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Evaluation of Cytotoxic Activity of *Cestrum nocturnum* Leaves Extract against A549 Cell Line using MTT Assay

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Abstract

Cancer remains a major global cause of death, necessitating ongoing research for effective treatments. Traditional herbal medicine is being explored for novel anti-cancer compounds. This study investigates the cytotoxic effects of Cestrum nocturnum ethanolic extract on A549 lung cancer cells, a type of non-small cell lung cancer (NSCLC). NSCLC is a significant health concern in India, affecting even non-smokers. Cestrum nocturnum, known as Night-blooming jasmine, has shown promise in inhibiting tumor growth in mice, and its active compound, apigenin, exhibits anti-cancer properties. Apigenin modulates epigenetic mechanisms involved in cancer development and progression, including DNA methylation, histone modification, signaling pathways, cell cycle arrest, and apoptosis. The ethanolic extract demonstrated concentration-dependent cytotoxicity, indicating potential anti-cancer properties. This study underscores Cestrum nocturnum's potential as an anti-cancer agent and provides insights into apigenin's mechanisms of action, warranting further research for therapeutic applications in NSCLC treatment.

Keywords: A549, MTT assay, Cestrum nocturnum, lung cancer

INTRODUCTION

Ongoing research efforts are imperative to unveil new and effective treatments, as cancer continues to persist as a prominent global cause of death. Traditional herbal medicine has gained attention as a potential source of novel anti-cancer compounds. Numerous herbs and natural products have been explored for their cytotoxic effects on cancer cells, with the aim of identifying promising candidates for further investigation and potential therapeutic development.

In India, lung cancer is the leading site in men [1]. Assessing the cytotoxic effects of herbs on the A549 cell line provides valuable insights into their potential anti-cancer properties and their viability as treatment options for NSCLC. According to recent estimates, a significant portion of lung cancer cases worldwide, specifically 15% in men and 53% in women, occurs in individuals who have never smoked [2]. The predominant risk factor for lung cancer is tobacco smoking, and the likelihood of developing the disease increases in correlation with both the number of cigarettes smoked and the duration of smoking. Tobacco smoking is the cause of more than 90% of lung cancers in men and between 74% to 80% of lung cancers in women. It is named "non-small cell" to distinguish it from small cell lung cancer, another major type of lung cancer. Non-small cell lung cancer (NSCLC) typically begins in the lung tissues and can potentially metastasize to other areas of the body if it remains undetected and untreated in its early stages. NSCLC includes various distinct subtypes, such as adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. Every subtype possesses unique characteristics and might exhibit varying responses to treatment strategies [3].

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Cestrum nocturnum, commonly referred to as Night-blooming jasmine or Lady of the Night, has a history of traditional use for various medicinal purposes. Limited scientific research has been conducted to specifically explore its potential anti-cancer effects against the A549 cell line or lung cancer. Zhong, Zhen-Guo, et al. experimentally proved that extracts of CN were able to inhibit tumor growth in mice [4]. Kumar, Pradeep, et al. demonstrated the mechanism of apigenin found in leaves of CN on hepatocellular carcinoma [5].

Apigenin is a natural compound found in various plants and has been studied for its potential anticancer properties, particularly its ability to modulate epigenetic mechanisms involved in cancer development and progression [5]. Epigenetic modifications refer to chemical changes that can impact gene expression without modifying the actual DNA sequence. They play a crucial role in regulating gene activity and can be disrupted in cancer cells, leading to abnormal gene expression patterns [6].

Apigenin has been found to exert its anticancer effects through the following mechanisms such as DNA methylation inhibition [7], histone modification, [8] modulation of signaling pathways [9] and induction of cell cycle arrest and apoptosis [10].

MATERIALS AND METHOD

Preparation of *Cestrum nocturnum* Ethanolic Extract

The dried leaves were grinded into a fine powder using a mixer. One gm of powder was submerged in 70% ethanol. After a 72-hour period, the supernatant was filtered and gathered. Subsequently, the supernatant was allowed to air dry at room temperature to yield the ethanolic extract. The extract transferred in an Eppendorf tube and DMSO was added to make a stock of 100mg/ml. The stock was stored at -20°C.

