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AN INVITRO APOPTOTIC SCREENING OF AQUEOUS SEED EXTRACT FROM SOLANUM VIRGINIANUM IN HUMAN LUNG CANCER CELLS

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ABSTRACT

AIM: The objective of the study was to examine the apoptotic activity of aqueous extract of *solanumvirginianum*on human lung cancer cells.

METHODS: 150g of S. virginianum was soaked in double distilled water and kept at 37°C for 3 days. The solution was well prepared and filtered by whatman II filter paper. Further it was concentrated into approx. 3g of plant extracted samples by rotary evaporation. The apoptotic potency of *S.virginianum* was measured by MTT assay against A549 cell line and it was confirmed by morphological evaluation using phase contrast microscopy.

RESULTS: The aqueous extract of *S.virginianum*showed significant dose dependent apoptotic potency by inhibiting 50% at 80µg/mL (IC_{50}) cell proliferation against MCF-7 cell line. which was further confirmed by morphological evaluation using phase contrast microscopy.

CONCLUSION: From the results, the extracts were apoptotic to lung cancer cells at this concentration and incubation period. However more research is needed to understand the mechanism of apoptotic properties of plants.

KEYWORDS: Apoptosis, MCF-7, *solanumvirginianum*, Cell proliferation.

INTRODUCTION

Solanumvirginianum, also known as Surattense nightshade, yellow-fruit nightshade, yellow-berry nightshade, Thai green eggplant, Thai striped eggplant (from unripe fruit), is also known as Indian nightshade or yellow berry nightshade, commonly referred to as Kantakari, SolanumsurattenseBurm.f. andSolanumxanthocarpumSchrad(1). andWendl. are synonyms of Solanumvirginianum L. It is also a medicinal plant primarily used in India.(2)

Lung cancer, also known as lung carcinoma, is a malignant lung tumourcharacterised by unregulated cell growth of lung tissue. This development will extend outside the lung by metastasizing to surrounding tissues or other areas of the body(3). The majority of cancers that begin in the lung, known as primary lung cancers, are carcinomas. The two major forms are small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC)(4) . The most frequent signs include coughing (including coughing up blood), weight loss, shortness of breath, and chest pain. (5)

The overwhelming majority (85 per cent) of cases of lung cancer are due to long-term consumption of tobacco. Nearly 10–15% of cases arise in individuals who have never smoked. These cases are mostly due to a combination of genetic causes and exposure to radon gas, asbestos, second-hand smoke or other sources of air pollution.Lung cancer can be seen on chest radiographs and computed tomography (CT) scans.(6) Diagnosis is confirmed by a biopsy normally done by a bronchoscopy or a CT-guidance.(7)

The main form of treatment is to avoid risk factors, including smoking and air quality. Treatment and long-term results depend on the type of cancer, the stage (degree of spread) and the general condition of the individual.(8) Any of the instances are not curable. Common care includes surgery, chemotherapy, and radiotherapy. NSCLC is often treated with surgery, while SCLC usually responds best to chemotherapy and radiotherapy.(9,10)

Lung cancer has occurred in 1.8 million people worldwide in 2012 and has resulted in 1.6 million deaths.(11) This is the most common cause of cancer-related death in men and the second most common cause of cancer-related death in women after human lung cancer(12)(13)(14)(15).The most common age at diagnosis is 70 years.(16)

Our team has extensive knowledge and research experience that has translate into high quality publications (17–21),(22),(23),(24),(25),(26),(27),((19,28,29),(30–34) .,(35),(36)

MATERIALS AND METHODS

CHEMICALS:

SDMEM 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 EXTRACT:

Solanumvirginianum herbal powder commercially purchased IMPCOPS - Chennai (Indian Medical Practitioners Co-operative Pharmacy and Stores Limited). 150g of sample was immersed in double distilled water for 3 days at 37°C temperature. The solution was placed in a rotary vacuum evaporator to concentrate fine filtered samples and leftover solvent was evaporated to dryness in a hot air oven. 3 grammes of material was obtained and immediately sorted at 4°C, for further experiments.

