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DETERMINATION OF THE ANTICANCER PROPERTIES OF LECTIN

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ABSTRACT

The growing demand for safe and effective therapeutic agents has intensified interest in natural bioactive compounds, particularly plant-derived proteins such as lectins. Lectins are known for their specific carbohydrate-binding properties and significant biological activities, including anticancer effects.

This study aimed to investigate the cytotoxic activity of lectin isolated from Phaseolus vulgaris seeds and evaluate its potential for pharmaceutical application. The lectin was extracted using ammonium sulfate fractionation, followed by dialysis and lyophilization. The obtained protein was tested for cytotoxic activity against the MCF-7 human breast cancer cell line using the CCK-8 assay.

The results demonstrated a clear concentration-dependent decrease in cell viability. At concentrations of 12.5, 25, 50, 100, and 200 μg/mL, cell viability decreased to 90.8%, 79.6%, 64.3%, 50.1%, and 32.6%, respectively. The IC_{50} value was determined to be approximately 100 μg/mL, indicating moderate cytotoxic activity. Morphological observations confirmed cell damage, including shrinkage and detachment at higher concentrations.

The findings suggest that lectin from Phaseolus vulgaris exhibits significant antiproliferative activity and may serve as a promising candidate for the development of new anticancer agents. However, further studies are required to investigate its mechanism of action and in vivo efficacy.

Introduction. In recent decades, the rapid increase in cancer incidence worldwide has become a major global health challenge, necessitating the development of new and more effective therapeutic strategies. Despite significant progress in chemotherapy, radiotherapy, and targeted treatments, many conventional anticancer drugs are associated with severe side effects, lack of selectivity, and the emergence of drug resistance. These limitations highlight the urgent need for safer and more

selective bioactive compounds derived from natural sources.

Plant-derived compounds have attracted considerable attention due to their structural diversity and wide range of pharmacological activities. Among them, lectins — carbohydrate-binding proteins — represent a unique class of bioactive molecules capable of specifically recognizing glycan structures on cell surfaces. This specificity makes lectins particularly interesting for biomedical applications, especially in cancer therapy, where abnormal glycosylation patterns are a hallmark of malignant cells.

Lectins have been reported to exhibit various biological activities, including antimicrobial, immunomodulatory, antiviral, and anticancer effects. Their anticancer activity is primarily attributed to their ability to bind selectively to glycoproteins and glycolipids on cancer cell membranes, leading to disruption of cellular signaling pathways, induction of apoptosis, inhibition of cell proliferation, and alteration of cell cycle progression. In addition, lectins may trigger mitochondrial dysfunction and reactive oxygen species (ROS) generation, further contributing to cancer cell death.

Phaseolus vulgaris (common bean) is a widely cultivated leguminous plant known to contain significant amounts of lectins with notable biological activity. Previous studies have demonstrated that lectins isolated from *Phaseolus vulgaris* can interact with tumor cells and influence their viability. However, the cytotoxic properties of these lectins and their potential pharmaceutical applications remain insufficiently explored, particularly in the context of standardized in vitro evaluation using modern analytical methods.

Therefore, this study aims to investigate the cytotoxic activity of lectin isolated from *Phaseolus vulgaris* seeds against the MCF-7 human breast cancer cell line and to evaluate its potential as a candidate for anticancer drug development. The findings of this research are expected to contribute to the growing body of knowledge on natural protein-based therapeutics and support the development of safer and more effective anticancer agents.

Materials and Methods

Plant material and chemicals. Seeds of *Phaseolus vulgaris* were used as the source of lectin. All chemicals and reagents, including ammonium sulfate, phosphate-buffered saline (PBS), and cell culture media components, were of analytical grade. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin/streptomycin, trypsin-EDTA, and Cell Counting Kit-8 (CCK-8) were obtained from standard commercial suppliers.

Extraction and purification of lectin. Lectin was extracted from *Phaseolus vulgaris* seeds using an aqueous extraction method followed by ammonium sulfate fractionation. The crude extract was subjected to stepwise ammonium sulfate precipitation (60–80% saturation) to isolate the protein fraction. The precipitated proteins were collected by centrifugation and subsequently dialyzed against distilled water to remove excess salts.

The dialyzed sample was then lyophilized to obtain a dry lectin powder. The purified lectin was stored at −20 °C

until further use. For experimental purposes, the lectin was dissolved in sterile phosphate-buffered saline (PBS, pH 7.4) to prepare working concentrations.

