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Unraveling the Role of Apoptosis in the Antiproliferative Activity of β-Glucan on A549 Cells

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ABSTRACT

Previous studies have reported the anticancer properties of β-glucan on various cancer cells. The objective of this research was to investigate the involvement of apoptosis in the cytotoxic action of β-glucan on the A549 cells. The cytotoxic impact of this drug on A549 cells was examined by subjecting them to various quantities of the substance, and the XTT assay was utilized to determine cell survival. Flow cytometry was performed to investigate apoptosis. A statistically significant and dose-dependent cytotoxic impact on A549 cells was observed upon treatment with β-glucan. The calculated IC50 value of β-glucan for A549 cells after a 24-hour treatment period was discovered to be 82.16 µg/mL. Further investigations carried out using the IC50 dose of β-glucan revealed a significant increase in the late apoptotic cells percentage. The capacity of β-glucan to trigger apoptosis is thought to be the cause of its cytotoxic action on lung cancer. The revelation of this discovery emphasizes the promising possibilities of β-glucan as an effective therapeutic choice.

Keywords: Anticancer, β-glucan, Apoptosis, A549.

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Introduction

According to incidence and cancer-related mortality rates worldwide, lung cancer is among the most widespread kind of malignant tumor [1]. It is still an incurable disease considering significant advancements in our knowledge of disease pathophysiology and the creation of cutting-edge treatments. Small- and non-small-cell lung cancers are the two main kinds of diverse illnesses known as lung cancer [2]. The A549 cell line is a cancerous cell line originating from human lung carcinoma. The lungs consist of various structures, including the respiratory bronchioles, and alveoli. Type I and type II pneumocytes are the two cell types that line the alveoli. Large, flat cells known as type I pneumocytes cover a sizable portion of the alveolar surface. Type I cells cannot divide and are extremely vulnerable to harm from many toxic substances, in contrast to type II pneumocytes. On the other hand, type II pneumocytes are smaller, cuboidal cells typically located at the alveolar septal junctions. These cells have the ability to replicate within the alveoli and play a crucial role in replacing damaged type I pneumocytes. A549 cells share several essential characteristics with type II pneumocytes, making them an ideal model for research purposes. They can be easily cultured due to their human origin and are widely used to study type II pneumocyte behavior and properties. Because of these advantageous features, the A549 cell line was selected for the present study [3]. A physiological mechanism called apoptosis results in cell death. In the organism, it serves as a crucial protective mechanism by eliminating damaged cells or cells that have overproliferated as a result of an incorrect mitotic stimulus. Cytoplasmic shrinkage, a lack of cell-cell connections, and active membrane blebbing are some of the early morphological changes in apoptotic cells. In recent years, the discovery of anticancer drugs has focused on the production of apoptosis, and an antitumor treatment that specifically induces apoptosis in tumor cells may be the best option [4,5]. Throughout history, natural components have been widely utilized as remedies for medical purposes, mainly due to the perception that they pose minimal adverse effects. Consequently, understanding the processes through which these natural substances can potentially provide health benefits is crucial for civilizations of humanity [6]. β-Glucans, which are plentiful in barley and oats, are a specific type of polysaccharide characterized by their location within the cell wall [7]. β-Glucans exhibit diverse properties that contribute to their beneficial effects in preventing and treating various illnesses. These properties, such as bile acid trapping and immune activation, have been found to be particularly helpful in conditions such as cancer, infectious diseases, diabetes, hyperlipidemia, and obesity [8]. β-Glucan therapy has demonstrated effectiveness in combating numerous types of cancers [9]. The anti-cancer properties of β-glucans are mediated through two essential receptors, receptor 3 (CR3) and lactosylceramide. These receptors are essential for immune system stimulation, cancer prevention, and direct suppression of tumor growth, all contributing to the overall anti-cancer effects of β-glucan

[10]. Numerous studies have showcased the anti-cancer properties of β-glucans on various cancer cell lines [9]. However, the specific mechanisms responsible for the anti-cancer effects of β-glucan against lung carcinoma, particularly on the A549 cell line, remain to be fully understood and require further investigation. This research aimed to explore the role of apoptosis in the cytotoxic action of β-glucan on the A549 cells.