Cancer cell lines and culture conditions

The A549 cell line, sourced from ATCC and originating from human lung adenocarcinoma, was acquired. These cells were cultured in a humidified incubator at 37°C with 5% CO2, using Dulbecco's Modified Eagle's Medium (DMEM, HIMEDIA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin.

Cytotoxicity Assay

Cytotoxicity studies were performed using the MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) assay. Exponentially growing A549 cells were plated in 96-well microtiter plates at a density of 10,000 cells per well using 180 µl of DMEM medium. After 24 hours of incubation at 37°C, stock solutions of the test samples dissolved in DMEM were added to the wells at serially diluted concentrations of 250, 100, 50, 25, 10, and 1 µg/ml in culture medium. As a vehicle control, dimethyl sulfoxide (DMSO) was included at a concentration of 0.25%.

Incubation and Cell Viability Assessment

After introducing the test samples, the plates underwent additional incubation for 24, 48, and 72 hours at 37°C with 5% CO2. At the conclusion of each respective incubation period, 20 µl of MTT solution (5 mg/ml in phosphate-buffered saline) was introduced to each well, followed by an additional 3-hour incubation. After 3 hours, the medium was removed, and the formazan crystals formed by viable cells were solubilized by adding 150 µl of dimethyl sulfoxide (DMSO) to each well. Absorbance was quantified at 570 nm utilizing a microplate reader, and cell viability was determined relative to the control group. The percentage cytotoxicity was calculated using the following formula,
% Cytotoxicity = ((mean OD of control – mean OD of individual group)/mean OD of control group)) *100

The percentage viability was calculated by subtracting cytotoxicity from 100.

RESULTS

The percentage viability was found to decrease in a trend from lower concentration to higher concentration. The inhibitory concentration for 24-, 48- and 72-hour incubation period was found to be 31.18 µg/ml, 24.8 µg/ml and 14.74 µg/ml respectively. *Cestrum nocturnum* showed % cytotoxicity in the range 23.9% - 92.8% with working concentration 1-250 µg/ml in A549 cell line (Figures 1-3).

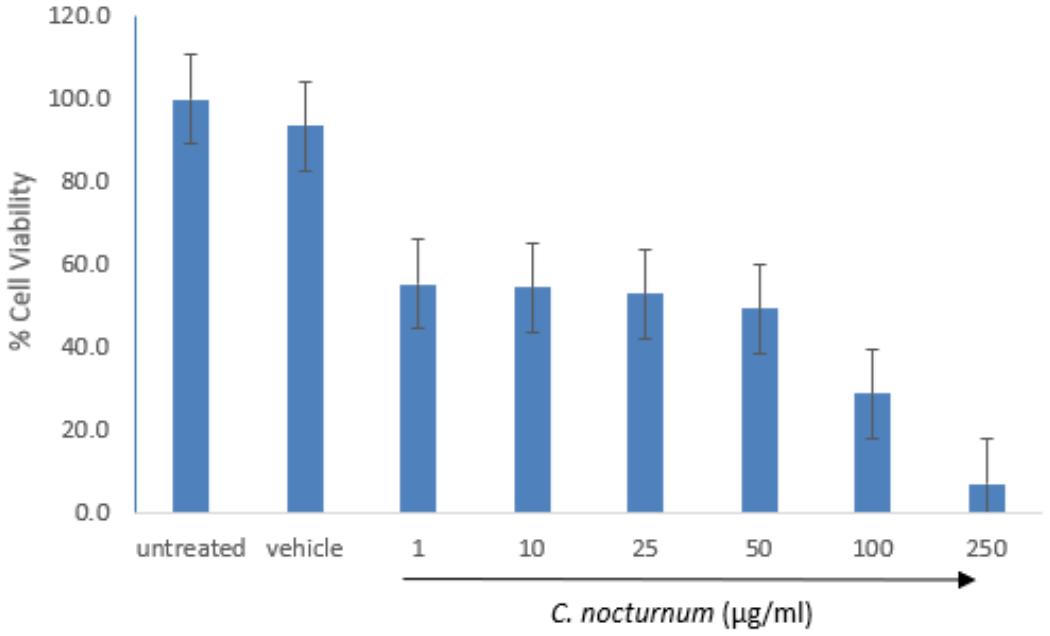

Figure 1. Percentage of cell viability in extract treated cells after 24 hours.