Cell culture conditions. The human breast cancer cell line MCF-7 was used to evaluate cytotoxic activity. Cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin under standard conditions (37 °C, 5% CO_2, humidified atmosphere).

Cells were maintained until they reached 80–90% confluence, after which they were detached using trypsin-EDTA solution, counted, and seeded into 96-well plates at a density of **5×10^4 cells per well**. The cells were allowed to adhere and stabilize for 24 hours before treatment.

Treatment with lectin. After the incubation period, cells were treated with different concentrations of lectin: **12.5, 25, 50, 100, and 200 µg/mL**. Control wells received only culture medium without lectin. All treatments were performed in triplicate to ensure reproducibility.

The treated cells were incubated for 24 hours under standard conditions.

Cell viability assay (CCK-8 test). Cell viability was determined using the Cell Counting Kit-8 (CCK-8) assay, which is based on the reduction of WST-8 to a water-soluble formazan dye by mitochondrial dehydrogenases in viable cells.

After 24 hours of lectin treatment, **10 µL of CCK-8 reagent** was added to each well, and the plates were incubated for an additional **2 hours at 37 °C**. The absorbance was measured at **450 nm** using a microplate reader.

Cell viability (%) was calculated relative to the control group using the formula:

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

Determination of IC_{50}. The half-maximal inhibitory concentration (IC_{50}) was determined from the dose–response curve by plotting cell viability against lectin concentration. The IC_{50} value corresponds to the concentration required to reduce cell viability by 50%.

Statistical analysis. All experiments were conducted in triplicate, and the results were expressed as **mean ± standard deviation (SD)**. Statistical analysis was performed using appropriate software (e.g., GraphPad Prism or Origin). Differences between groups were considered statistically significant at **$p < 0.05$**.

Results. The cytotoxic activity of lectin isolated from Phaseolus vulgaris was evaluated against the MCF-7 human breast cancer cell line using the CCK-8 assay. The results demonstrated a clear concentration-dependent inhibitory effect on cell viability.

Quantitative analysis of cell viability revealed a progressive decrease with increasing lectin concentration. The control group showed 100 ± 3.2% viability with an optical density (OD_{450}) value of 0.98 ± 0.04. Upon treatment with lectin, the following results were obtained:

1-table

Activity of citotokcity -7 (Test CCK-8)

Lektin konsentratsiyasi (µg/mL)	Optik zichlik (OD 450 nm)	Hujayra hayotiyligi (%)
Nazorat (0)	0.98 ± 0.04	100 ± 3.2
12.5	0.89 ± 0.05	90.8 ± 4.1
25	0.78 ± 0.06	79.6 ± 4.8
50	0.63 ± 0.04	64.3 ± 3.9
100	0.49 ± 0.03	50.1 ± 3.1
200	0.32 ± 0.02	32.6 ± 2.7

These results clearly indicate that lectin exhibits a strong dose-dependent cytotoxic effect on MCF-7 cells.

IC_{50} determination.Based on the dose–response relationship, the IC_{50} value (the concentration required to reduce cell viability by 50%) was calculated to be approximately 100 µg/mL. This value suggests a moderate but significant cytotoxic potency of the lectin.

Morphological observations. Visual examination of the treated cells supported the quantitative findings. In the control group, MCF-7 cells exhibited normal morphology, including adherence to the plate surface and typical epithelial-like structure.

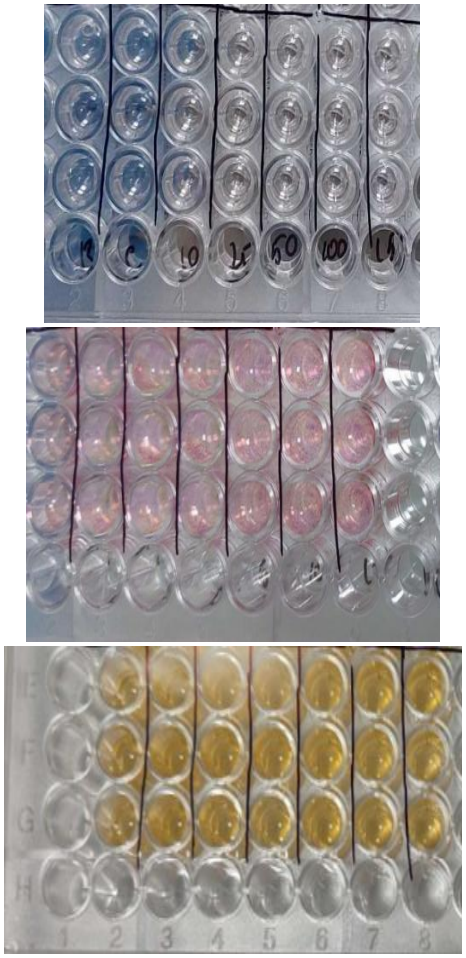

Figure 1.Cytotoxic properties of lectin:*1 – MCF-7 cells; 2 – MCF-7 cells treated with lectin solution; 3 – Determination of MCF-7 cells viability using the CCK-8 assay.*

