

Bergenin Inhibits Cell Viability and Induces Apoptotic Cell Death in Leukemia MOLT-4 Cells

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ABSTRACT

Background: Leukemia, a hematological malignancy resulting from impaired blood cell formation in the bone marrow, is a considerable global health challenge, inflicting major burdens on individuals and healthcare systems. Current therapeutic strategies for leukemia, while exhibiting efficacy in certain contexts, frequently encounter significant limitations such as severe adverse effects and the development of drug resistance. **Objectives:** The current work was committed to investigating the anticancer mechanisms of bergenin against leukemia cells. **Materials and Methods:** The cytotoxic level of bergenin at various concentrations of 5-60 μ M against leukemia MOLT-4 cells was studied using the MTT assay. The cell cytotoxic nature of bergenin against MOLT-4 cells was evaluated using the LDH release assay. The dual staining assay was performed to scrutinize the apoptosis-inducing potential of the bergenin against MOLT-4 cells. The interaction of LDHA and LDHB with the bergenin was evaluated using AutoDock Vina and Discovery Studio Visualizer (DSV). In addition, the drug-likeness properties and the toxicity profile were identified by accessing the ProTox v3.0 server. **Results:** The growth of leukemia MOLT-4 cells was markedly reduced after treatment with bergnin at elevated doses (5-60 μ M). The bergenin administration significantly elevated the LDH activity in MOLT-4 cells, indicating cellular damage and cytotoxicity in response to bergenin treatment. Moreover, the bergenin treatment significantly increased apoptotic cell deaths in MOLT-4 cells, as evidenced by the dual staining results. The underlying mechanisms of this apoptosis were evaluated through computational analysis by targeting LDHA and LDHB, a significant enzyme in the glycolytic pathway, contributing to the rapid cell proliferation in leukemia. The molecular docking results revealed that the bergenin exhibited better interactions with the LDHA (Binding Affinity (BA) = -6.7 kcal/mol; RMSD = 2.392Å) and LDHB (BA = -7.1 kcal/mol; RMSD = 1.420Å). Meanwhile, the predicted drug-likeness properties and toxicity profile of the bergenin revealed that it could serve as a key compound to develop as a drug to treat leukemia in the future. **Conclusion:** The findings of this work demonstrate that bergenin treatment notably suppresses proliferation and triggers apoptosis in leukemia MOLT-4 cells, which may occur due to the inhibition of LDHA and LDHB. These findings provide evidence that bergenin has the capacity to function as an anticancer candidate for the treatment of leukemia.

Keywords: Apoptosis, Bergenin, Cytotoxicity, Leukemia, MOLT-4 cells.

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INTRODUCTION

Leukemia is a heterogeneous group of hematologic malignancies characterized by the uncontrolled growth of anomalous hematopoietic cells in the bone marrow. This malignant process subsequently leads to diffuse infiltration of the bone marrow,

often displacing normal hematopoiesis. This results in a gathering of undeveloped leukocytes in the blood and/or bone marrow, disrupting normal physiological functions.¹ The abnormal cells can then metastasize to other organs, including the lymph nodes, spleen, and liver, leading to extramedullary hematopoiesis. Leukemia is primarily classified into lymphoid and myeloid types, further delineated by their rate of progression into acute or chronic forms. Globally, leukemia affected 643.58 thousand individuals in 2019 and is categorized as a hematopoietic stem cell malignant clonal disease.² Specific chromosomal abnormalities frequently observed in acute leukemias often lead to altered gene expression, contributing to increased cell numbers due to impaired differentiation, resistance to programmed cell death, or enhanced



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proliferation. The principal types of leukemia encompass acute lymphoblastic, acute myeloid, chronic lymphocytic, and chronic myeloid leukemias. These classifications are based on the cell lineage involved and the maturity of the cells, impacting the disease's progression and requiring immediate attention.³

Acute lymphoblastic leukemia represents a clonal hematopoietic stem cell disorder affecting B or T cells, classified under precursor lymphoid neoplasms. This malignancy, particularly B-cell lymphoblastic leukemia, is the most prevalent childhood cancer globally, although its precise origins remain largely unelucidated.⁴ However, several factors have been implicated in its pathogenesis, including genetic predispositions, specific molecular aberrations, and environmental exposures. A significant characteristic of acute leukemias is the frequent observation of specific chromosomal abnormalities, which lead to altered gene expression within the affected cells. Despite these insights, the etiology of acute leukemia remains largely unknown, as a universally applicable cause has not been identified.⁵ However, ongoing research continues to uncover a complex interplay of genetic mutations and environmental influences that contribute to its development, including critical roles for specific transcription factors. The highest case of acute lymphoblastic leukemia is found between 1 and 4 years of age, yet in most instances, it arises in otherwise healthy individuals with no apparent predisposing factors. Nevertheless, a subset of patients exhibits identifiable risk factors, including inherited genetic susceptibilities and environmental exposures, which contribute to disease development.⁶ While the precise mechanisms remain elusive, a "two-hit" theory was proposed to explain the development of lymphoblastic leukemia, suggesting that a combination of genetic alterations and environmental triggers is necessary for leukemogenesis. Most cases of lymphoblastic leukemia, particularly in children, are considered idiopathic, arising without a clear precipitating cause.⁷

Current therapeutic strategies for leukemia, while exhibiting efficacy in certain contexts, frequently encounter significant limitations such as severe adverse effects and the onset of drug resistance. This conventional chemotherapeutic often indiscriminately targets proliferating cells, leading to collateral damage in healthy cells, which can be dose-restricting and contribute to poor clinical results.⁸ This non-specific targeting frequently manifests as gastrointestinal disturbances, alopecia, and myelosuppression, severely impacting patient quality of life. Moreover, the compromised immune systems of leukemia patients are particularly vulnerable to these toxicities, exacerbating their already delicate health status.⁹ The emergence of drug-resistant subclones further complicates treatment, as a small population of cells with resistance genes can survive initial chemotherapy regimens, leading to relapse and more aggressive, inoperable tumors. This therapeutic challenge necessitates the development of novel strategies that maximize leukemia cell

apoptosis before the onset of resistance.¹⁰ Consequently, there is an urgent demand for alternative therapies that offer selective cytotoxicity towards leukemic cells while minimizing harm to normal cells and tissues. This pressing need has driven extensive research into natural products and plant-derived compounds, which often exhibit diverse pharmacological properties and a less toxic profile compared to synthetic agents.¹¹ Bergenin, a C-glucoside of 4-O-methyl gallic acid, is a natural bioactive compound occur in numerous plants, such as *Bergenina crassifolia*, *Bergenina purpurascens*, *Rodgersia sambucifolia*, *Ardisia japonica*, and *Ardisia creanata*. Bergenin shows several biological activities, including antidiabetic,¹⁰ neuroprotective,¹¹ and antiarthritic properties.¹² In addition, several previous studies have already described that bergenin showed anticancer activities against numerous cancers, including colon cancer,¹³ bladder cancer,¹⁴ and cervical cancer¹⁵ cells. Nevertheless, there is no previous literature available to claim its anticancer properties against leukemia. Therefore, the current work was committed to examining the anticancer mechanisms of bergenin against leukemia cells and to elucidate the underlying mechanisms of apoptosis induction by targeting LDHA and LDHB, as these enzymes are the key regulators of glycolytic metabolism, which are critically involved in leukemia cells' proliferation and survival.¹⁶⁻¹⁸