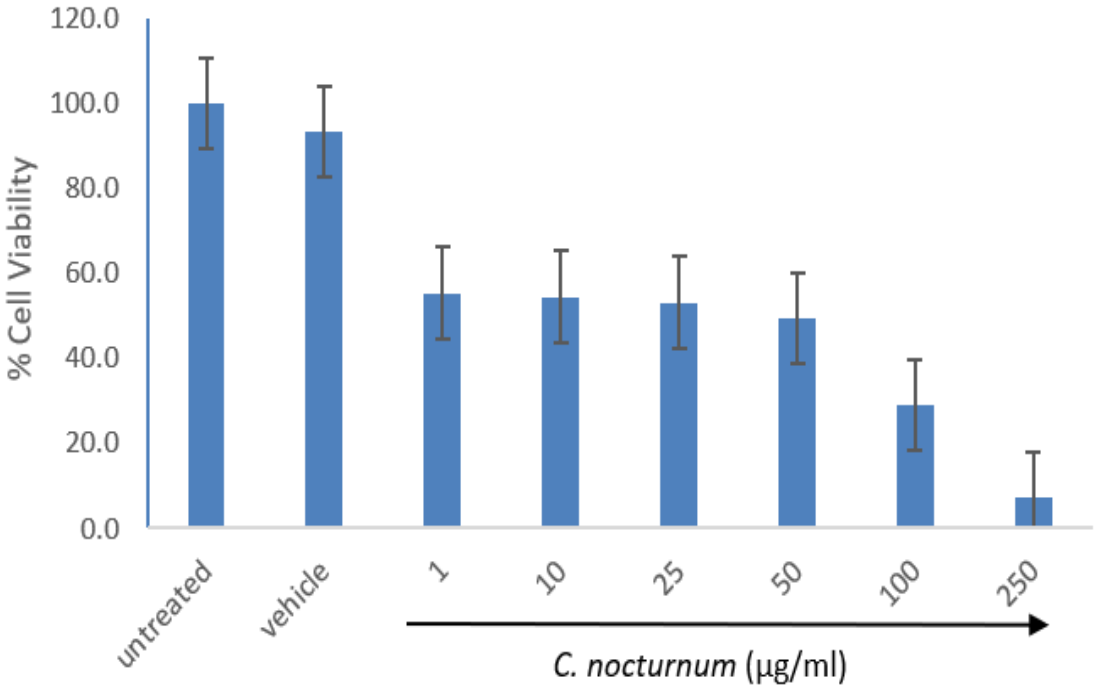

Figure 2. Percentage of cell viability in extract treated cells after 48 hours.