CCK-8 assay interpretation. The decrease in absorbance at 450 nm reflects reduced mitochondrial dehydrogenase activity, indicating a decline in metabolic activity of the cells.

This confirms that lectin affects not only cell number but also cellular metabolic function.

Summary of findings

Overall, the obtained data demonstrate that lectin from Phaseolus vulgaris:

exhibits significant dose-dependent cytotoxicity

reduces cell viability by more than 65% at high concentrations

has an IC_{50} value of approximately 100 µg/mL

induces morphological and metabolic changes in cancer cells

These findings confirm the biological activity of the lectin and support its potential application in anticancer research.

Discussion. The present study demonstrates that lectin isolated from *Phaseolus vulgaris* exhibits a significant cytotoxic effect against the MCF-7 human breast cancer cell line in a concentration-dependent manner. The observed decrease in cell viability, along with morphological alterations, confirms the antiproliferative potential of this natural protein.

The IC_{50} value of approximately **100 µg/mL** indicates a moderate level of cytotoxicity. Compared to highly potent cytotoxic agents, which often exhibit IC_{50} values in the micromolar or nanogram range, the lectin studied here shows a relatively milder but stable biological effect. This characteristic may be advantageous, as compounds with moderate cytotoxicity often demonstrate better safety profiles and lower toxicity toward normal cells, making them suitable candidates for further pharmacological development.

The cytotoxic activity of lectins is primarily associated with their specific carbohydrate-binding properties. Cancer cells are known to exhibit altered glycosylation patterns on their surface, including overexpression of glycoproteins and glycolipids. This allows lectins to selectively recognize and bind to malignant cells. Such interactions can trigger multiple intracellular pathways, including disruption of signal transduction, inhibition of cell cycle progression, and activation of programmed cell death mechanisms such as apoptosis.

The morphological changes observed in this study — including cell shrinkage, detachment, and reduced cell density — are consistent with previously reported features of apoptotic cell death. Additionally, the reduction in CCK-8 absorbance values suggests impaired mitochondrial activity, which may indicate mitochondrial dysfunction, a key event in apoptosis induction.

Previous studies have also reported that plant lectins can induce reactive oxygen species (ROS) generation, activate caspases, and disrupt mitochondrial membrane potential. Although these mechanisms were not directly investigated in the present study, the observed cytotoxic effects suggest that similar pathways may be involved.

Furthermore, the dose-dependent nature of the effect confirms that the biological activity of lectin is directly related to its concentration. This is an important characteristic for potential therapeutic agents, as it allows controlled modulation of biological effects.

Despite these promising findings, several limitations should be noted. First, the study was conducted on a single cancer cell line (MCF-7), which limits the generalizability of the results. Second, the selectivity of lectin toward cancer cells compared to normal cells was not evaluated. Third, the exact molecular mechanisms underlying the observed cytotoxicity remain unclear.

Conclusion

In this study, lectin isolated from *Phaseolus vulgaris* seeds was successfully obtained and its cytotoxic activity was evaluated against the MCF-7 human breast cancer cell line. The results demonstrated a clear concentration-dependent inhibitory effect on cell viability, with an IC_{50} value of approximately 100 µg/mL.

The observed decrease in cell viability, along with morphological alterations such as cell shrinkage, detachment, and reduced cell density, confirms the antiproliferative activity of the lectin. The reduction in metabolic activity, as indicated by the CCK-8 assay, suggests that lectin affects mitochondrial function and cellular viability.

These findings highlight the potential of plant-derived lectins as promising candidates for anticancer drug development. Their ability to selectively interact with cancer cell surface glycoconjugates and induce cytotoxic effects makes them valuable bioactive compounds.

However, for further pharmaceutical application, additional studies are required to: elucidate the molecular mechanisms of action; evaluate selectivity toward normal cells; investigate in vivo efficacy and toxicity; improve stability and bioavailability.

Overall, lectin from *Phaseolus vulgaris* represents a natural compound for the development of new anticancer therapeutics.

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