

Apoptotic Effect of Cissus Quadrangularis Ethanolic Extract on A549 Human Lung Cancer Cells

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ABSTRACT

Cissus quadrangularis (CQ) extracts are used as various remedies for pain relief, bone regeneration, ulcerations, diuretics, mitigation and treatment of cancer. In addition to antiinflammatory and osteoporotic benefits, the ethanolic extract also has considerable apoptotic effects which effectively suppresses tumor cells upon activation by the mitotic signalling pathways. The present study aimed to investigate whether the ethanolic extract of Cissus quadrangularis possessed apoptotic effects against human lung cancer cells. For the present study, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay was employed to determine the viability of the human lung cancer cells upon treatment with different concentrations of the ethanolic extract of Cissus quadrangularis. The percentage of viable cells was calculated, at 570 nm, based on the absorbance of the resultant formazan crystals after treatment with 0.1% DMSO solution. From the results of the MTT assay, it was concluded that when the concentration of the plant extract was increased, the viability of the lung cancer cells significantly decreased, and the IC₅₀ values of CQ was found to be 25 µg/ml which inhibits 50% viability of the human lung cancer cells at this concentration. Thus, the ethanolic extract of Cissus quadrangularis had significant cytotoxic effects on the human lung cancer cells and can be used as a potent treatment modality for the same.

KEYWORDS:

Lung cancer, CQ, apoptosis, cytotoxicity, innovative technique.

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INTRODUCTION

Lung cancer is one of the world's top most leading causes of death, affecting men and women of all age groups equally. Every one in seventeen women possess a risk towards this disease. The most common cause of lung cancer is the excess consumption of tobacco, obtained from the Nicotiana tabacum plant. Parallel to this, the economic development of the country, exposure to radiation, air pollution, inhalation of asbestos, age, gender, race, geography, socioeconomic status, use of marijuana, biomass burning, infections,

inappropriate diet and other genetic factors also contribute to the onset of lung cancer (1). In depth, lung cancer can be subdivided into various types or classes based on the molecular and histological variations. The most abundant ones include small cell carcinoma, squamous cell carcinoma, adenocarcinoma and large cell carcinoma. Molecular markers present in the human body can help in early detection and diagnosis of these tumour cells (2). Chronic use of tobacco leads to the formation of N-nitrosamines whose main mechanism of action is the nitrosation of nicotine. This in turn paves way for the formation of DNA adducts, thus

inducing damage to cells by free radical mechanisms. The mortality rates for lung cancer can be improved by early screening through methods such as computed tomography (CT) and computerized axial tomography (CAT). Large scale molecular profiling helps in identification of molecular targets to cancer in the body (3). In cases independent of nodal involvement, surgical confirmation can be employed. Staging of cancer is based on two major methods i.e. TNM staging and AJC staging which depends on the severity of the disease (4).

Cissus quadrangularis Linnaeus, belonging to the Vitaceae family, is a tropical or subtropical xeric wood plant. Its extracts are used as various remedies such as pain relief, bone regeneration, ulcerations, diuretics and mitigation (5). The ethanolic extract of this plant possesses less antioxidant activity but affects growth kinetics in a dose dependent manner. In addition, anabolic and catabolic effects are exercised for relatively inferior concentration. The antiapoptotic effect of *Cissus quadrangularis* occurs by interfering with cellular processes after the mitotic signalling pathway activation. The activation of MAP kinase signaling in human lung cancer cells increases the expression of Bcl2 during apoptosis (6). The analgesic effect is based on the anti-osteoporotic activity of the ethanolic extract whose effect was due to the activation of alkaline phosphatase enzyme thus decreasing the osteoclastic activity of the bone (7). Some of the general effects include reduction in body pain and weight, maintenance of cholesterol level and blood sugar level (8,9). The *cissus* plant has significant free radical scavenging potential which is analogous to some of the anti cancer drugs present in the market and is hence used for the treatment of various tumours (10)

Despite previous researches conducted on the ethanolic extract of *cissus quadrangularis*, its effects were in relation to skin cancer and only the antiinflammatory property was taken into account. Our team has extensive knowledge and research experience that has translate into high quality publications (11-15),(16),(17),(18),(19),(20),(21),(13,22,23),(24-28)),(29),(30)). The present study aims at determining the apoptotic effect of the ethanolic extract of *Cissus quadrangularis* on human lung cancer cells and to predict its use in advanced research on the same(31) (32) (33) (34).

MATERIALS AND METHODS

CHEMICALS USED

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.

Preparation of the herbal extract

For the present study, about 50g of *Cissus quadrangularis* (CQ) stem powder commercially obtained from IMPCOPS (Chennai, India) was soaked in 500ml of 95% ethanol. A static condition was maintained for 3 days at room temperature. The fine filtrate, obtained by filtering the solution with crude filter paper followed by whatmann filter paper, was subjected to rota evaporation from which 3g of the material was derived. The total ethanolic extract was stored at 4°C immediately after concentration in vacuum evaporate.