MATERIALS AND METHODS

In vitro approach

Chemicals

The following chemicals, including bergenin, Fetal Bovine Serum (FBS), Dimethyl Sulfoxide (DMSO), Dulbecco's Modified Eagle Medium (DMEM), and antibiotics, were obtained from Sigma-Aldrich, USA. The test kit for measuring Lactate Dehydrogenase (LDH) activity was obtained from Elabscience, USA.

Collection and maintenance of cell lines

The T-lymphoblastic leukemia MOLT-4 cells were acquired from ATCC, USA, and planted in DMEM with 10% FBS and 1% antimycotic mixture in 5% CO₂ environment. Cells were collected upon achieving 80% confluency and employed for subsequent assessments.

MTT assay

The cytotoxic potential of bergenin on the growth of MOLT-4 cells was examined using the MTT assay. Cells were cultivated on a 96-well plate for 24 hr. Subsequently, cells were subjected to bergenin treatment for an additional 24 hr at varying dosages of 5, 10, 20, 30, 40, 50, and 60 µM. Following the bergenin treatment, MTT (20 µL) was added to DMEM (100 µL) in the wells and incubated for 4 hr. Following the dissolution of formazan deposits in 100 µL of DMSO, the absorbance was determined at 570 nm.

LDH release assay

The cellular membrane was degraded in response to the drug treatment, and subsequently, LDH enzyme activity was released into the extracellular space. The increased activity of the LDH enzyme indicates severe cellular damage. In this work, the activity of LDH enzyme in the growing media of both untreated and bergenin-treated cells was assessed utilizing a kit (Elabscience, USA), in accordance with the manufacturer's guidelines.

Dual staining

A dual staining assay was performed on both untreated and bergenin-treated MOLT-4 cells to examine apoptotic cell death levels. MOLT-4 cells were cultured in a 24-well plate and exposed to 30 μM of bergenin for an additional 24 hr. A solution of 100 $\mu\text{g}/\text{mL}$ AO/EB dyes was then administered to the wells for 5 min in a light-protected environment. The stained cells were examined using a fluorescence microscope to evaluate apoptotic levels.

Statistical analysis

The statistical tests were conducted using the Graphpad tool, and the values were illustrated as mean $\pm$ SD of triplicates. The findings were assessed using one-way ANOVA and Tukey's *post hoc* tests, with a significance threshold of $p < 0.05$.

In silico approach

The dataset retrieval and its pre-processing for molecular docking analysis

The targets, such as LDHA (PDB ID: 4QT0) (Kolappan *et al.*, 2015) 19 and LDHB (PDB ID: 7DBK),¹⁸ were retrieved from the Protein Data Bank (PDB) to perform the molecular docking analysis.^{19,20} Meanwhile, the bergenin 3D atomic coordinates were obtained from the PubChem compound database in SDF format²¹ was then converted into PDB format using Discovery Studio Visualizer (DSV) v19.1.0.18287 (www.accelerys.com) (DSV). Likely, the retrieved targets, LDHA and LDHB, were pre-processed by removing the associated water molecules and the ligands using DSV.

Molecular docking and visualization

The molecular docking process was carried out for the defined targets LDHA and LDHB with bergenin using Autodock Vina with an advanced scoring algorithm integrated in the PyRx software.²² The Autodock vina uses a new scoring mechanism,

image1

C- Sum of intermolecular and intramolecular distance; Σ - Over all of the pairs of atoms; f_{tij} - Symmetric set of interaction functions; r_{ij} - Interatomic distance

Primarily, the preprocessed LDHA, LDHB, and bergenin were converted into PDBQT format. Subsequently, grid boxes were

assigned for the target proteins LDHA (size_x = 53.14 Å, size_y = 95.60 Å, size_z = 63.85 Å) and LDHB (size_x = size_y = size_z = 25 Å), and the docking runs were completed, resulting in multiple docking poses. From these, the best-docked pose for each complex was selected based on binding affinity and RMSD values and visualized in both 3D and 2D using Discovery Studio Visualizer (DSV).

The evaluation of drug-likeness and toxicity profile

The drug-likeness and the toxicity of the bergenin were identified using the ProTox v3.0 (<https://tox.charite.de/protox3/index.php?site=home>).²³

RESULTS

Effect of Bergenin on the growth of leukemia MOLT-4 cells

Figure 1 depicts the results of the MTT cytotoxicity assay, highlighting the cytotoxic impact of bergenin on the leukemia MOLT-4 cell growth. The bergenin treatment at increasing concentrations (5-60 μM) significantly diminished the growth of MOLT-4 cells at dose-dependently. The increased levels of bergenin demonstrated a marked decrease in the viability of MOLT-4 cells. The IC₅₀ concentration of bergenin was established at 30 μM against MOLT-4 cells, and this dosage was chosen for subsequent tests.

Effect of the bergenin on the LDH activity in MOLT-4 cells

Figure 2 illustrates the findings of LDH activity in untreated and bergenin-treated MOLT-4 cells. The treatment of various concentrations (5-60 μM) of bergenin significantly increased the activity of LDH enzyme in MOLT-4 cells, which indicates the cellular damage in response to the bergenin treatment.

Effect of Bergenin on the apoptotic level in the MOLT-4 cells

Figure 3 illustrates the results of the dual staining assay, which validates the apoptotic effects of bergenin on the leukemia cells. The results demonstrate that treatment with bergenin at a dosage of 30 μM led to a higher number of red fluorescent cells in comparison to the untreated cells. The green fluorescent cells in the control signify the presence of normal viable cells, whereas the increased number of red fluorescent cells in the bergenin-treated cells suggests the presence of apoptotic events.

The interactions of LDHA and LDHB with the bergenin

The targets, including LDHA and LDHB, were selected, and molecular docking with bergenin was performed. The results, including binding affinity, RMSD, and bond types, are tabulated in Table 1.

