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Anticancer Activity of a Poly-Herbal formulation 'Tulsi & Licorice Herbal Green Tea' on A549 Human Lung Carcinoma Cell Line

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A B S T R A C T

Smoking tobacco has emerged as a growing concern among young adults in both urban and rural populations, with well-established correlations to lung cancer and respiratory diseases such as chronic bronchitis and Chronic Obstructive Pulmonary Disorders (COPD). Additionally, non-smokers face risks through passive smoking in their environments. In this context, traditional folklore medicine offers potential solutions, utilizing certain herbs known for their efficacy in treating bronchitis and other lung ailments.

This research investigates the therapeutic potential of a polyherbal formulation, 'Tulsi-Licorice Herbal Green Tea,' composed of green tea leaves, Ocimum sanctum, Glycyrrhiza glabra, Adhatoda vasica, Solanum xanthocarpum, Zingiber officinale, Terminalia bellirica, Piper longum, Curcuma longa, and Quercetin. The study aims to explores its potential anticancer properties using A549 Human Lung Carcinoma cell lines and L132 Lung cell lines.

Cell line studies revealed a notable toxicity of the 'Tulsi-Licorice Herbal Green Tea' on A549 Human Lung Carcinoma cells, with an IC50 value of 32 µg/ml, while L132 cells exhibited an IC50 of 40.4 µg/ml. Morphological examinations further demonstrated the formulation's ability to disrupt lung cancer cell structures, suggesting potential anticancer properties. Subsequent DNA degradation assays confirmed characteristics of apoptosis at the IC50 concentration.

Tulsi & Licorice herbal green tea can be considered as potential anti-cancer agent. Further testing, preclinical and clinical trial needs to be done to for confirmation of therapeutic utility.

Keywords: Smoking, Carcinoma, Lungs, Anticancer, herbal, green tea

Introduction

Lung cancer remains a global health concern, with its incidence steadily rising across diverse demographic landscapes, presenting a formidable challenge to public health systems worldwide. According to recent epidemiological data, lung cancer is a leading cause of cancer-related deaths globally, with an alarming increase in its prevalence, particularly among young adults. In 2020, approximately 2.2 million new cases were diagnosed, accounting for nearly 11.4% of all cancer diagnoses worldwide, and 1.8 million succumbed to the disease, making up 18% of all cancer-related deaths [1].

The demographic distribution of lung cancer demonstrates a complex interplay of factors such as age, gender, socioeconomic status, and geographical location. While historically perceived as predominantly affecting older individuals, there is a rising trend in the incidence of lung cancer among younger populations, underscoring the need for comprehensive strategies that transcend traditional risk factors. Disparities in lung cancer incidence and mortality are evident globally, with higher rates reported in developed regions compared to developing nations [2].

Risk factors associated with lung cancer are multifaceted and include tobacco smoking, environmental pollution, occupational exposures to carcinogens, genetic predisposition, and increasingly, exposure to secondhand smoke [3]. Tobacco smoking, in particular, continues to be the predominant causative factor, accounting for a substantial proportion of lung cancer cases. The prevalence of smoking is influenced by cultural practices, socioeconomic factors, and marketing strategies employed by the tobacco industry.

As the challenges posed by lung cancer intensify, there is a growing interest in exploring unconventional therapeutic approaches, with natural products gaining attention as potential sources of anticancer agents. Traditional herbal medicine, deeply rooted in various cultures, has historically been a rich reservoir of bioactive compounds with diverse pharmacological properties. Herbs are increasingly recognized for their potential in cancer prevention and treatment due to their ability to modulate various cellular processes involved in carcinogenesis [4].

This research article seeks to delve into the anticancer activity of an herbal formulation 'Tulsi & Licorice herbal green tea' using A549 Human Lung Carcinoma cell lines and L132 Lung cell lines and explore novel avenues using herbal ingredients for prevention and management of lung carcinoma. The herbal formulation is comprised of green tea leaves, *Ocimum sanctum* [5], *Glycyrrhiza glabra* [6], *Adhatoda vasica* [7], *Solanum xanthocarpum* [8],

Zingiber officinale [9], *Terminalia bellirica* [10], *Piper longum* [11], *Curcuma longa* [12], and Quercetin. These herbs are known to be useful for respiratory ailments in folklore medicine and Ayurveda system of medicine.

Experiment 1: Study of morphological changes in the cells post Tulsi & Licorice herbal green tea treatment.

Aim: To study morphological changes in the carcinoma cells, post drug treatment.