CELL CULTURE REAGENTS

Dulbeccos Minimal Essential Media (DMEM)

Commercially available DMEM consists of 7.5% sodium bicarbonate solution. 5ml of penicillin/ streptomycin solution and 0.5ml of amphotericin B solution was added to 500ml of DMEM. The medium was then stored in a sterile container at a temperature of 4°C after sterile filtration (0.22 μ) inside the hood.

Growth medium (DMEM with 10% FBS)

Using sterile DMEM, 10ml of FBS was made up to 100ml. The solution was stored in a cool and aseptic condition with the help of a sterile container.

Phosphate Buffered Saline (PBS; pH 7.4)

To 500ml of double autoclaved milliQ water, 0.63g of sodium phosphate monobasic (NaH₂PO₄), 0.17g of sodium phosphate dibasic (NaHPO₄) and 4.5g of sodium chloride (NaCl) were dissolved. With the help of 1N HCl and 1N NaOH, the pH was adjusted to 7.4 followed by using sterile filtration (0.22 μ) and storage in a sterile container.

Trypsin-EDTA Solution

Trypsin was purchased as 1x 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).

0.89% Physiological Saline

To 100ml of double autoclaved milliQ water, 890 mg of sodium chloride was dissolved. Following the addition of sodium chloride, the solution was filtered for sterility.

Cell Line

From the National Centre for Cell Science (NCCS, Pune), India, the A549 Human lung adenocarcinoma cell line was obtained. The growth of cells occurred in T25 culture flasks containing DMEM medium supplemented with 10% FBS. After confluence, Trypsin-EDTA solution was employed for the detachment of the cells.

Cytotoxicity Analysis

In order to determine the anticancer activity of the ethanolic extract of *Cissus quadrangularis*, MTT (3-(4,5-dimethylthiazol-2yl)-2, 5-diphenyl tetrazolium bromide) assay was employed to test the cytotoxic activity according to the method describes by Koka P et al, 2018 (35). The cells were seeded in 96-well microtiter plates at a density of 5x10³/100µl. The cells were treated with ethanolic extract of *Cissus quadrangularis* after 24 hours of incubation at concentrations of 50,100,150,200,250 and 300 µg/ml. The negative controls were cells with extract free medium. 10µl of MTT reagent was added to each well after incubation at 37oC for 24hours followed by subsequent incubation at the same temperature for 4h in dark. For solubilizing the formazan crystals, 100µl of Sorenson glycerine buffer consisting of 0.1M glycerine, 0.1M NaCl, pH 10.5 with 0.1N NaOH was added. The absorbance was subsequently measured at 570 nm. The experiment was repeated thrice and each concentration was tested. The percentage of cell viability was calculated as follows:

$$\text{Cell viability \%} = \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Inhibition %= 100- cell viability %

Statistical Analysis

All data obtained were analyzed by Students-t-test using MS-Excel, represented as mean ± SD for six animals in each group. Statistical analysis utilizing one way ANOVA (SPSS/10 Software Package; SPSS Inc., Chicago, IL, USA) was performed. For intercomparison including LSD, post-hoc testing was recruited. p<0.05 was rooted as the statistically significant value.

RESULTS

The ethanolic extract of *Cissus quadrangularis* induces apoptosis in dose and time dependent manner effects against the human lung cancer cells. Based on the MTT assay, we observed the cell proliferation was inhibited as 20% at 50 µg/ml, 35% at 100 µg/ml, 40% at 150 µg/ml, 60% at 200 µg/ml, 70% at 250 µg/ml and 90% at 300 µg/ml at 24hrs treatment respectively. The number of viable lung cancer cells were gradually decreased as the concentration of the extract increased.

The morphological examination of A549 lung cancer cells treated with *Cissus quadrangularis* 25µg/ revealed the morphological changes indicating cell death via apoptosis. 5r`

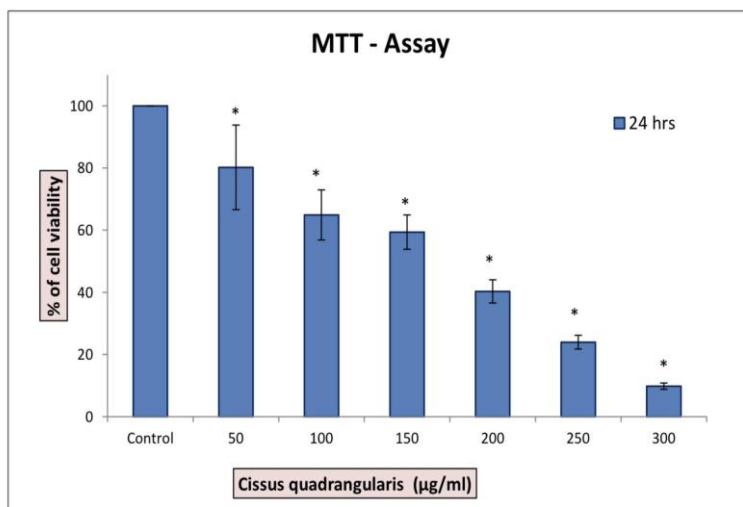


Fig.1: Graph depicting the apoptotic effect of *Cissus quadrangularis* ethanolic extract on human lung cancer cells. The x-axis represents the concentration of the plant extract and the y-axis represents the percentage of cell viability. We infer that as the concentration of the extract increases, the percentage of cell viability decreases non-uniformly. Data are shown as means ± SD (n = 3). * compared with the control-blank group, p < 0.001.

