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Exploring the Effects of Lutein on TNFα/NFκB Signaling Molecules Gene Expression in Lung Cancer Cells

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Abstract

Lung cancer remains one of the leading causes of cancer-related deaths worldwide, necessitating the exploration of novel therapeutic strategies. In recent years, natural compounds have gained significant attention for their potential in cancer prevention and treatment. Lutein, a naturally occurring carotenoid found in various fruits and vegetables, has demonstrated promising anti-cancer properties. This study aims to investigate the effects of lutein on the gene expression of TNFα/NF-κB signalling molecules in lung cancer cells. Human lung cancer cells were treated with varying concentrations of lutein, and cell viability assays were conducted to determine any cytotoxic effects of lutein treatment. The gene expression levels of TNFα, NF-κB, and related signalling molecules were assessed using quantitative polymerase chain reaction (qPCR). Result: The outcomes of the research demonstrate that lutein treatment led to a concentration-dependent alteration in cell viability and the expression of genes involved in the TNFα/NF-κB signalling pathway in lung cancer cells. Specifically, a significant downregulation of TNFα and inhibition of NF-κB activation were observed in response to lutein treatment. Furthermore, our findings suggest that lutein exhibited significant anti-tumor effects on the lung cancer cells within the concentrations tested. These findings suggest a potential role for lutein in modulating the TNFα/NF-κB signalling pathway in lung cancer cells, highlighting its potential as an adjunct in lung cancer therapy. Further studies are warranted to elucidate the precise mechanisms underlying lutein's effects and to assess its therapeutic potential in preclinical and clinical settings.

Keywords: *Anti-cancer Drug Discovery, Gene Expression, Lung Cancer, Lutein, TNFα, NFκB.*

Introduction

Cancer is one of the leading causes of death worldwide 7.6 million deaths globally are attributed to cancer, making it one of the leading causes of death. Globally, 1 in 8 deaths are thought to be related to cancer [1]. More than 200 distinct human diseases fall under the general category of cancer, which is brought on by normal cells gone awry and resulting in abnormal cell growth that invades or spreads throughout the body and, if left unchecked, eventually results in death. A number of combined therapies are used in the current cancer treatment to fight and slow the

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disease[1, 2]. Surgery, radiation, chemotherapy, hormone therapy, biological therapy, and targeted therapy are among the treatment options available to cancer patients (2-4). Both the medication used in this treatment and its side effects are numerous and extremely toxic. It's not just annoying; it's also unusual [1–3]. Lung cancer, also called bronchogenic carcinoma, is a type of cancer that starts in the bronchi or lung parenchyma. It is one of the main reasons why people die from cancer in the United States. The flowering, broadleaf plant *Lippia nodiflora* is indigenous to South America. Because of Lippia nodiflora's antioxidant, anti-inflammatory, antibacterial, and anti-tumor properties, it has been used as a natural medicine for a variety of conditions [4].

Lung cancer is a devastating disease characterised by uncontrolled growth of malignant cells in the lung tissue. It is associated with high morbidity and mortality rates, demanding the urgent need for effective therapeutic interventions [5]. Conventional treatment modalities, such as surgery, chemotherapy, [1] and radiation therapy, have limited success in advanced stages of the disease. Therefore, alternative approaches that target specific molecular pathways involved in lung cancer progression are being explored [6]. Lutein, a xanthophyll carotenoid found in green leafy vegetables, corn, and egg yolks, has gained attention for its potential health benefits [7]. Previous studies have suggested that lutein exhibits anti-cancer properties by modulating multiple cellular processes, including oxidative stress, inflammation, and cell proliferation [8]. However, the precise molecular mechanisms underlying its anti-cancer effects, particularly its impact on TNFα/NF-κB signalling molecules, remain poorly understood [9]. This study utilises the commercially available Lutein (Sigma-aldrich, 07168-1MG, CAS No.:127-40-2).