LDHA interaction with bergenin

The LDHA residues, such as ASN164, HIS186 (2), GLY187, and ARG269, as well as TRP188, interacted well with bergenin via hydrogen and pi-alkyl bonds, with a binding affinity of -6.7 kcal/mol and an RMSD of 2.392 Å. Additionally, the hydrophobic residues, such as SER167, ALA168, ARG171, VAL270, HIS271, and PRO272, surrounded the LDHA-bergenin complex and helped stabilise the interactions (Figures 4A, 4B).

LDHB interaction with bergenin

Likely, the bergenin interacted well with the LDHB residues, which include ARG170, THR249(2), and ASN139(2) via the hydrogen bonds. Meanwhile, the residues such as HIS194 and ALA239 interacted with the bergenin via pi-cation and pi-alkyl bonds. Also, the hydrophobic residues such as VAL32, ARG100, GLN101, SER138, GLY195, ASP196, TYR240, ILE243, TYR248, ASN250, and ILE253 surrounded the LDHB-bergenin complex with the binding affinity of -7.1 kcal/mol, and RMSD was

observed as 1.42Å (Figures 4C, 4D). Meanwhile, the hydrogen bonds were validated and confirmed using LigPlot⁺ v2.2.9. The results confirmed that the stable hydrogen bond interactions are formed with the residues HIS186(2), GLY187, and ASN164(2), and an additional hydrogen bond interaction was observed between HIS271 and bergenin (Figure 4E).

LigPlot⁺ analysis further revealed that bergenin formed an additional hydrogen bond with the LDHA residue HIS271 (Figure 4E), while also confirming stable hydrogen bond interactions with LDHB (Figure 4F).

Drug-likeness and toxicity profile

The drug-likeness properties of the bergenin hold numerous beneficial physicochemical and pharmacological characteristics, such as the molecular weight of 328.27 Da (<500 Da), nine hydrogen bond acceptors, five hydrogen bond donors, and the octanol/water partition coefficient (logP) of -1.2, which indicate

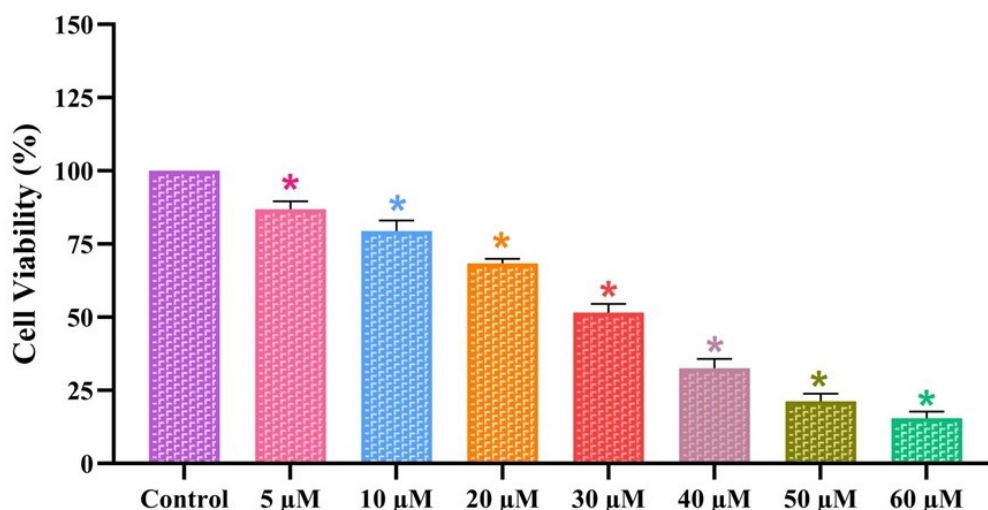


Figure 1: Effect of bergenin on the growth of leukemia MOLT-4 cells. The bergenin treatment at increasing concentrations (5-60 μM) significantly reduced the viability of MOLT-4 cells at dose-dependently. The IC₅₀ concentration of bergenin was established at 30 μM against MOLT-4 cells. The data were displayed as the mean±SD of triplicates. The values were assessed using one-way ANOVA and Tukey's *post hoc* tests using GraphPad Prism software. A symbol "*" denotes that the data were significant at *p*<0.05 from the control group.

Table 1: Targets, PDB ID, binding affinity (kcal/mol), RMSD (Å), and their interacted residues with monotropein and its type of interactions

Targets Name	Targets (PDB ID)	Binding affinity (Kcal/mol)	RMSD (Å)	Hydrogen bonds	Hydrophobic residues	Other types of bonds
LDHA	4QT0	-6.7	2.392	ASN164, GLY187, HIS186(2), ARG269CH	SER167, ALA168, ARG171, VAL270, HIS271, PRO272	TRP188PA
LDHB	7DBK	-7.1	1.42	ARG170, THR249(2), ASN139(2)	VAL32, ARG100, GLN101, SER138, GLY195, ASP196, TYR240, ILE243, TYR248, ASN250, ILE253	HIS194PC, ALA239PA

Note: Types of bonds: CH-Carbon-hydrogen; PA-Pi-alkyl; PC-Pi-cation.

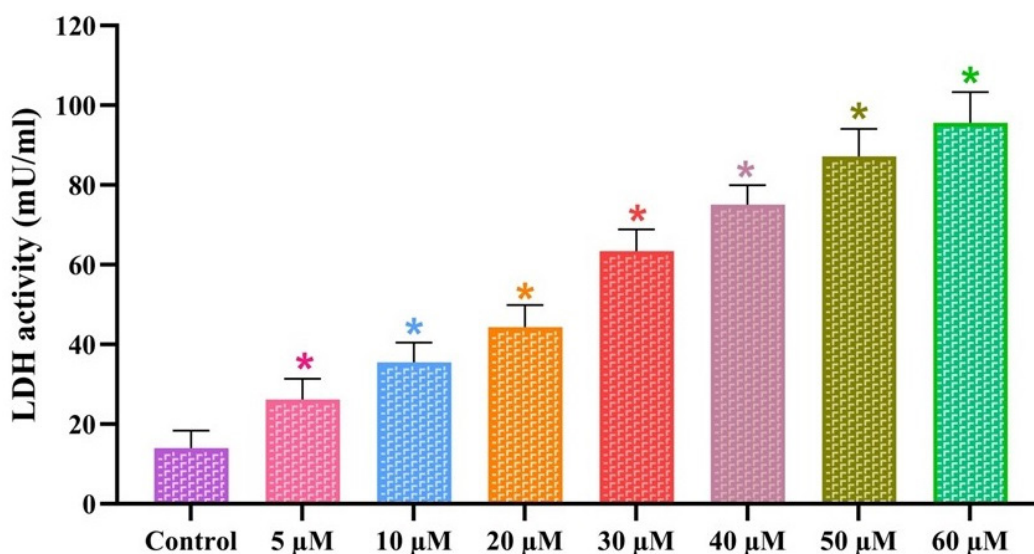


Figure 2: Effect of the bergenin on the LDH activity in MOLT-4 cells. The treatment of various concentrations (5-60 μ M) of bergenin significantly increased the activity of the LDH enzyme in MOLT-4 cells. The data were displayed as the mean $\pm$ SD of triplicates. The values were assessed using one-way ANOVA and Tukey's *post hoc* tests using GraphPad Prism software. A symbol '*' denotes that the data were significant at $p < 0.05$ from the control group.

