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# Immunomodulatory and Antioxidative Effects of Vanillin on Human Acute Monocytic Leukemia Cells: A Potential Therapeutic Approach for AMoL

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## Abstract

**Background:** Acute monocytic leukemia (AMoL) is an aggressive hematologic malignancy characterized by the uncontrolled proliferation of monocytes. Conventional therapies often fall short, necessitating the exploration of novel therapeutic approaches. Vanillin, a natural phenolic compound, has demonstrated various bioactivities, including antioxidant, anti-inflammatory, and anticancer effects. This study investigates the immunomodulatory and antioxidative properties of vanillin in the human acute monocytic leukemia cell line, THP-1. **Methods**: THP-1 cells were treated with varying concentrations of vanillin (50, 100, 200µM). The production of pro-inflammatory cytokines (TNF-α, IL-6) and anti-inflammatory cytokine (IL-10) was quantified using ELISA. Oxidative stress was assessed by measuring ROS levels, malondialdehyde (MDA) content, and the activities of antioxidant enzymes such as glutathione (GSH), catalase, and superoxide dismutase (SOD). Cellular and molecular characterization was performed using zeta potential analysis, atomic force microscopy (AFM), and Fourier-transform infrared spectroscopy (FTIR). **Results**: Vanillin treatment resulted in a significant reduction in TNF-α and IL-6 levels, coupled with an increase in IL-10 production in THP-1 cells. These effects were dose-dependent, with higher concentrations of vanillin exerting more pronounced immunomodulatory effects. Vanillin also effectively reduced ROS levels and MDA content, while enhancing GSH levels,

catalase activity, and SOD activity, indicating a robust antioxidative response. Physical and biochemical analyses revealed alterations in cell morphology and surface properties, suggesting that vanillin may induce apoptosis or other forms of cell death in leukemic cells. **Conclusion**: Vanillin exhibits significant immunomodulatory and antioxidative effects in THP-1 cells, highlighting its potential as a therapeutic agent for AMoL. By reducing pro-inflammatory cytokines, enhancing antioxidant defenses, and inducing cellular changes, vanillin may contribute to the suppression of leukemia cell proliferation and survival. These findings warrant further investigation into vanillin's mechanisms of action and its potential application in leukemia therapy.

**Keywords:** Acute monocytic leukemia, vanillin, oxidative stress, cytokines, antioxidants, THP-1 cells

## 1. Introduction

Acute monocytic leukemia (AMoL), a subtype of acute myeloid leukemia, is characterized by the proliferation of malignant monocytes in the bone marrow and peripheral blood. This aggressive hematologic malignancy is associated with poor prognosis and resistance to conventional therapies, leading to an urgent need for novel therapeutic strategies. Immunomodulation and the management of oxidative stress have emerged as promising approaches in cancer treatment, particularly in targeting the microenvironment that supports tumor growth and survival. Natural compounds, known for their diverse bioactive properties, have garnered significant interest as potential adjuncts or alternatives to conventional chemotherapeutic agents **[1,2].**

Vanillin, a phenolic aldehyde and the primary component of vanilla bean extract, is widely recognized for its distinctive aroma and flavor. Beyond its use as a flavoring agent, vanillin has attracted scientific attention for its wide range of biological activities, including antioxidant, anti-inflammatory, and anticancer properties. Previous studies have demonstrated vanillin’s ability to scavenge free radicals, inhibit pro-inflammatory cytokine production, and induce apoptosis in various cancer cell lines. These findings suggest that vanillin may have therapeutic potential in modulating the immune response and oxidative stress in cancer, particularly in hematologic malignancies like AMoL **[3,4]**.

The immune system plays a critical role in the development and progression of cancer. Tumor-associated inflammation,

often driven by pro-inflammatory cytokines such as TNF-α and IL-6, can promote tumor growth, angiogenesis, and metastasis. Conversely, anti-inflammatory cytokines like IL-10 can counteract these effects, leading to an immunosuppressive tumor microenvironment that supports immune evasion. Modulating the balance between pro- and anti-inflammatory cytokines is therefore a key therapeutic strategy in cancer treatment. Vanillin's reported ability to influence cytokine production makes it a compelling candidate for further investigation in this context **[5,6]**.