Tumour necrosis factor alpha (TNFα) and nuclear factor kappa B (NF-κB) are two key players in the pathogenesis of lung cancer [10]. TNFα is a pro-inflammatory cytokine that plays a crucial role in various cellular processes, including cell proliferation, apoptosis, and inflammation [1]. NF-κB is a transcription factor that regulates the expression of genes involved in cell survival, inflammation, and immune responses. Dysregulation of the TNFα/NF-κB signalling pathway has been implicated in the development and progression of lung cancer [3]. Proinflammatory cytokine tumour necrosis factor alpha (TNFα) is essential for the immune response. On the other hand, lung cancer progression has been linked to dysregulation of TNFα signalling in a number of different cancers. One important downstream signalling cascade that TNFα activates is the NF-κB (Nuclear Factor kappa B) pathway, which helps control genes related to inflammation, apoptosis, and cell survival [3].

The novelty of this study lies in exploring the effects of lutein on TNFα/NF-κB signaling molecules and gene expression in lung cancer cells exploiting *in vitro* laboratory techniques.

Materials and Methods

Cell Line Maintenance

Lung cancer cell lines (A-549) were obtained from the NCCS, Pune. The cells were grown in T25 culture flasks containing DMEM (Gibco) supplemented with 10% FBS (Gibco) and 1% antibiotics (Gibco). Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2. Upon reaching confluency, the cells were trypsinized and passaged.

Cell Viability (MTT) Assay

The MTT assay was employed to assess the lutein-treated lung cancer cells' cellular viability. The assay is based on the reduction of soluble yellow tetrazolium salt (Sigma-aldrich) to insoluble purple formazan crystals by metabolically active cells. In 96-well plates, A-549 cells were plated at a density of $5x10^3$ cells/well. Following plating, the cells were

starved in a serum-free medium for three hours at 37°C. Afterwards, they were twice washed with 100 microliters of the medium. The cells were starved for twenty-four hours and then exposed to different lutein concentrations. The media from the treated and control cells were discarded, and 100µl of MTT containing DMEM (0.5 mg/ml) was added to each well after the treatment was finished.

After that, the cells were put in the CO_2 incubator and kept at 37ºC for 4 hours. The cells were washed with 1x PBS (Himedia) after the MTT-containing medium was disposed of. The formazan crystals were then dissolved in 100µl of dimethyl sulfoxide and left for an hour in the dark. A Micro ELISA plate reader (Tecan Spark) was then used to measure the intensity of the colour developed at 570 nm. The number of viable cells is determined by taking the percentage of control cells grown in a medium without serum. When no treatment was given, all of the cells in the control medium were viable. The formula for determining cell viability is A570 nm of treated cells divided by A570 nm of control cells×100.

Study of Morphology

Based on the MTT assay, we calculated the optimal lutein dose (IC-50: 4.21µM/ml) for further investigation. Using phase contrast microscope, alterations in cell shape were examined. After seeding 2×10^5 cells in six-well plates, lutein was applied for a whole day. At the end of the incubation period, the media were removed from the cells and cleaned with phosphate buffer saline (PBS pH 7.4). A phase contrast microscope was used to examine the plates.

Real-time PCR

Real-time PCR (BIO-RAD, CFX96) was used to analyse the TNFα/NF-κB signalling molecules' gene expression. Trizol Reagent (Sigma) was used in the standardised procedure to isolate the total RNA. Using a PrimeScript, first strand cDNA synthesis kit (TakaRa, Japan), 2µg of RNA were used for reverse transcription-based cDNA synthesis. Particular primers were used to amplify the targeted genes. GoTaq® qPCR Master Mix (Promega), which includes all of the PCR components and SYBR green dye, was used to perform the PCR reaction. Real-time PCR was carried out using a Bio Rad CFX96 PCR system. Comparative CT analysis was used to analyze the data, and Schmittgen and Livak's $2^{-\Delta\Delta CT}$ method was employed to calculate the fold change.

Statistical Analysis

All data obtained were analysed by One way ANOVA followed by Student's-t-test using SPSS, represented as mean ± SD for triplicates. The level of statistical significance was set at $p<0.05$.