that the bergenin is highly hydrophilic in nature and has limited passive membrane permeability.

Toxicity profiling of bergenin indicated a low risk of hepatotoxicity (0.86), neurotoxicity (0.87), respiratory toxicity (0.51), cardiotoxicity (0.50), carcinogenicity (0.87), mutagenicity (0.56), and cytotoxicity (0.64). In addition, bergenin was predicted to be unable to cross the Blood-Brain Barrier (BBB), suggesting limited central nervous system exposure. However, a moderate risk of nephrotoxicity (0.73) and a high probability of immunotoxicity (0.99) were observed. Even though bergenin exhibited nephrotoxicity and immunotoxicity, its toxicity class was defined as VI, and the lethal dose was predicted as LD₅₀ 10000 mg/kg, which described that the bergenin toxicity profile was highly suitable to develop as a drug (Table 2).

DISCUSSION

Leukemia, a hematologic malignancy developing from disrupted blood cell production in the bone marrow, poses a pivotal global health challenge, imposing substantial burdens on individuals and healthcare systems. Characterized by the uncontrolled proliferation of abnormal white blood cells, leukemia's global prevalence is estimated at 6.3 and 4.5 per 100,000 people each year in both sexes.²⁴ This pervasive disease is notably the most common cancer in children, responsible for 28% of all pediatric cancer incidences and demonstrating a concerning rise in incidence rates within certain regions. Leukemia's classification into lymphoid and myeloid types, further delineated by acute or chronic progression, underscores the disease's heterogeneity, with acute forms typically advancing rapidly and chronic forms evolving more gradually.²⁵ The global problem of leukemia, encompassing incidence, mortality, and disability-adjusted life years, has shown a considerable decline in age-standardized rates,

yet an increase in prevalent cases and overall prevalence demands urgent attention. Despite advancements in medical treatments that have contributed to a reduction in the global leukemia burden, certain demographic groups, particularly males and the elderly, and specific geographical regions continue to experience disproportionately high rates.²⁶ Current therapeutic modalities for leukemia, predominantly chemotherapy, often yield suboptimal outcomes in specific patient populations, particularly geriatrics, with overall survival rates as low as 5-10%. Such restrictions highlight the immediate need for new therapeutic strategies that not only enhance efficacy but also mitigate the severe toxicities associated with conventional regimens, especially in vulnerable populations.²⁷

The evaluation of cytotoxic level is paramount for identifying compounds with selective toxicity towards malignant cells while sparing healthy tissues, a crucial step in developing effective and safe therapeutic agents. This selectivity is critical for minimizing adverse effects, a significant challenge in conventional chemotherapy, where inadequate tumor specificity often leads to toxicity in normal, rapidly dividing cells.²⁸ Consequently, early and thorough cytotoxicity testing is indispensable for discerning drug candidates that exhibit a favorable therapeutic window, thus guiding the optimization of formulations to enhance both efficacy and safety. The *in vitro* cytotoxicity assays are foundational for early-stage drug discovery, toxicology, and biomedical research, providing vital insights into a compound's impact on cellular health and viability.²⁹ Furthermore, these initial screenings play a pivotal role in prioritizing compounds that demonstrate promising cytotoxic activity against cancer cells with minimal impact on non-cancerous cells, thereby streamlining the drug discovery pipeline. The objective of these cytotoxicity assays is to ascertain the extent to which a sample drug compound can harm

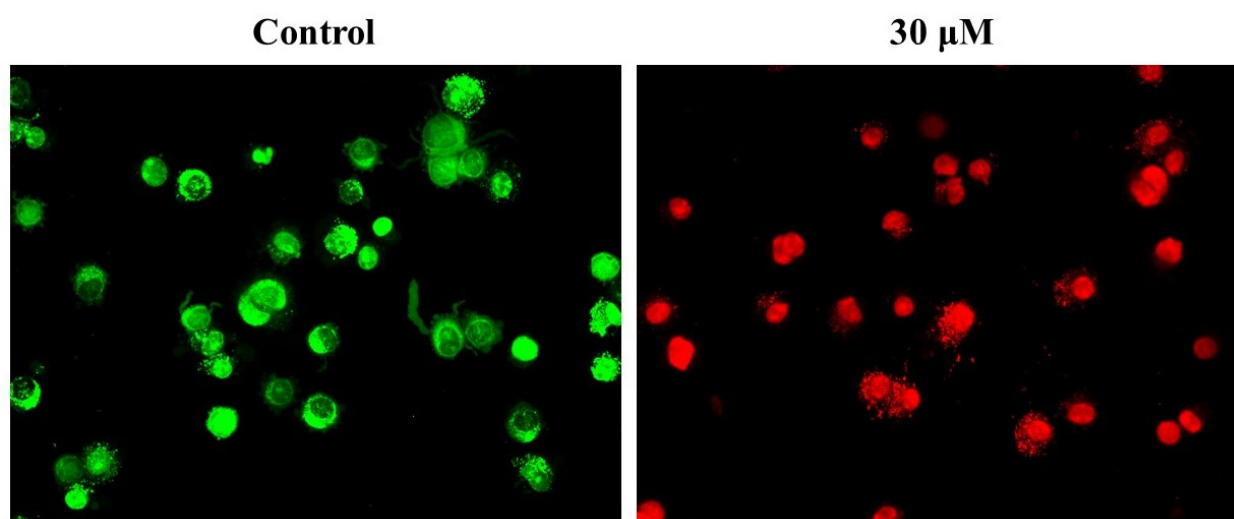


Figure 3: Effect of bergenin on the apoptotic level in the MOLT-4 cells. The results of the dual staining assay demonstrate that bergenin treatment at a 30 μ M dosage resulted in a higher number of red fluorescent cells compared to the untreated cells, which suggests the presence of apoptotic events.