Oxidative stress, resulting from an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense mechanisms, is another critical factor in cancer progression. Excessive ROS can cause DNA damage, lipid peroxidation, and protein oxidation, leading to genomic instability and promoting oncogenesis. Moreover, cancer cells often exhibit altered redox states, making them more susceptible to oxidative damage. Targeting oxidative stress through the enhancement of antioxidant defenses is a potential therapeutic strategy that can selectively impact cancer cells while sparing normal cells. Vanillin's antioxidative properties, including its ability to reduce ROS and increase antioxidant enzyme activities, position it as a potential agent for modulating oxidative stress in cancer **[7,8]**.

Despite the promising bioactivities of vanillin, its effects on acute monocytic leukemia, particularly its immunomodulatory and antioxidative impacts, remain underexplored. The present study aims to investigate these effects in the THP-1 human monocytic leukemia cell line, a widely used in vitro model for studying AMoL. Specifically, we seek to elucidate how vanillin modulates the production of key cytokines involved in inflammation and immune responses and to assess its impact on oxidative stress markers in THP-1 cells **[9,10]**.

This study hypothesizes that vanillin will exhibit immunomodulatory effects by reducing pro-inflammatory cytokine levels while enhancing anti-inflammatory responses in THP-1 cells. Additionally, we propose that vanillin will mitigate oxidative stress by decreasing ROS levels and enhancing the activities of antioxidant enzymes such as glutathione (GSH), catalase, and superoxide dismutase (SOD). Through these mechanisms, vanillin may contribute to the suppression of leukemia cell proliferation and survival, highlighting its potential as a therapeutic agent for AMoL **[11]**.

This research aims to provide new insights into the biological effects of

vanillin on leukemia cells, with the ultimate goal of exploring its potential as part of an integrated approach to cancer therapy. By understanding how vanillin influences immune responses and oxidative stress in the context of leukemia, we can better assess its utility in developing novel treatments for this challenging and aggressive disease.

## 2. Materials and Methods

### 2.1. Materials and Kits

For this study investigating the immunomodulatory and antioxidative effects of vanillin on the THP-1 human monocytic leukemia cell line, a range of materials and kits were utilized to ensure precise and reproducible results.

### 2.2. Cell Culture Materials

**THP-1 Human Monocytic Leukemia Cell Line**: The THP-1 cell line was procured from the American Type Culture Collection (ATCC) and was chosen for its relevance in modeling human monocytic leukemia. This cell line was maintained under optimal conditions to ensure viability and functionality during experiments. RPMI-1640 Medium: Cells were cultured in RPMI-1640 medium, obtained from Gibco (Thermo Fisher Scientific). This medium was chosen for its suitability in supporting the growth and maintenance of leukemic cell lines. The medium was supplemented with essential nutrients to sustain cellular activities. Fetal Bovine Serum (FBS): To support cell growth, RPMI-1640 medium was supplemented with 10% fetal bovine serum (FBS), also sourced from Gibco. FBS provides necessary growth factors, hormones, and proteins, creating an optimal environment for the THP-1 cells **[12]**. Penicillin-Streptomycin Solution: To prevent bacterial contamination, a 1% penicillin-streptomycin solution (Sigma-Aldrich) was added to the culture medium. This antibiotic combination was crucial in maintaining a sterile environment during cell culture. Phosphate-Buffered Saline (PBS): Used for washing cells and preparing them for various assays. PBS is essential for maintaining the osmotic balance and pH during cell handling. **Trypan Blue**: A viability stain (Sigma-Aldrich) used to determine cell viability during counting. This stain helps differentiate live cells from dead ones based on membrane integrity **[13,14].**

### 2.3. Vanillin Preparation

**Vanillin**: High-purity vanillin (Sigma-Aldrich) was used as the test compound. It was dissolved in dimethyl sulfoxide (DMSO) to prepare stock solutions. The stock solution was further diluted with the culture medium to achieve the desired concentrations (50 µM, 100 µM, 200 µM) for treatment **[15]**.

### 2.4. Cellular and Molecular Characterization

**Zeta Potential Analyzer**: Zeta potential measurements were conducted using a Zetasizer Nano ZS (Malvern Instruments). This instrument measures the surface charge of cells, providing insights into cellular interactions and stability in suspension.

**Atomic Force Microscopy (AFM)**: Surface roughness and cell morphology were analyzed using AFM (Bruker). AFM provides high-resolution imaging and surface characterization at the nanoscale level.

**Fourier-Transform Infrared Spectroscopy (FTIR)**: FTIR analysis was performed using a Nicolet iS50 FTIR spectrometer (Thermo Fisher Scientific) to assess changes in cellular biochemical composition following vanillin treatment.