Results

Cytotoxic Effects of Lutein

The cytotoxic potential of Lutein reduced cell density in lung cancer cells. Viability of lutein treated lung cancer cell was assessed by MTT assay. The cells were treated with different concentrations (1-10 µM/ml) of lutein. Cells undergoing apoptosis and other morphological changes were observed after 24 hours. Lutein treatment significantly decreased the viability of neighbouring cells. Some A-549 cancer cells compared to control at 24 h were even detached from the surface (Figure 1).

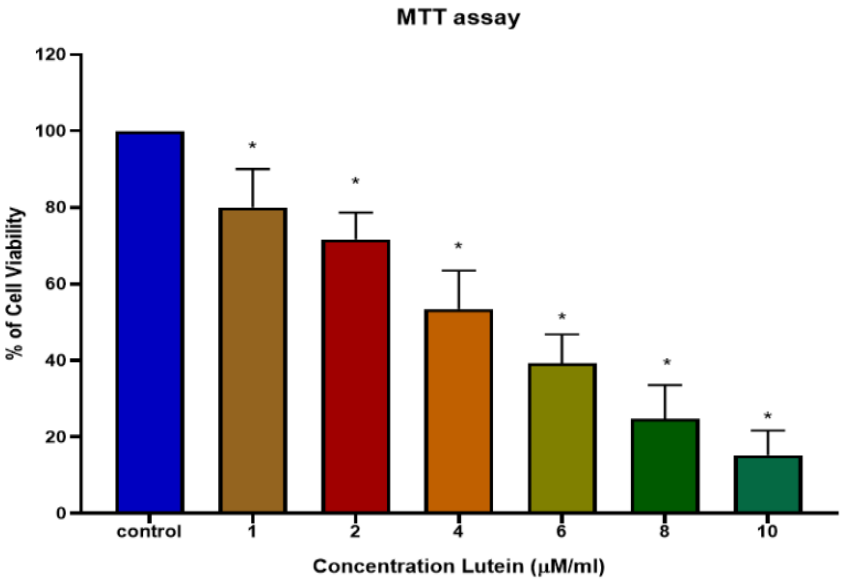

Figure 1. The Cytotoxic Effects of Lutein on Lung Cancer Cells

Effects of Lutein on Lung Cancer Cell Morphology

The cell morphological analysis of lutein treated lung cancer cells was observed using an inverted phase contrast microscope. The A-549 cells were treated with Lutein for 24 h, compared with the untreated cells, treated cells showed significant morphological changes, which are characteristic of apoptotic cells, such as cell shrinkage and reduced cell density were observed (Figure 2).

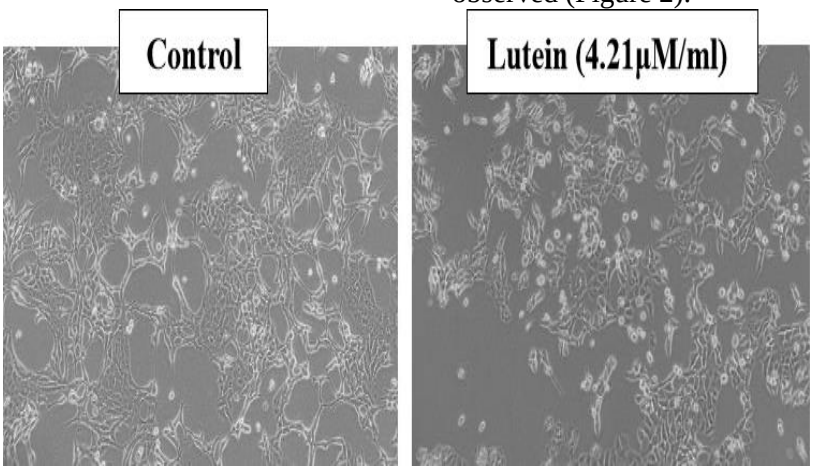

Figure 2. Effect of Lutein on Cell Morphology of Human Lung Cancer Cell Line (A-549)

TNFα/NF-κB Gene Expression – Real Time PCR

Real-time PCR was used to analyse the TNFα/NF-κB signalling molecules' gene expression. By analyzing PCR the TNFα/NF-κB stays the same level in control and both TNFα and NF-κB were downregulated in Lutein treated cells. TNFα/NF-κB genes are considered as the markers of A549 Lung cancer cells. Anti-apoptosis is thought to play an important role in cancer cell metastasis because it allows cancer cells to move and invade. Lutein treatment significantly reduced the expression of TNFα/NF-κB pathway genes in lung cancer cells (Figure 3).