Table 2: The drug-likeness and toxicity profile of bergenin.

Properties	Probability
Drug-likeness	
Molecular weight	328.27
Number of hydrogen bond acceptors	9
Number of hydrogen bond donors	5
octanol/water partition coefficient (logP)	-1.2
Toxicity profile	
Hepatotoxicity	0.86(-)
Neurotoxicity	0.87(-)
Nephrotoxicity	0.73(+)
Respiratory toxicity	0.51(-)
Cardiotoxicity	0.50(-)
Carcinogenicity	0.87(-)
Immunotoxicity	0.99(+)
Mutagenicity	0.56(-)
Cytotoxicity	0.64(-)
BBB-barrier	0.59(-)
Toxicity class	Class VI
LD ₅₀	10000 mg/kg

Note: (-) indicates no toxicity; (+) indicates toxicity.

or induce cell death.³⁰ The MTT assay serves as a fundamental cell-based high-throughput screening method to assess cytotoxic and cytostatic effects. This colorimetric assay, which quantifies cellular metabolic activity, is particularly valuable in the initial screening of potential anticancer agents to identify their capacity to trigger apoptosis or necrosis in tumor cell lines. This makes the

MTT assay a cornerstone in early-stage drug discovery, offering a rapid and cost-effective approach to prioritize promising compounds for further investigation.³¹ Here, we evaluated the cytotoxic level of bergenin against leukemia MOLT-4 cells using the MTT assay. The present findings clearly showed that the bergenin treatment dose-dependently inhibited the growth of MOLT-4 cells, which suggests the cytotoxic effects of bergenin against leukemia cells.

The LDH release assay offers a robust and reliable technique for quantifying cellular cytotoxicity, a critical parameter in assessing the efficacy of potential chemotherapeutic agents against various cancer cell lines. This assay leverages the principle that LDH, a stable cytosolic enzyme, is rapidly released into the extracellular medium upon compromise of the plasma membrane, directly correlating with the extent of cellular damage and mortality. The assay's mechanism involves the conversion of lactate to pyruvate by LDH, which subsequently decreases a tetrazolium salt into a quantifiable formazan product.³² This formazan product can then be measured colorimetrically using a spectrophotometer, providing a direct correlation between absorbance and the amount of LDH released, thus reflecting the level of cytotoxicity. This method is particularly valuable for evaluating drug candidates against aggressive and invasive cancer cell lines, which are frequently employed in anticancer research. The versatility of the LDH release assay makes it an indispensable tool for high-throughput screening of novel compounds, providing an initial assessment of cellular toxicity before more detailed mechanistic studies are conducted.³³ Furthermore, its simplicity, high sensitivity, and cost-effectiveness render it a preferred choice over other cytotoxicity assays for initial drug screening. This methodology assesses membrane integrity, a common indicator of cell viability and cytotoxic effects, by monitoring the efflux of intracellular components like LDH into the extracellular space. This makes it a particularly useful indicator for necrotic cell death,

where plasma membrane permeabilization is a hallmark event. Consequently, the quantification of released LDH activity in cell culture supernatants serves as a direct measure of cell death, providing a distinct advantage over metabolic assays, which may reflect altered cellular activity rather than outright membrane damage.³⁴ Here, the results of the LDH release assay displayed that the treatment of numerous concentrations of bergenin remarkably elevated the activity of LDH enzyme in MOLT-4 cells, which indicates the cellular damage and cytotoxicity in response to the bergenin treatment.

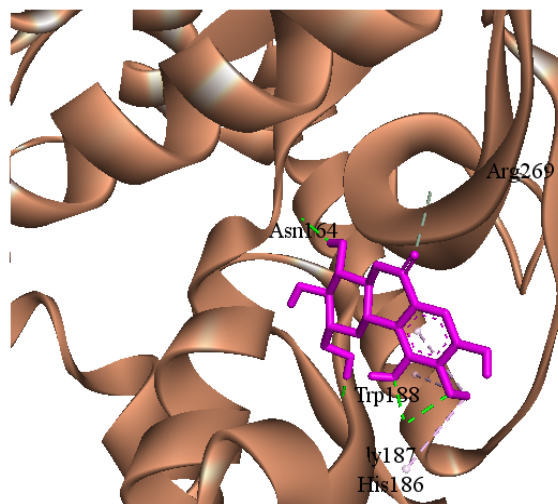
Apoptosis, or programmed cell death, is an essential biological mechanism critical for maintaining cellular homeostasis and discarding unwanted cells, playing a pivotal role in normal development and tissue remodeling. Dysregulation of apoptosis is frequently observed in cancer, where impaired cell death pathways contribute to tumor growth, progression, and resistance to therapy.³⁵ Cancer cells often exhibit an apoptosis-resistant phenotype due to alterations in the intrinsic and extrinsic signaling cascades, which normally promote cell death. This resistance often stems from mutations in key regulatory proteins or aberrant expression of pro-survival factors, thereby circumventing the cell's natural apoptotic mechanisms. Consequently, inducing apoptosis in tumor cells is a highly attractive strategy for successful cancer therapy.³⁶ Many anti-cancer agents, including natural products, are thus designed to exploit or restore these compromised apoptotic pathways to selectively eliminate malignant cells. This therapeutic approach frequently involves leveraging compounds, particularly those derived from natural sources that can modulate critical apoptotic proteins such as the Bcl-2 family, leading to cytochrome c discharge and caspase activation.³⁷ A majority of anticancer natural compounds enhance apoptosis to trigger tumor cell death via either the mitochondrial (intrinsic) or death

receptor (extrinsic) pathways. A significant reduction in apoptosis is frequently linked to malignant diseases, often due to genetic alterations that promote prolonged cell survival. An imbalance favoring anti-apoptotic proteins, rather than the mere expression of a single family member, often confers drug resistance in cancer cells.³⁸