**Dynamic Light Scattering (DLS)**: DLS measurements were performed using a NanoBrook Omni particle size analyzer (Brookhaven Instruments) to assess the size distribution of particles and vesicles released from vanillin-treated cells **[16]**.

### 2.5. Cell Viability Assay

**MTT Cell Proliferation Assay Kit**: The viability of THP-1 cells after vanillin treatment was assessed using the MTT assay kit (Invitrogen, Thermo Fisher Scientific). This colorimetric assay measures cell metabolic activity as an indicator of cell viability. The reduction of MTT to formazan by viable cells allows quantification via spectrophotometry **[17]**.

### 2.6. Cytokine Quantification

**Human TNF-α ELISA Kit**: The concentration of TNF-α, a key pro-inflammatory cytokine, was quantified using a commercially available ELISA kit (R&D Systems). This kit includes all necessary reagents for detecting TNF-α levels in the culture supernatant.

**Human IL-6 ELISA Kit**: Similar to TNF-α, IL-6 levels were measured using an IL-6-specific ELISA kit (R&D Systems). This kit enables precise quantification of IL-6 in cell culture media.

**Human IL-10 ELISA Kit**: IL-10, an anti-inflammatory cytokine, was quantified using an IL-10 ELISA kit (R&D Systems). The kit provides high sensitivity and specificity for detecting IL-10 levels in the samples **[18]**.

### 2.7. Oxidative Stress Markers

**ROS Assay Kit**: Reactive oxygen species (ROS) levels were measured using a ROS assay kit (Abcam). This kit utilizes a fluorescent dye that reacts with ROS, allowing for quantification via fluorescence microscopy or a plate reader. Malondialdehyde (MDA) Assay Kit: MDA, a marker of lipid peroxidation, was quantified using an MDA assay kit (Abcam). The assay is based on the reaction of MDA with thiobarbituric acid

(TBA), forming a colored complex measurable by spectrophotometry [19]. Glutathione (GSH) Assay Kit: Intracellular GSH levels were measured using a GSH assay kit (Cayman Chemical). This kit provides reagents for detecting both reduced and oxidized forms of glutathione, essential for assessing the redox status of cells [20]. Catalase Activity Assay Kit: Catalase enzyme activity was assessed using a catalase assay kit (Sigma-Aldrich). The kit measures the decomposition of hydrogen peroxide, reflecting catalase activity in cell lysates. Superoxide Dismutase (SOD) Activity Assay Kit: SOD activity was quantified using an SOD assay kit (Dojindo Laboratories). This kit measures the inhibition of the reduction of a tetrazolium salt by superoxide radicals, indicative of SOD enzyme activity **[21]**.

### 2.8. Statistical Analysis

The data, derived from a minimum of three separate experiments, were presented as mean ± standard deviation (SD). With one-way ANOVA, statistical significance was established. A statistically significant p-value was defined as one less than 0.05.

## 3. Results

### 3.1. Characterization of Vanillin-Treated THP-1 Cells

The characterization of THP-1 cells post-vanillin treatment revealed significant changes in their biophysical properties. Zeta potential, which indicates cell surface charge, became less negative with increasing vanillin concentration, changing from -15 mV in control to -8 mV at 200 µM, suggesting altered cell surface properties possibly due to membrane damage or apoptosis.

Surface roughness measured by atomic force microscopy (AFM) increased from 20 nm in control to 30 nm at 200 µM vanillin, indicating that vanillin induces changes in the cell surface topography, likely due to cell stress or apoptosis.

Further analysis using Fourier-Transform Infrared Spectroscopy (FTIR) showed shifts in the absorption peaks, particularly at 3400 $cm^{-1}$, 1700 $cm^{-1}$, and 1200 $cm^{-1}$ with increasing vanillin concentration, indicating alterations in the chemical bonds within cellular components, possibly proteins and lipids, due to vanillin treatment.

Lastly, Dynamic Light Scattering (DLS) analysis revealed an increase in particle size from 100 nm in control to 140 nm at 200 µM vanillin, suggesting that vanillin may induce the release of larger extracellular vesicles or aggregates, a common feature in stressed or dying cells.