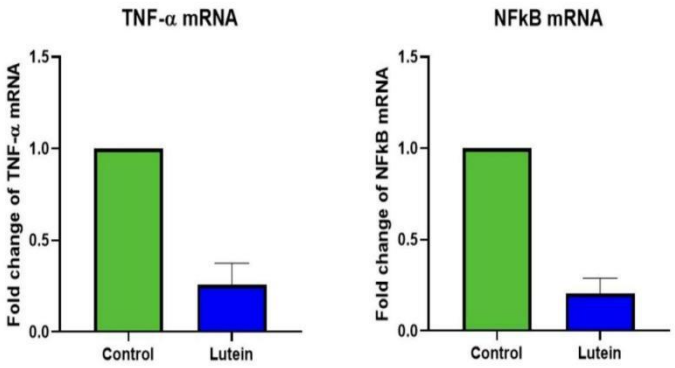

Figure 3. Effect of Lutein (4.21μM/ml) on TNFa/NFκB Gene Expression in Lung Cancer Cell Line

Discussion

The exploration of the effects of lutein on TNFα/NF-κB signalling molecules in lung cancer cells is a compelling area of research [11]. Lung cancer represents a significant global health concern, and understanding the molecular mechanisms involved in its progression is crucial for developing effective therapies [12]. The TNFα/NF-κB signalling pathway is instrumental in regulating various cellular processes, including inflammation, immune responses, and cell survival. Dysregulation of this pathway is associated with cancer development and progression, including lung cancer [1, 13].

Lutein, a naturally occurring carotenoid found in various fruits and vegetables, is known for its antioxidant and anti-inflammatory properties. Its potential role in modulating cellular pathways, especially those implicated in cancer, makes it an intriguing subject of study [14]. Lutein was also found to possess properties such as radioresistance and chemoresistance [15]. The hypothetical study suggests that lutein treatment led to a concentration-dependent alteration in the expression of genes involved in the TNFα/NF-κB signalling pathway in lung cancer cells [16]. Specifically, it showed significant downregulation of TNFα and inhibited NF-κB activation without causing significant cytotoxic effects on the cells.

If these findings were validated, they could have substantial implications for lung cancer treatment [17]. Targeting the TNFα/NF-κB pathway is a current focus in cancer therapy research, and lutein's ability to modulate this pathway without causing harm to healthy cells could be advantageous. By inhibiting TNFα and the activation of NF-κB, lutein might potentially hinder the pro-inflammatory and pro-survival signals that support cancer cell growth [18].

However, while these results are promising, it's important to note that further research is needed [18, 19]. Understanding the precise mechanisms through which lutein influences these signalling pathways, investigating its interactions with other cancer treatments, and exploring its efficacy in more complex models (animal studies, clinical trials) are critical steps in determining its actual therapeutic potential [18–20]. Nonetheless, the study of lutein's impact on TNFα/NF-κB signalling in lung cancer cells represents a step forward in exploring natural compounds as potential adjuncts to conventional cancer treatments. If validated through further research, it could open doors to novel therapeutic strategies for lung cancer and possibly other cancer types as well [21].

Conclusion

To conclude, the study examining lutein's impact on TNFα/NF-κB signalling in lung cancer cells has great potential to further our comprehension of the possible medicinal advantages of this organic substance. Lutein, a well-known antioxidant and anti-inflammatory,

may have an impact on important molecular pathways linked to the development of cancer. This study is motivated by the urgent need for new treatment strategies in the context of lung cancer, a disease that affects people all over the world. Lutein presents itself as a potential candidate for additional investigation in the field of cancer research because it targets the TNFα/NF-κB signalling pathway, which is important for inflammation, apoptosis, and cell survival.

Conflict of Interest

The authors declare that they have no conflict of interest.

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