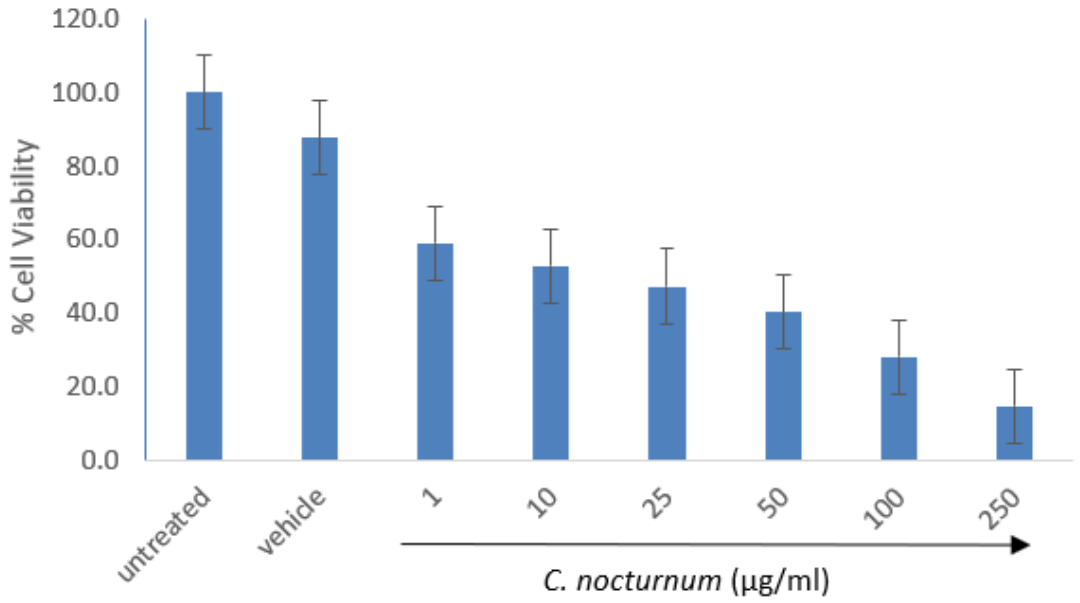

Figure 3. Percentage of cell viability in extract treated cells after 72 hours.

CONCLUSIONS

The present study evaluated the cytotoxic effects of the ethanolic extract of *Cestrum nocturnum* leaves on the A549 cell line, a type of lung cancer cell, at three different time points: 24, 48, and 72 hours. The inhibitory concentration for each time point was found to be 31.18 µg/ml, 24.8 µg/ml, and 14.74 µg/ml, respectively. These findings indicate that the ethanolic extract of *Cestrum nocturnum leaves* possesses potential cytotoxic properties against lung cancer cells, with stronger inhibitory effects observed at longer incubation periods.

Cestrum nocturnum, commonly referred to as Night-blooming jasmine or Lady of the Night, has a history of traditional use for various medicinal purposes.While there is limited scientific research specifically investigating its anti-cancer potential against lung cancer, previous studies have demonstrated the anti-tumour and anti-cancer effects of *Cestrum nocturnum* extracts. Zhong, Zhen-Guo, et al. conducted an experiment showing the inhibition of tumor growth in mice by CN extracts, supporting the notion that this plant has promising anti-cancer properties [4]. Furthermore, Kumar, Pradeep, et al. explored the mechanism of apigenin, a natural compound found in *Cestrum nocturnum leaves*, on hepatocellular carcinoma [5]. Apigenin has been studied extensively for its potential anticancer properties, particularly in modulating epigenetic mechanisms involved in cancer development and progression [5].

Epigenetic modifications, such as DNA methylation and histone modifications, play a crucial role in regulating gene expression and can be disrupted in cancer cells, leading to abnormal gene expression patterns [6]. Apigenin has been found to exert its anticancer effects through several mechanisms involving epigenetic regulation.

Primarily, apigenin inhibits DNA methyltransferase enzymes, which are responsible for adding methyl groups to DNA molecules. By inhibiting DNA methylation, apigenin may help restore normal gene expression patterns that could be altered in cancer cells [7]. Secondly, apigenin impacts histone proteins, which are involved in packaging DNA and regulating gene expression. It inhibits histone deacetylase enzymes, leading to increased acetylation of histones. This modification can loosen the chromatin structure and promote the expression of tumor-suppressor genes, ultimately inhibiting cancer cell growth [8]. Furthermore, apigenin has demonstrated the ability to modulate various signaling pathways that are implicated in cancer development and progression. For instance, it can

block the PI3K/Akt pathway in breast cancer cells, thereby inhibiting metastasis [9]. These findings suggest that apigenin's effects on signaling pathways may contribute to its overall anticancer properties. Lastly, apigenin has been documented to induce cell cycle arrest and facilitate apoptosis in cancer cells [10]. These effects can help prevent uncontrolled cell growth and facilitate the elimination of cancer cells.

In conclusion, the findings of this study demonstrate the cytotoxic effects of the ethanolic extract of *Cestrum nocturnum* leaves on lung cancer cells. The inhibitory concentration of the extract was noted to decrease with extended incubation periods. This study adds to the growing body of evidence supporting the potential anti-cancer properties of *Cestrum nocturnum* and its active compound, apigenin. The mechanism of action of apigenin, particularly its role in modulating epigenetic processes, provides valuable insights into its anticancer effects. Further research and clinical studies are warranted to explore the full potential of *Cestrum nocturnum* as a promising candidate for lung cancer therapy and to elucidate the underlying molecular mechanisms involved.

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