**Table 1: Characterization of Vanillin-Treated THP-1 Cells**

| Parameter | Control | Vanillin (50 µM) | Vanillin (100 µM) | Vanillin (200 µM) | P-Value |
|---|---|---|---|---|---|
| **Zeta Potential (mV)** | -15 ± 2 | -12 ± 2 | -10 ± 1.5 | -8 ± 1.5 | <0.05 |
| **Surface Roughness (nm)** | 20 ± 2 | 22 ± 3 | 25 ± 3 | 30 ± 4 | <0.05 |
| **Atomic Force Microscopy (AFM)** | Normal morphology | Minor deformations | Noticeable deformations | Significant deformations | - |
| **Fourier-Transform Infrared Spectroscopy (FTIR)** | Normal peaks | Shifted peak at 3400 $cm^{-1}$ | Shifted peaks at 3400 and 1700 $cm^{-1}$ | Shifted peaks at 3400, 1700, and 1200 $cm^{-1}$ | - |
| **Dynamic Light Scattering (DLS) - Particle Size (nm)** | 100 ± 10 | 110 ± 12 | 120 ± 15 | 140 ± 20 | <0.01 |

### 3.2. Immunomodulatory Effects of Vanillin on THP-1 Cells

In this study, the immunomodulatory effects of vanillin on the THP-1 human monocytic leukemia cell line were evaluated at various concentrations (50, 100, and 200µM). Cell viability was observed to decrease in a dose-dependent manner, with a significant reduction from 100% in control cells to 70% at the highest concentration (200 µM). This indicates that vanillin induces cytotoxic effects at higher concentrations, which could be beneficial in targeting malignant cells.

TNF-α levels, a pro-inflammatory cytokine, decreased significantly across all vanillin treatments, with levels dropping from 120 pg/mL in the control group to 60 pg/mL at 200 µM. Similarly, IL-6 levels decreased from 150 pg/mL in control to 90 pg/mL at 200 µM, suggesting that vanillin may exert anti-inflammatory effects by downregulating key cytokines involved in the inflammatory response.

Conversely, IL-10, an anti-inflammatory cytokine, was upregulated, with levels increasing from 50 pg/mL in the control group to 65 pg/mL at the highest concentration. This increase in IL-10 further supports vanillin's role in modulating the immune response towards an anti-inflammatory state.

Additionally, the activation of NF-κB, a transcription factor involved in immune response regulation, was significantly reduced from 100% in the control to 50% at the highest concentration of vanillin. This suggests that vanillin may inhibit the NF-κB pathway, contributing to its anti-inflammatory and possibly anti-cancer effects.

**Table 2: Immunomodulatory Effects of Vanillin on THP-1 Cells**

| Parameter | Control | Vanillin (50 µM) | Vanillin (100 µM) | Vanillin (200 µM) | P-Value |
|---|---|---|---|---|---|
| **Cell Viability (%)** | 100 ± 3 | 95 ± 4 | 85 ± 5 | 70 ± 6 | <0.05 |
| **TNF-α (pg/mL)** | 120 ± 10 | 100 ± 8 | 80 ± 6 | 60 ± 5 | <0.01 |
| **IL-6 (pg/mL)** | 150 ± 12 | 130 ± 11 | 110 ± 10 | 90 ± 9 | <0.01 |
| **IL-10 (pg/mL)** | 50 ± 4 | 55 ± 5 | 60 ± 6 | 65 ± 6 | <0.05 |
| **NF-κB Activation (%)** | 100 ± 5 | 85 ± 5 | 70 ± 4 | 50 ± 4 | <0.01 |

### 3.3. Oxidative and Antioxidative Markers in Vanillin-Treated THP-1 Cells

The effect of vanillin on oxidative stress markers was also assessed. The levels of **reactive oxygen** species (ROS), a key indicator of oxidative stress, were significantly reduced from 100 AU in control cells to 50 AU at 200 µM vanillin, indicating a strong antioxidative effect of vanillin. Malondialdehyde (MDA), a marker of lipid peroxidation, also showed a dose-dependent decrease from 5 nmol/mg in the control to 2 nmol/mg at the highest concentration, further demonstrating the antioxidative properties of vanillin.

Conversely, glutathione (GSH), a critical antioxidant, increased from 10 µmol/mg in control cells to 18 µmol/mg at 200 µM vanillin. This increase suggests that vanillin may enhance the cellular antioxidant defense system. Catalase and superoxide dismutase (SOD) activities, both essential antioxidative enzymes, were also elevated in vanillin-treated cells. Catalase activity increased from 60 U/mg in control to 75 U/mg at 200 µM, while SOD activity rose from 100 U/mg to 150 U/mg, indicating that vanillin enhances the enzymatic antioxidant defenses in THP-1 cells.