The required quantity of the herbal extract was weighed and dissolved in DMSO with concentration of 1mg/ml as a stock solution. This solution was subsequently diluted to a series of concentrations ranging from 20 to 300 µg/ ml for cell viability assay.

MTT ASSAY:

The cytotoxic effect of S.virginianum on MCF-7, were measured with MTT (3-(4, 5-dimethyl thiazol-2 yl)-2, 5-diphenyl tetrazolium bromide) assay by Alam(37) Cells were seeded in 96-well plates at the density of $5x10^3$/100µl and treated with different concentrations (0, 20, 40, 80, 100, 200 and 300 µg) of S.virginianum for 24hrs. After 24hrs incubation, 20 µl of 5 mg/ml MTT stock solution was added to each well and incubated for 4 h at 37 °C. 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 (A570) in control cells (0.1 % DMSO). The IC_{50} 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:

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

Inhibition (%)= 100- cell viability (%)

STATISTICAL ANALYSIS:

All data obtained were analyzed and 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 $p<0.05$

RESULTS

An invitro antiapoptotic screening of aqueous seed extract from *Solanum virginianum* in human lung cancer cells

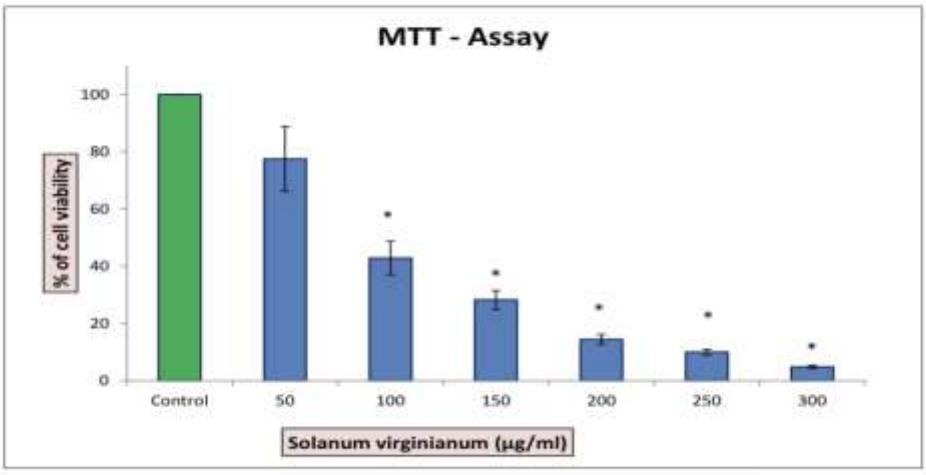

Figure 1: The apoptotic effects of S.virginianum on MCF-7 cells was determined by MTT assay. The Cells were treated with different concentrations (0, 50, 100, 150, 200, 250 and 300 µg) for 24hrs. The 50% of cancer cell growth inhibition was observed at 80 µg/ml concentration, which has been considered the IC50 value of that herbal extract and fixed for morphological evaluation.
Data are shown as means ± SD (n = 3). * compared with the control-blank group, $p < 0.001$