Materials and Methods

Cell Culture

The American Type Culture Collection, a research institution with headquarters in the US, provided the A549 lung cancer cell line (CCL-185) for use in research. The A549 lung cancer cell line was cultured for cell culture purposes using Dulbecco's modified Eagle's medium (DMEM) and kept at a temperature of 37°C. The cells were grown in a humidified atmosphere with the addition of 15% CO_2. In this research, the culture medium used comprised 10% fetal bovine serum (FBS), 1% L-glutamine, and 1% penicillin/streptomycin. The substance of interest, β-glucan, was first dissolved in dimethyl sulfoxide (DMSO) 1and then further diluted in the culture medium. Before treating the cells, significant care was taken to ensure that the culture medium's DMSO concentration did not exceed 0.1%. All materials utilized in the experiment, with the exception of the cells, were acquired from Sigma-Aldrich.

Cell Viability Assay

The XTT test was employed to evaluate how β-glucan affected the A549 cell line's viability. Some experimental works used the XTT test to measure the cell proliferation [11,12]. Before commencing the experiment, the cells were first seeded at a density of $1x10^4$ cells per well and left to incubate for 24 hours. The A549 cells were then treated with β-glucan at different doses (10, 25, 50, 100, 200, and 400 µg/mL). There was also a control group made up of untreated cells. Following the incubation period, a 50 µL XTT mixture was introduced to each well, and the cells were further incubated for 4 hour. Fisher Scientific, based in Altrincham, United Kingdom, provided a microplate reader for use in measuring the absorbance at 450 nm after the cells had been incubated. To assure accuracy and reliability, the experiment was performed three times. By quantifying the proportion of living cells in comparison to the control cells that were not treated, the viability of the cells was evaluated [13,14]. Beta glucan does not interact with the chemicals used in cell culture studies. Since beta glucan containing solutions are removed from the wells before the XTT test and the wells are washed three times with buffer solution, it does not cause any color interference in the XTT test. The XTT test has been frequently used to measure cell proliferation.

Annexin V Binding Assay

A total of around 5×10^5 A549 cells were placed in 6-well plates and given full night to adhere. The next day, the A549 cells were exposed to β-glucan at dose of 82.16 µg/mL (IC_{50}) and incubated for an additional 24 hours. Following the duration of incubation, the cells were detached using trypsin, gathered, and resuspended in PBS containing at least 1% FBS. Following the manufacturer's directions, the cell suspension was then mixed with an equivalent volume of Annexin V & Dead Cell reagent (Merck, Millipore). To quantify the various cell types, including dead, live, early apoptotic, and late apoptotic cells, the Muse Cell Analyzer (Millipore) was employed [15].

Statistical Analysis

The laboratory results were reported as the mean ± standard error. The XTT assay results were analyzed using one-way analysis of variance (ANOVA), followed by post-hoc Tukey tests for multiple comparisons. The Student's t-test was used to assess the flow cytometry results. To identify statistically significant modifications, $p < 0.05$ was utilized as the significance threshold. Version 22.3 of the SPSS Statistics Program was used to conduct the statistical analysis.

Results and Discussion

The Antiproliferative Activity of β-glucan in A549 Cells

The A549 cells were subjected to various amounts of β-glucan ranging from 10 to 400 µg/mL for a duration of 24 hours. The XTT assay was used to examine the total amount of cells that survived. β-glucan did not demonstrate any significant effect at quantities of 10, 25, and 50 µg/mL. However, at quantities of 100, 200, and 400 µg/mL, β-glucan displayed a dose-dependent antiproliferative action on A549 cells ($p < 0.001$; Fig. 1). The IC_{50} value of β-glucan, identified using GraphPad Prism, was determined to be 18.47 µg/mL.

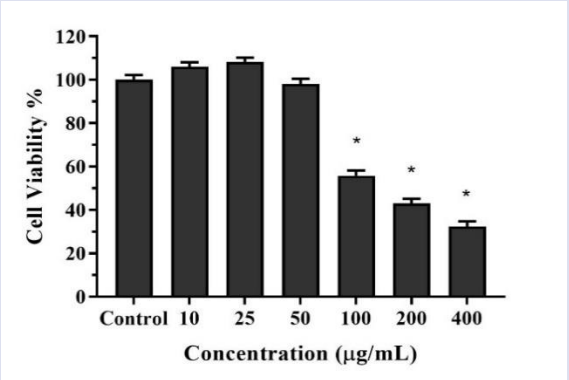

Figure 1: The cytotoxicity activity of β-glucan in A549 cells. The outcomes are expressed as the mean ± SEM of six samples.

*$p<0.001$ as compared to the control group.