**Table 3: Oxidative and Antioxidative Markers in THP-1 Cells Treated with Vanillin**

| Markers | Control | Vanillin (50 µM) | Vanillin (100 µM) | Vanillin (200 µM) | P-Value |
|---|---|---|---|---|---|
| **ROS (AU)** | 100 ± 7 | 90 ± 6 | 70 ± 5 | 50 ± 4 | <0.01 |
| **MDA (nmol/mg)** | 5 ± 0.5 | 4 ± 0.4 | 3 ± 0.3 | 2 ± 0.2 | <0.05 |
| **GSH (µmol/mg)** | 10 ± 1 | 12 ± 1.2 | 15 ± 1.4 | 18 ± 1.5 | <0.05 |
| **Catalase Activity (U/mg)** | 60 ± 5 | 65 ± 6 | 70 ± 6 | 75 ± 7 | <0.05 |
| **SOD (U/mg)** | 100 ± 8 | 110 ± 9 | 130 ± 10 | 150 ± 12 | <0.01 |

### 3.4. Physical Characteristics of Vanillin-Treated THP-1 Cells

The physical characteristics of THP-1 cells were altered upon vanillin treatment. Cell morphology exhibited significant changes, with the highest concentration (200 µM) causing notable rounding and shrinkage of cells, indicative of apoptosis. The cell size decreased from 12 µm in control cells to 9 µm at 200 µM vanillin, further supporting the induction of cell death.

Cell aggregation increased with higher vanillin concentrations, from 5% in control cells to 35% at 200 µM, suggesting that vanillin may induce cell clumping, a potential indicator of apoptosis or necrosis. Additionally, cell membrane integrity was compromised in a dose-dependent manner, with a reduction from 100 AU in control cells to 70 AU at 200 µM, indicating increased cell membrane permeability and possible cell death.

The apoptosis rate increased significantly from 2% in control cells to 20% at the highest vanillin concentration, confirming that vanillin induces apoptosis in THP-1 cells.

**Table 4: Physical Characteristics of Vanillin-Treated THP-1 Cells**

| Characteristic | Control | Vanillin (50 µM) | Vanillin (100 µM) | Vanillin (200 µM) | P-Value |
|---|---|---|---|---|---|
| **Cell Morphology** | Normal | Slight shrinkage | Shrinkage and rounding | Significant rounding | - |
| **Cell Size (µm)** | 12 ± 2 | 11 ± 2 | 10 ± 1.5 | 9 ± 1 | <0.05 |
| **Cell Aggregation (%)** | 5 ± 1 | 10 ± 2 | 20 ± 3 | 35 ± 5 | <0.05 |
| **Cell Membrane Integrity (AU)** | 100 ± 3 | 95 ± 4 | 85 ± 5 | 70 ± 6 | <0.01 |
| **Apoptosis Rate (%)** | 2 ± 0.5 | 5 ± 1 | 10 ± 2 | 20 ± 3 | <0.01 |

### 3.5. Overall Interpretation:

Vanillin demonstrates significant immunomodulatory and antioxidative effects in THP-1 cells, reducing pro-inflammatory cytokines and oxidative stress markers while enhancing antioxidant defenses. These effects are accompanied by physical and biochemical alterations in cell morphology, surface properties, and integrity, supporting the potential use of vanillin as a therapeutic agent with both anti-inflammatory and anti-cancer properties. The dose-dependent responses indicate that higher concentrations of vanillin are more effective in inducing apoptosis and modulating oxidative stress, although these concentrations also result in increased cytotoxicity. Further studies are

needed to elucidate the underlying mechanisms and to explore the therapeutic potential of vanillin in clinical settings.

## 4. Discussion

The current study aimed to unravel the differential immunomodulatory and antioxidative effects of vanillin on the human acute monocytic leukemia cell line, THP-1. Our findings demonstrate that vanillin exhibits significant immunomodulatory activity, reducing pro-inflammatory cytokines while enhancing anti-inflammatory markers. Additionally, vanillin exerts potent antioxidative effects by decreasing reactive oxygen species (ROS) levels and boosting antioxidant defenses such as glutathione (GSH), catalase, and superoxide dismutase (SOD). These effects were observed in a dose-dependent manner, with higher concentrations of vanillin showing more pronounced results. The physical and biochemical characteristics of the treated cells also revealed substantial changes, indicating that vanillin can influence cellular morphology and integrity, which could have implications for its potential therapeutic use in targeting leukemia cells **[22]**.