Materials: Sample of Tulsi & Licorice herbal green tea and Test Cell line A549 (Human Lung Carcinoma cell lines)

Method: Sample was taken in a powdered form. 10mg of the sample was dissolved in 1mL DMSO to give a stock concentration of 10mg/mL. The working concentration of 1mg/mL was used for the studies. A549 cells were revived and 0.05 million cells were seeded in 6 well plate. The cells were incubated in CO_2 incubator at 37° C, 5% CO_2 overnight. After observing the fully confluent cells under microscope the cells were treated with IC50 value (3.2 µL from 1mg/mL) of the drug to observe morphological changes. The cells were incubated 48h in the presence of sample in CO_2 incubator at 37° C, 5% CO_2. Post 48h, media was discarded from the plate. The monolayer was rinsed with DPBS and discarded. Methanol was added to all wells and incubated for 5 minutes. The wells were then air dried. The cells were stained with 0.1% of Crystal violet and incubated for 5 minutes. Cells were mounted and photographed using florescence microscope (EVOS Life Technologies FLC) at 20X magnification under transmitted light.

Observations:

Cells that undergo cell death lose their adherence and are subsequently lost from the population during the staining procedure. The untreated control cells appeared healthy with majority of cells intact while the cells treated with the sample appeared damaged along with evident debris were noticed. The Tulsi & Licorice herbal green tea damages the A549 lung carcinoma cells, suggestive of anticancer property.

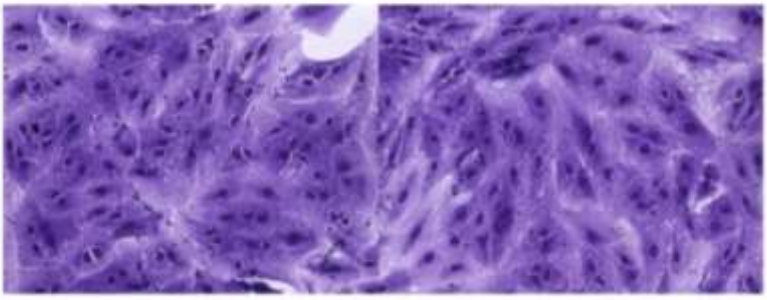

Image 1: Lung carcinoma Control cells (untreated) stained with 0.1% Crystal Violet and viewed under 20X magnification

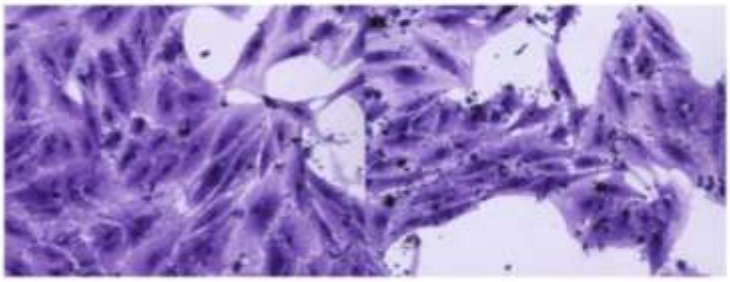

Image 2: lung carcinoma cells (treated) stained with 0.1% Crystal Violet and viewed under 20X magnification

Experiment 2: MTT assay for cytotoxicity - IC 50

Aim: To carry out MTT assay for the Tulsi & Licorice herbal green tea

Materials: Sample of Tulsi & Licorice herbal green tea, A549 Cell Line, L132 Cell Line. A549 (Lung Carcinoma) – Human epithelial lung carcinoma cells. This line was initiated in 1972 by D.J. Giard, et al. through explant culture of lung carcinomatous tissue from a 58-year-old Caucasian male. L132 (Lung cell line)- Human epithelial cells. This line was originally thought to be derived from embryonic lung tissue, but was subsequently found, based

on isoenzyme analysis, HeLa marker chromosomes, and DNA fingerprinting, to have been established via HeLa cell contamination. The cells are positive for keratin by immunoperoxidase staining.

Method: Sample preparation**:** Sample was taken in a powdered form. 10mg of the sample was dissolved in 1mL DMSO to give a stock concentration of 10mg/mL. The working concentration of 1mg/mL was used for the studies. A549 cells were revived and 0.05 million cells were seeded in 96 well plate. The cells were incubated in CO_2 incubator at 37° C, 5% CO_2 overnight. After observing the fully confluent cells under microscope the cells were treated with the given samples at 6 different concentrations. The cells were incubated overnight in the presence of sample in CO_2 incubator at 37° C, 5% CO_2. After observing the cells under microscope 10µL of 5mg/mL MTT reagent was added in the wells and incubated for 4 hours. The media was discarded and the formazan crystals were dissolved by adding 100µL of DMSO. The absorbance was measured at 570 nm, the values are as follow. The study was performed in triplicates.

