it could be used for a healthy and environmentally sustainable synthesis of selenium nanoparticles for inflammatory conditions (37,38). Our team has done extensive research and published many scientific work (39–46). The cytotoxic effect of coriander oleoresin on lung cancer cell line was proved; the drug concentration increased, the percentage of cell viability decreased proving its cytotoxic effect. Many studies shows dose dependent activity (45,47- 49). Limitation seen in the present study is that the mechanism of cytotoxicity of coriander oleoresin could be understood better for future studies. The study may be extended in animal models in the future.

CONCLUSION

The use of coriander in daily life is quite common and it also has no side effects. The therapeutic value of coriander such as cytotoxic, antioxidant, antimicrobial activity plays a very important role in improving human health. From the present study, coriander oleoresin shows a dose-dependent cytotoxic effect on lung cancer cell line which may be helpful in treatment of lung cancer. However more research is needed to understand the underlying mechanisms of the cytotoxicity property of the plants.

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CONFLICT OF INTEREST: Nil

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