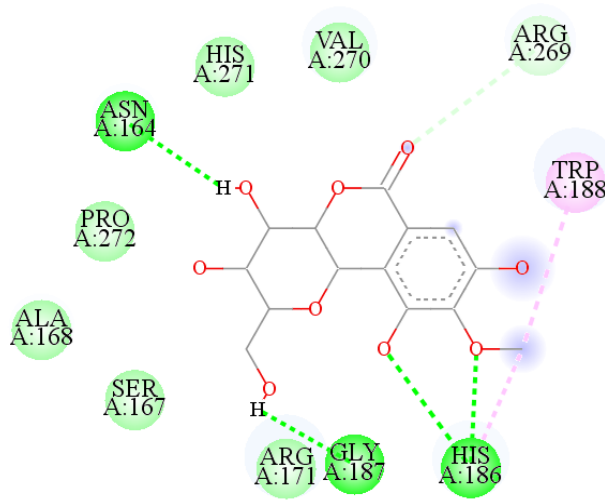
The evasion of apoptosis is considered a hallmark of tumors, allowing for sustained cell survival, proliferation, and the accumulation of mutations that drive tumor progression. Consequently, pharmacological strategies aimed at inhibiting these anti-apoptotic proteins represent a promising avenue for restoring apoptotic sensitivity in cancer cells and enhancing therapeutic efficacy.³⁹ The ability of a drug to selectively trigger programmed cell death, or apoptosis, in malignant cells is a crucial indicator of its therapeutic efficacy and potential as an anticancer agent. This distinction between desirable programmed cell death and indiscriminate necrosis is paramount in early drug evaluation. Understanding the mechanisms through which novel compounds trigger apoptosis provides critical insights into their mode of action and helps predict potential side effects and resistance pathways.⁴⁰ The modulation of apoptosis in cancer tumor cells is renowned for its enormous therapeutic values in oncology, making the initiation of apoptosis a critical mode of action for anti-tumor drugs. Consequently, evaluating the apoptotic activity of novel compounds against cancer cell lines provides a crucial benchmark for their development as effective anticancer therapies.⁴¹ Here, we evaluated the apoptosis-inducing potentials of bergenin against leukemia cells. The present findings demonstrated that the bergenin treatment led to a higher number of red fluorescent cells, signifying the presence of apoptotic events. These findings demonstrate the apoptotic properties of bergenin against leukemia cells.

LDHA (PDB ID: 4QT0)

(A)



(B)



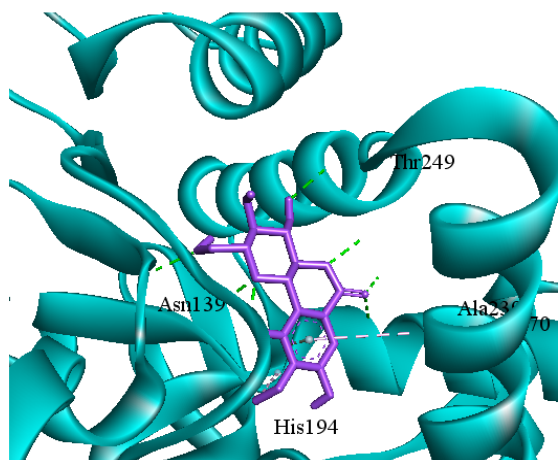
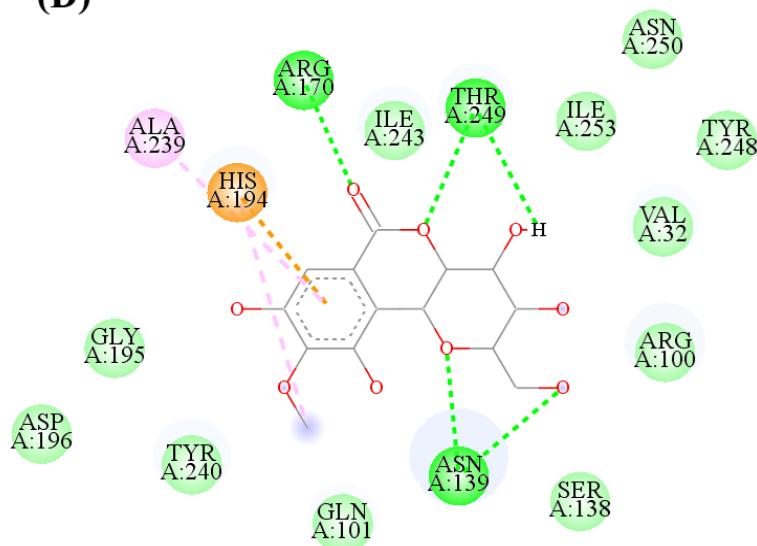
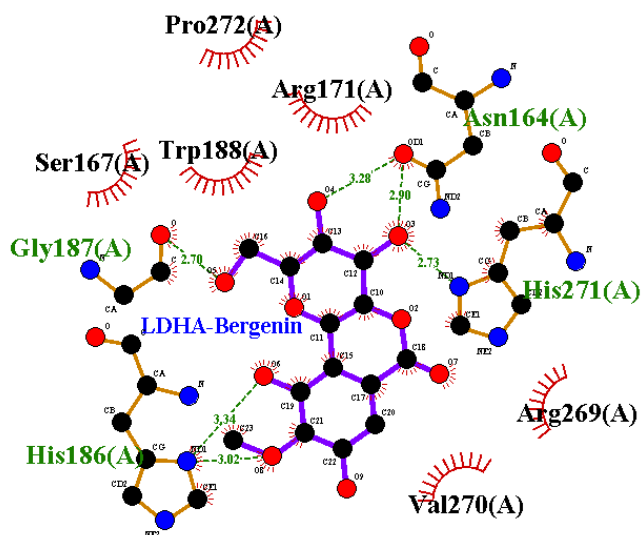
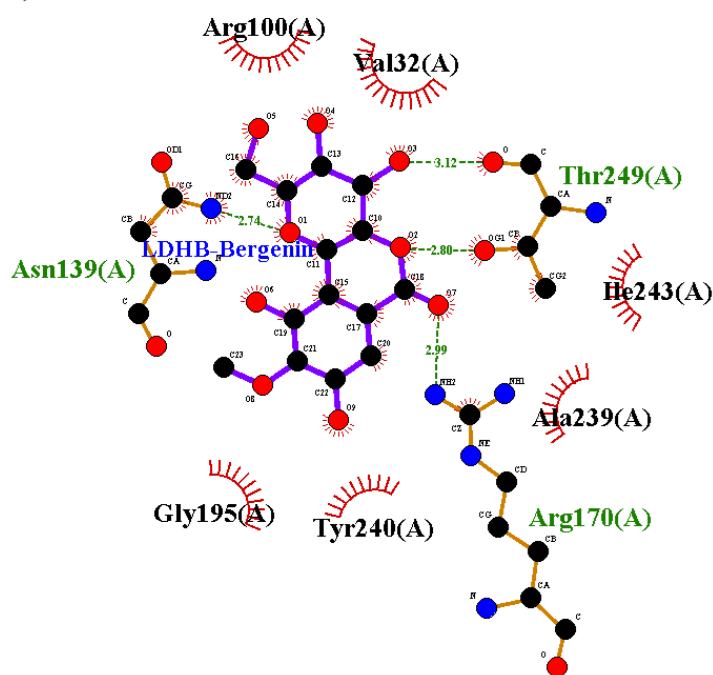
LDHB (PDB ID: 7DBK)**(C)****(D)****(E)****(F)**

Figure 4: The docking poses of targets LDHA and LDHB with the bergenerin and its validation using LigPlot+. (A, B) The 3D and 2D docked poses of the LDHA with bergenerin (C, D). The 3D and 2D docked poses of the LDHB with bergenerin (E, F). The LigPlot+ validation for the LDHA and LDHB interacted with the monotropein to confirm the hydrogen bonds.