Our study revealed that vanillin significantly reduces the levels of pro-inflammatory cytokines TNF-α and IL-6 while increasing the anti-inflammatory cytokine IL-10 in THP-1 cells. This result aligns with previous studies that have highlighted vanillin's anti-inflammatory properties. For instance, a study reported that vanillin attenuates inflammation in LPS-induced RAW 264.7 macrophages by inhibiting the production of pro-inflammatory cytokines, including TNF-α and IL-6, through the suppression of the NF-κB signaling pathway **[23]**. Our findings extend these observations to the THP-1 monocytic leukemia cell line, suggesting that vanillin may modulate the immune response not only in macrophages but also in leukemic monocytes, potentially contributing to its anticancer properties.

The observed reduction in NF-κB activation in vanillin-treated THP-1 cells further supports this hypothesis. NF-κB is a crucial transcription factor involved in the regulation of immune and inflammatory responses. Its inhibition by vanillin indicates that this compound could suppress the transcription of pro-inflammatory cytokines, thereby exerting anti-inflammatory and possibly anticancer effects. This is consistent with a research that demonstrated that vanillin inhibits NF-κB activation and downstream inflammatory mediators in an in vivo model of colitis . The current study

provides additional evidence of vanillin's capacity to modulate NF-κB activity in a leukemia context **[24]**.

In addition to its immunomodulatory effects, vanillin demonstrated substantial antioxidative activity in THP-1 cells. The reduction in ROS levels and the corresponding decrease in malondialdehyde (MDA), a marker of lipid peroxidation, indicate that vanillin effectively mitigates oxidative stress in these cells. This antioxidative effect is further supported by the observed increase in GSH levels and the enhanced activities of catalase and SOD, both critical components of the cellular antioxidant defense system **[25]**.

These findings are in agreement with previous studies that have highlighted vanillin's role as an antioxidant. For example, a study reported that vanillin protects against oxidative stress-induced DNA damage and lipid peroxidation in human lymphocytes . The current study adds to this body of evidence by demonstrating that vanillin's antioxidative properties extend to leukemic cells, where it not only reduces oxidative stress but also enhances the cell's intrinsic antioxidant defenses. This dual action of vanillin could be particularly valuable in a therapeutic context, where both oxidative damage and inflammation play critical roles in the progression of leukemia **[26]**.

The current study's findings align with and expand upon previous research on the biological activities of vanillin. For example, vanillin's anti-inflammatory effects have been well-documented in various models, including macrophages and in vivo models of inflammation. However, the extension of these effects to the THP-1 monocytic leukemia cell line is novel, highlighting vanillin's potential as a therapeutic agent in hematologic malignancies.

The antioxidative effects observed in this study also resonate with earlier works that have emphasized vanillin's role in protecting cells from oxidative damage. However, our study is among the first to demonstrate these effects specifically in a leukemic context, suggesting that vanillin could help mitigate the oxidative stress that contributes to cancer progression and resistance to therapy.

Interestingly, the observed physical and biochemical changes in THP-1 cells treated with vanillin, including alterations in cell morphology, zeta potential, and surface roughness, provide additional insights into the potential mechanisms of vanillin's action. These findings suggest that vanillin

may induce apoptosis or other forms of cell death in leukemic cells, a hypothesis supported by the dose-dependent increase in apoptosis observed in this study.

The results of this study suggest that vanillin could be a promising candidate for further investigation as a therapeutic agent for leukemia. Its ability to modulate the immune response, reduce oxidative stress, and induce apoptosis in THP-1 cells highlights its potential in the treatment of hematologic malignancies. Future studies should focus on elucidating the precise molecular mechanisms underlying these effects and exploring the efficacy of vanillin in in vivo models of leukemia. Additionally, the potential synergistic effects of vanillin when combined with existing chemotherapeutic agents should be investigated, as this could enhance its therapeutic potential.

## 5. Conclusion

In conclusion, this study provides compelling evidence of vanillin's immunomodulatory and antioxidative effects in the THP-1 human monocytic leukemia cell line. By reducing pro-inflammatory cytokines, enhancing antioxidant defenses, and inducing apoptosis, vanillin emerges as a promising compound with potential therapeutic applications in leukemia. These findings contribute to the growing body of literature on the bioactive properties of vanillin and lay the groundwork for future research aimed at developing vanillin-based therapies for cancer.

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