The apoptotic impact of β-glucan on A549 Cells

The apoptosis profile of β-glucan in A549 lung cancer cells was assessed using a flow cytometry device along with suitable kits. The control group had a late apoptotic cell percentage of 5.67%. However, in the group treated with β-glucan, there was a remarkable and statistically significant increase in late apoptotic cells, with the percentage measuring 14.78%. The percentage of early apoptotic cells did not significantly differ between the two groups. Based on our findings, it was observed that the percentage of dead cells showed an increase from 1.93% in the control group to 6.06% in the β-glucan group. Conversely, there was a considerable reduction in the percentages of live cells, declining from 90.03% to 77.47% after treatment with β-glucan ($p < 0.05$ Fig. 2). The findings indicate that β-glucan significantly contributes to the induction of apoptosis in A549 lung cancer cells, resulting in a remarkable reduction in cell proliferation.

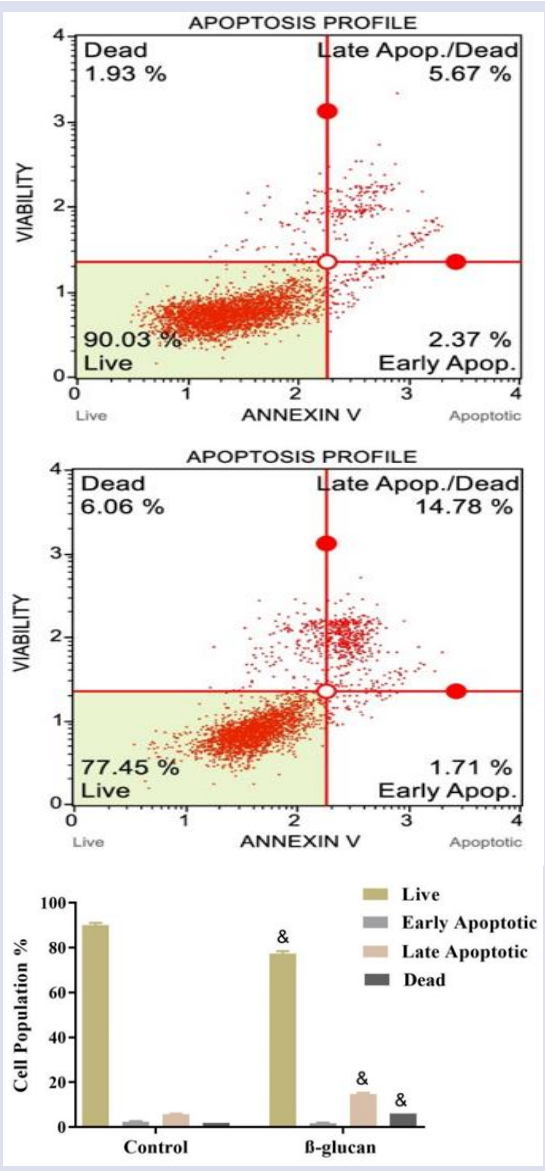

Figure 2: The apoptotic action of β-glucan on A549 1cells. The results are expressed as the mean ± SEM1 of six samples.

&p<0.05 as compared to the control group.