Additionally, the possible molecular mechanisms underlying these biological effects were evaluated through molecular docking studies targeting LDHA and LDHB, two key enzymes predominantly involved in glycolysis that support the rapid proliferation and survival of leukemia cells.⁴²⁻⁴⁴ Hence, inhibition of LDHA and LDHB could induce programmed cell death, which might be the causative reason for the increased LDH release observed from damaged leukemia cells. Accordingly, bergenerin exhibited a better binding affinity with LDHA and LDHB,

indicating stable and energetically favourable interactions, and the optimal RMSD value was observed for both complexes. Meanwhile, the drug-likeness properties and toxicity profile of the bergenerin described that the bergenerin was classified as a class VI drug, and the LD₅₀ values were identified as 10000 mg/kg, which clearly stated that bergenerin is considered to be a potential compound in treating leukemia after being validated with enormous *in vitro* and *in vivo* studies. Also, our study was

correlated with the earlier report that bergenin cannot penetrate into the BBB.⁴⁵

CONCLUSION

The results of this study demonstrate that bergenin treatment effectively suppresses proliferation and triggers apoptosis in leukemia MOLT-4 cells. The administration of bergenin increased LDH release and triggered cell death in MOLT-4 cells. Meanwhile, the computational analysis indicated that the bergenin exhibits better binding affinity with the LDHA and LDHB, which may suppress their enzymatic activity, and thus disrupt glycolytic metabolism, and subsequently induce apoptosis. Altogether, the results strongly stated that bergenin has the potential to function as an anticancer agent for the treatment of leukemia. Furthermore, additional *in vitro* and *in vivo* experiments are necessary to confirm the identified specific molecular mechanisms underlying bergenin-induced apoptosis in leukemia cells.

ABBREVIATIONS

DSV: DiscoveryStudio Visualizer; **BA:** Binding Affinity; **FBS:** Fetal Bovine Serum; **DMSO:** Dimethyl Sulfoxide; **DMEM:** Dulbecco's Modified Eagle Medium; **LDH:** Lactate Dehydrogenase; **BBB:** Blood-Brain Barrier; **AO:** Acridine Orange; **EB:** Ethidium Bromide; **IC₅₀:** Half maximal inhibitory concentration; **RMSD:** Root-mean-square deviation; **SD:** Standard Deviation; **ATCC:** American Type Culture Collection; **PDB:** Protein Data Bank; **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

J.Y. and L.Y. conceived and designed the study. A.B. performed the experiments and collected the data. Y.L. and S.X. conducted the data analysis and interpretation. C.H. contributed to methodology development and provided technical support. R.G. assisted in statistical analysis and validation of results. S.X. supervised the project, drafted the manuscript, and provided critical revisions to the manuscript; all authors reviewed, edited, and approved the final version of the manuscript.

ANIMAL APPROVAL

The experiment has been approved by the animal committee of Affiliated Hospital of Hebei University.

SUMMARY

The present study investigated the anticancer potential of bergenin against leukemia MOLT-4 cells. The findings revealed that bergenin treatment significantly inhibited cell proliferation

and promoted apoptotic cell death in MOLT-4 cells. Increased Lactate Dehydrogenase (LDH) release following bergenin exposure indicated enhanced cytotoxicity and membrane damage, confirming the induction of cell death. In addition, computational molecular docking analysis demonstrated that bergenin exhibits strong binding affinity toward LDHA and LDHB, key enzymes involved in cellular glycolysis. This interaction may inhibit their enzymatic activity, leading to disruption of glycolytic metabolism, which is essential for the survival and growth of cancer cells. The impairment of this metabolic pathway likely contributes to the activation of apoptosis in leukemia cells.