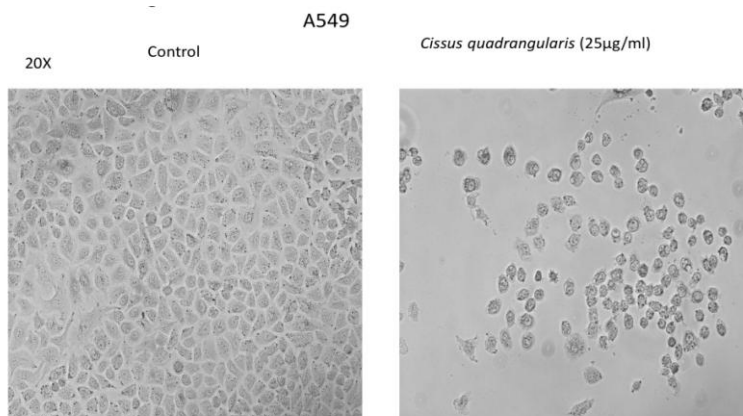


Fig.2: Assessment of cell morphology of A549 cells treated without and with *Cissus quadrangularis*. Cells were treated with *Cissus quadrangularis* (25µg) for 24 h along with the control group. Images were obtained using an inverted Phase contrast microscope (20x).

DISCUSSION

In the present day, extracts of naturally occurring herbal plants have become an increasingly essential for the development of multi treatment strategies against different types of cancer. The ethanolic extract of *Cissus quadrangularis* shows potent apoptotic effects against human lung cancer cell lines in addition to toxicity towards skin cancer (36). The present study indicates that *Cissus quadrangularis* can induce apoptotic effects and at a concentration of 25µg/ml, suppress the growth of the cancer cells (37), (38), (39), (40), (40,41), (42), (43), (44), (45), (46), (47), (48), (49), (50), (51), (52), (53).

The term cancer refers to the uncontrolled growth and proliferation of cells in the body which lose their normal functioning and spread to different parts of the body. The process of apoptosis in general acts as a protective mechanism against tumour cells. For the cancer cells to progress, certain mechanisms to avoid apoptosis must be employed (54). The current treatment modalities for cancer such as chemotherapy, radiotherapy and certain drugs work on the principle of inducing apoptosis in tumour cells but however provide toxic side effects. The use of natural plant extracts can significantly reduce these toxic effects and are hence becoming a popular treatment regime.

Induction of apoptosis by the ethanolic extract of *Cissus quadrangularis*, leads to membrane damage in the mitochondria of cells in turn causing release of the enzyme cytochrome c (55). The extract was known to trigger the production of free radicals which activated the apoptotic pathway and led to breakdown of the genetic material (56). Thus apoptosis in lung cancer cells occurs through a series of reactions including cytochrome c enzyme, Bcl-2 gene and Bax gene. In addition to apoptotic effects, the extracts of *Cissus quadrangularis* can be used as a potent anti-inflammatory agent and antioxidants. These effects are effective against various other cancer types and other diseases (8)

Based on previous researches conducted, the major components of *Cissus quadrangularis* were found to be esters, phenols and phytosteroids. These compounds collectively were responsible for damaging the cancer cells through

apoptotic mechanisms (8,9,57). The ethanolic extract of this plant can also be useful for bone regeneration, inhibition of cell proliferation and angiogenesis. Parallel to the variations seen in cancer cells, the osteoblastic activity of *Cissus quadrangularis* also varies inversely with the concentrations of the extract (58).

Despite these positive effects of *Cissus quadrangularis*, the consumption of its extract in the long run poses several side effects. Some of these include drop in body weight, low cholesterol levels and decreased fasting and random blood sugar levels. During the initial days of intake of the plant extract, the toxicity levels remain low and subsequent consumption generates higher concentrations of toxic free radicals in the body (59). On consumption of the extract in pregnant women, the ossification of the fetal long bones was found to be elevated by the action of the phytochemical steroid component of this plant (8,9,57,60).

The modern, synthetic drugs used for the diagnosis and treatment of cancer produce other responses in the body such as vomiting, anemia, constipation, hair loss and skin reactions(27) (61) (62) (63) (64) (65) (66) (67) (68) (69) (70) (71) (72) (73) (74). These effects proved to be less significant when extracts such as *Cissus quadrangularis* were employed. However, the present study provided certain limitations such as restriction to in vitro analysis and limited tests to determine the cytotoxic effects of the extract.

CONCLUSION

The present study proved to be useful in determining the apoptotic effects of the ethanolic extract of *Cissus quadrangularis* on human lung cancer cells and provides scope for future research which may use the present findings on engineering natural drugs of *Cissus quadrangularis* against lung cancer.

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CONFLICT OF INTEREST

The author declares that there was no conflict of interest in the present study.

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