In recent times, β-glucan has attracted considerable interest as a conceivable nominee for cancer cure. While scientists are working to confirm its cancer-fighting abilities, they are still committed to figuring out the particular pathways by which it exerts its effects. In this particular investigation, our focus was on assessing the influences of β-glucans on proliferation and apoptosis using in vitro models. For our experiments, we employed the A549 cell line, which originates from a type II pneumocyte lung tumor. Initially, XTT test was conducted to assess the dose-dependent cytotoxicity of β-glucan on A549 cells. The findings from the experiments demonstrated a notable decrease in the growth of A549 cells, indicating that β-glucan's impact was related to its concentration. After a 24-hour exposure to β-glucan, the IC_{50} value was discovered to be 82.16 µg/mL. In line with our results, earlier research has demonstrated that β-glucan reduces MCF-7 cell survival in a a way that depends on concentration [16]. Furthermore, Kim et al. reported that β-glucan induced cell death in colon cancerous cells [17]. The proliferation of B16 melanoma cells was inhibited by β-glucans extracted from S. Cerevisae [18]. According to Sadeghi et al., the isolated β -glucan demonstrated a substantial cell death in both the sphere and parental cells [19]. Upadhyay's study revealed a remarkable deceleration in the multiplication of the HeLa cancer cells following a 24-hour treatment with different quantities of β-glucan [20]. In our earlier investigation, we observed a concentration-dependent suppression of SH-SY5Y cell reproduction by β-glucan, with a value for the IC_{50} of 94.6 g/mL following 24 hours. Concerns regarding the toxicity of antiproliferative agents on normal cells remain a significant limitation in treatment options. Therefore, during our previous investigation, we looked at how β-glucan affected non-cancerous L929 cells. The findings showed that β-glucan had no detectable cytotoxic consequences on L929 cells [21]. Cell development and proliferation are significantly regulated by the process of apoptosis, which is a type of controlled cell death. When cells receive specific signals triggering apoptosis, they activate internal mechanisms leading to distinct physiological modifications. The alterations involve several processes, including the external presentation of phosphatidylserine (PS) on the outer layer of cells, the breaking down of certain cellular proteins through fragmentation and cleavage, the condensation and fragmentation of nuclear chromatin, and eventually, the deterioration of membrane integrity during later stages [22]. Annexin V is a protein with a selective affinity for phosphatidylserine (PS), a phospholipid mainly situated on the inner surface of the cell membrane. In the early stages of apoptosis, PS molecules are relocated to the external surface of the cell membrane, making them accessible for Annexin V binding. Due to its capability to attach to externalized PS molecules on the cell membrane surface, Annexin V is commonly used as a marker to detect apoptotic cells [23]. Apoptosis is widely recognized as a pivotal factor in the molecular progression of cancer and exerts a

substantial influence on the efficacy of chemotherapy and radiation remedies [24]. In this investigation, we conducted a flow cytometry-based experiment to analyze the potential induction of apoptosis by β-glucan exposure. Our results demonstrated a significant increase in Annexin V binding of A549 cells following β-glucan treatment, indicating that β-glucan treatment effectively triggered apoptosis in these cells. Consistent with our findings, a study demonstrated that β-glucan exhibited a notable suppression rate of 75% on the viability of S-180 cells [25]. Notably, this inhibition rate surpassed that of the positive control, cytoxan, which recorded a 54% inhibition rate. β-glucan enhanced the immune cell recruitment towards cancerous tumors, leading to subsequent cell apoptosis and suppression of cell survival. Moreover, β-glucan induced an increase in the expression of pro-apoptotic proteins Bax and caspase 3/9, as well as the tumor suppressor p53, while concurrently reducing the levels of the anti-apoptotic protein Bcl-2 in cancer cells [25]. The β-glucan compound effectively inhibited the proliferation of C6 glioma cells through several mechanisms. It induced apoptosis, disrupted the cell cycle, and notably augmented the number of cells in the G0/G1 stage, accompanied by a reduction in the proportion of cells in the S-stage [26]. In a separate study, the efficacy of (1-6,1-3)-β-glucan was observed in combination with oxaliplatin in Hep-G2 cells (human hepatocyte carcinoma) implanted in mice with H22 tumors (mouse hepatoma). The results revealed synergistic effects, as the combination treatment effectively inhibited the expression of NF-kB, STAT3, and survivin in the cancer cells [27]. Treatment with (1-6,1-3)-β-glucan induces programmed cell death and autophagy in human osteosarcoma cells [26]. This effect is achieved by up-regulating miR-340 expression and inhibiting the MAPK/ERK signaling pathway in MG63 cells. Moreover, (1-6,1-3)-β-glucan treatment leads to reduced levels of cyclin D1, while increasing the levels of Beclin-1, LC3B-II/LC3B-I, and caspase-3, -9 in these cells [28]. In our previous study, we observed a noteworthy effect of β-glucan treatment on pro-apoptotic factors, including increased expressions of Bax, cleaved caspase 3, and cleaved PARP proteins. These findings strongly suggest that apoptosis plays a vital role in the drug's ability to inhibit cell proliferation [21]. However, it is thought that performing apoptosis experiments in 200 and 400 ug/mL beta glucan may provide more comprehensive results

In summary, our research showed that treatment with β-glucan suppressed the proliferation of A549 cells in a way that was dependent on concentration. This inhibitory impacts was associated with notable programmed cell death. These promising results suggest that β-glucan could hold promise as a therapeutic agent for lung cancer treatment. Nevertheless, it is crucial to emphasize that additional validation through in vivo and clinical investigations is required to corroborate these results and comprehensively evaluate the safety and efficacy of β-glucan as a potential treatment option.

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