Observations:

The given sample was tested on A549 Cell line for toxicity. The IC50 of the given sample was found to be 3.2 µL i.e. 32µg/mL

µL of sample	**Average OD**	**% viability**	**% cytotoxicity**
2.5	1.632	59.9394	40.0606
5	0.892	32.761	67.239
10	0.213	7.822973	92.17703
20	0.275	10.10008	89.89992
40	0.267	9.806262	90.19374
60	0.286	10.50409	89.49591
80	0.219	8.043339	91.95666

Table 1: MTT assay for cell line A-549

The given sample was tested on L132 Cell line for toxicity. The IC50 of the given sample was found to be 4.03µL i.e. 40.4µg/mL

µL of sample	**Average OD**	**% viability**	**% cytotoxicity**
2.5	1.977	70.607	29.393
5	0.817	29.179	70.821
10	0.279	9.952	90.048
20	0.254	9.083	90.917
40	0.266	9.500	90.500
60	0.233	8.321	91.679
80	0.229	8.190	91.810

Table 2: MTT assay for cell line L132

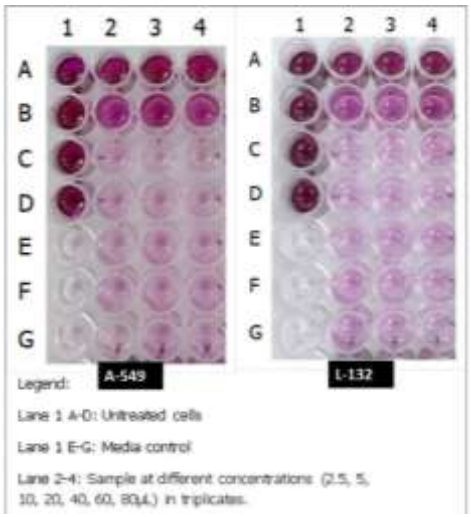

Image 3: MTT assay of A549 and L132 cells for toxicity.

Experiment 3: DNA degradation studies of A549 with Tulsi & Licorice herbal green tea

Aim: To carry out DNA degradation studies for the given samples

Materials: Tulsi & Licorice herbal green tea, Test Cell line: A549 (Human Lung Carcinoma cell lines)

Method: Sample preparation: Sample was taken in powdered form. 10mg of the sample was dissolved in 1mL DMSO to give a stock concentration of 10mg/mL. The working concentration of 1mg/mL was used for the studies. A549 cells were revived and 0.05 million cells were seeded in 6 well plate. The cells were incubated in CO2 incubator at 37° C, 5% CO2 overnight. After observing the fully confluent cells under microscope the cells were treated with the given samples at IC10 and IC25 of the drug to study DNA degradation. The cells were incubated overnight in the presence of sample in CO2 incubator at 37° C, 5% CO2. All the cells were harvested by trypsinization and washed with DPBS. Cells were lysed with lysis buffer containing 10Mm Tris (Ph 7.4), 150Mm NaCl, 5Mm EDTA and 0.5% Triton X-100 for 30 mins on ice. Lysates were vortexed and cleared by centrifugation at 10,000g for 20mins. Fragmented DNA in the supernatant was extracted with an equal volume of neutral phenol: chloroform: isoamyl alcohol mixture (25:24:1) and analysed electrophoretically on 1% agarose gel containing 0.1ug/ml ethidium bromide.

Observations:

In order to delineate the mechanism of cell death mediated by Tulsi & Licorice herbal green tea it was tested on A549 Cell line for DNA degradation assay, which is characteristic of apoptosis. The results show DNA degradation at IC50.

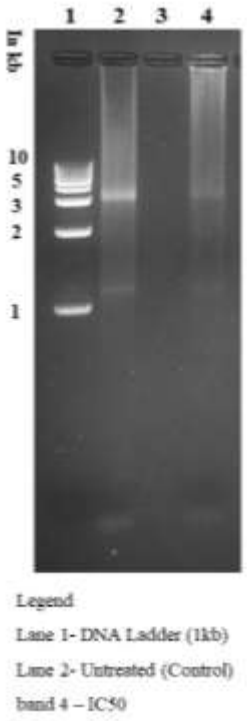

Image 4: electrophoresis showing DNA degeneration in lung cancer cells post treatment

Results and Conclusion:

The Tulsi & Licorice herbal green tea damages the A549 lung carcinoma cells, suggestive of its anticancer properties. In order to delineate the mechanism of cell death mediated by Tulsi & Licorice herbal green tea it was tested on A549 Cell line for DNA degradation assay, which is characteristic of apoptosis. The results show DNA degradation at IC50. The Tulsi & Licorice herbal green tea showed difference in the IC50 values for L132 lung epithelial cells and A549 lung carcinoma cells. That means the Tulsi & Licorice herbal green tea is more toxic to lung cancer cells than normal cells, as the IC50 for the sample for A549 and L132 was found to be 32µg/mL and 40.4µg/mL respectively. Tulsi & Licorice herbal green tea can be considered as potential anti-cancer agent. But the difference in the IC50 values is marginal hence further testing needs to be done.

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