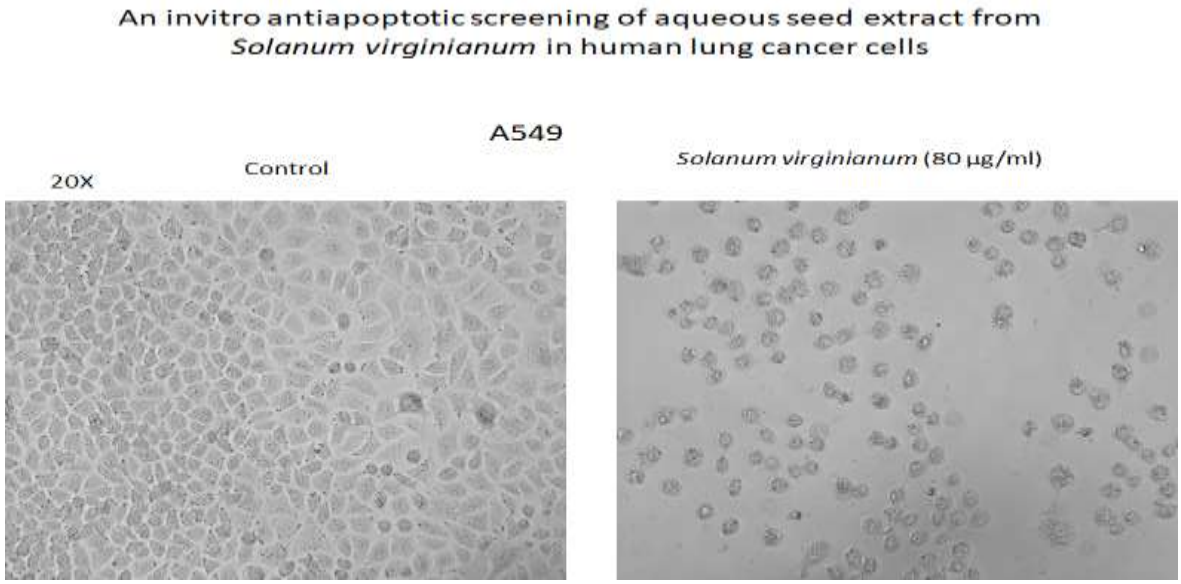

Figure 2: Represents the morphological alteration in human lung cancer cell line upon treatment with *S.virginianum*(80µg/mL) for 24hrs was compared with control cells by phase contrast microscope at 20x magnification.

Discussion:
Lung cancer is a disease with high morbidity and mortality rates. As a result, it is often associated with a significant amount of suffering and a general decrease in the quality of life(38). Herbal medicines are recognized as an attractive approach to lung cancer therapy with little side effects and are a major source of new drugs.(39). Lung cancer is actually the malignant tumour with the highest mortality rate in the world, owing to the fact that it is often not diagnosed until the disease has progressed significantly, resulting in a drastic reduction in the patient's quality of life(10). Lung cancer is the leading cause of cancer-related death globally. Despite many advanced approaches to treat cancer, they are often ineffective due to resistance to classical anti-cancer drugs and distant metastases. Currently, alternative medicinal agents derived from plants are the major interest due to high bioavailability and fewer adverse effects. Tannins are polyphenolic compounds existing as specialized products in a wide variety of vegetables, fruits, and nuts. Many tannins have been found to possess protective properties, such as anti-inflammatory, anti-fibrotic, anti-microbial, anti-diabetic, and so on. Plants have long been used to treat cancer, and they continue to be a significant source of new drugs(8,10) Herbal medicines have been described as one of the most appealing approaches for lung cancer treatment since they have been shown to be useful and effective in sensitising traditional agents, extending patient recovery time, reducing chemotherapy side effects, and improving quality of life (40). In the study done by (6)Tv-AgNPs displayed potential antibacterial, and anti-proliferative activities by inducing the ROS, oxidative stress, DNA division, nucleus damage, and apoptosis in both cancer and bacterial cells.

CONCLUSION
The present study revealed an inhibitory concentration of the aqueous extract of solanumvirginianum on lung cancer cells(33)(41)(42)(43)(44)(45)(46)(47)(48)(49)(50)(51)(52)(53)(54). The results from MTT assay and morphological evaluation have clearly indicated that *S.virginianum* significantly inhibits cell growth by inducing apoptosis in cancer cells. 50 percent of the cells, and concluded that had a marginal effect on the cell viability of the lung cancer cells. In other words, it has an apoptotic influence on MCF-7.

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