REFERENCES

- Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2024;74(3):229-63.
- Daltveit DS, Morgan E, Colombet M, Steliarova-Foucher E, Bendahhou K, Marcos-Gragera R, et al. Global patterns of leukemia by subtype, age, and sex in 185 countries in 2022. *Leukemia.* 2025;39(2):412-9.
- Bispo JAB, Pinheiro PS, Kobetz EK. Epidemiology and etiology of leukemia and lymphoma. *Cold Spring Harb Perspect Med.* 2020;10:a034819.
- Lamb M, Painter D, Howell D, Barrans S, Cargo C, de Tute R, et al. Lymphoid blood cancers, incidence and survival 2005-2023: A report from the UK's Haematological Malignancy Research Network. *Cancer Epidemiol.* 2024;88:102513.
- Solans M, Osca-Gelis G, Comas R, Roncero JM, Gallardo D, Marcos-Gragera R, et al. Challenges in assessing the real incidence of chronic lymphocytic leukemia: 16 years of epidemiological data from Girona, Spain. *Cancer Causes Control.* 2018;29(3):379-82.
- Trallero J, Sanvisens A, Almela Vich F, Jekhalef El Karoni N, Saez Lloret I. Incidence and time trends of childhood hematological neoplasms: a 36-year population-based study in the southern European context, 1983-2018. *Front Oncol.* 2023;13:1197850.
- Schmidt JA, Hornhardt S, Erdmann F, Sánchez-García I, Fischer U, Schüz J, et al. Risk factors for childhood leukemia: radiation and beyond. *Front Public Health.* 2021;9:805757.
- Morcos-Sandino M, Quezada-Ramírez SI, Gómez-De León A. Advances in the treatment of acute myeloid leukemia: implications for low- and middle-income countries. *Biomedicine.* 2025;13(5):1221.
- Döhner H, Wei AH, Appelbaum FR, Craddock C, DiNardo CD, Dombret H, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. *Blood.* 2022;140(12):1345-77.
- Pandya BJ, Chen CC, Medeiros BC, McGuinness CB, Wilson SD, Walsh EH, et al. Economic and clinical burden of acute myeloid leukemia episodes of care in the United States: a retrospective analysis of a commercial payer database. *J Manag Care Spec Pharm.* 2020;26:849-59.
- Huggar D, Knoth RL, Copher R, Cao Z, Lipkin C, McBride A, et al. Economic burden in US patients with newly diagnosed acute myeloid leukemia receiving intensive induction chemotherapy. *Future Oncol.* 2022;18:3609-21.
- Kumar R, Patel DK, Prasad SK, Laloo D, Krishnamurthy S, Hemalatha S. Type 2 antidiabetic activity of bergenin from the roots of *Caesalpinia digyna* Rottler. *Fitoterapia.* 2012;83(2):395-401.
- Rao K, Roome T, Aziz S, Razzak A, Abbas G, Imran M, et al. Bergenin-loaded gum xanthan stabilized silver nanoparticles suppress synovial inflammation through modulation of the immune response and oxidative stress in adjuvant-induced arthritic rats. *J Mater Chem B.* 2018;6:4486-501.
- Gan Y, Li X, Han S, Zhou L, Li W. Targeting Mcl-1 degradation by bergenin inhibits tumorigenesis of colorectal cancer cells. *Pharmaceuticals (Basel).* 2023;16(2):241.
- Liu J, Zhang Y, Yu C, Zhang P, Gu S, Wang G, et al. Bergenin inhibits bladder cancer progression via activating the PPAR γ /PTEN/Akt signaling pathway. *Drug Dev Res.* 2021;82(2):278-86.
- Chauhan R, Malhotra L, Gupta A, Dagar G, Mendiratta M, Masoodi T, et al. Bergenin inhibits growth of human cervical cancer cells by decreasing Galectin-3 and MMP-9 expression. *Sci Rep.* 2024;14(1):15287.
- Feng Y, Xiong Y, Qiao T, Li X, Jia L, Han Y. Lactate dehydrogenase A: a key player in carcinogenesis and potential target in cancer therapy. *Cancer Med.* 2018;7(12):6124-36.
- Shibata S, Sogabe S, Miwa M, Fujimoto T, Takakura N, Naotsuka A, et al. Identification of the first highly selective inhibitor of human lactate dehydrogenase B. *Sci Rep.* 2021;11(1):21353.
- Erdem A, Kaye S, Caligiore F, Johanns M, Leguay F, Schuringa JJ, et al. Lactate dehydrogenase A-coupled NAD⁺ regeneration is critical for acute myeloid leukemia cell survival. *Cancer Metab.* 2025;13(1):22.

20. Kolappan S, Shen DL, Mosi R, Sun J, McEachern EJ, Vocadlo DJ, *et al.* Structures of lactate dehydrogenase A (LDHA) in apo, ternary and inhibitor-bound forms. *Acta Crystallogr D Struct Biol.* 2015;71(Pt 2):185-95.
21. Saeed M, Tasleem M, Shoib A, Kausar MA, Sulieman AME, Alabdallah NM, *et al.* Identification of putative plant-based ALR-2 inhibitors to treat diabetic peripheral neuropathy. *Curr Issues Mol Biol.* 2022;44(7):2825-41.
22. Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. *Methods Mol Biol.* 2015;1263:243-50.
23. Banerjee P, Kemmler E, Dunkel M, Preissner R. ProTox 3.0: a webserver for the prediction of toxicity of chemicals. *Nucleic Acids Res.* 2024; 52(W1):W513-W520.
24. Miranda-Filho A, Piñeros M, Ferlay J, Soerjomataram I, Monnereau A, Bray F. Epidemiological patterns of leukaemia in 184 countries: a population-based study. *Lancet Haematol.* 2018;5:e14-e24.
25. Zhou Y, Huang G, Cai X, Liu Y, Qian B, Li D. Global, regional, and national burden of acute myeloid leukemia, 1990-2021: a systematic analysis for the Global Burden of Disease Study 2021. *Biomark Res.* 2024;12:101.
26. Han X, Yun Z, Liu Z, Si Y, Tian S, Zhang Y, *et al.* Global, regional, and national burden of acute leukemia and its risk factors from 1990 to 2021 and predictions to 2040. *Biomed Eng Online.* 2025;24(1):72.
27. Gómez-De León A, Demichelis-Gómez R, Da Costa-Neto A, Gómez-Almaguer D, Rego EM. Acute myeloid leukemia: challenges for diagnosis and treatment in Latin America. *Hematology.* 2023;28:2158015.
28. Hernández Martínez X, Contreras-Ochoa CO, Mir-García M, Aguilar-García N, Cortés Martínez H, Morales-Hipólito EA, *et al.* Development and in vitro cytotoxicity evaluation of individual and combined injectable solutions of curcumin and resveratrol against lung cancer cells. *Antioxidants (Basel).* 2025;14(8):983.
29. Wang C, Song X, Shang M, Zou W, Zhang M, Wei H, *et al.* Curcumin exerts cytotoxicity dependent on reactive oxygen species accumulation in non-small-cell lung cancer cells. *Future Oncol.* 2019;15:1243-53.
30. Harutyunyan K, Nersesova L, Ayvazyan V, Avagyan E, Melkumyan M, Sargsyan M, *et al.* *In silico* and *in vitro* evaluation of drug-like properties and anticancer potential of novel 5-fluorouracil derivatives. *Sci Rep.* 2025;15(1):39689.
31. Sazonova EV, Chesnokov MS, Zhivotovsky B, Kopeina GS. Drug toxicity assessment: cell proliferation versus cell death. *Cell Death Discov.* 2022;8(1):417.
32. Chan FKM, Moriwaki K, De Rosa MJ. Detection of necrosis by release of lactate dehydrogenase (LDH) activity. *Methods Mol Biol.* 2013;979:65-70.
33. De Hamer PCW, Jonker A, Leenstra S, Ruijter JM, Van Noorden CJF. Quantification of viability in organotypic multicellular spheroids of human malignant glioma using lactate dehydrogenase activity: a rapid and reliable automated assay. *J Histochem Cytochem.* 2005;53:23-34.
34. Cox MC, Mendes R, Silva F, Mendes TF, Zelaya-Lazo A, Halwachs K, *et al.* Application of LDH assay for therapeutic efficacy evaluation of ex vivo tumor models. *Sci Rep.* 2021;11(1):18571.
35. Singh AK, Kushwaha PP, Singh AK, Pandey AK. Overcoming cancer drug resistance: insights into apoptotic pathway modulation by plant-based nanoparticles and natural products. *Front Oncol.* 2025;15:1677380.
36. Chaudhry GS, Md Akim A, Sung YY, Sifzizul TMT. Cancer and apoptosis: the apoptotic activity of plant and marine natural products and their potential as targeted cancer therapeutics. *Front Pharmacol.* 2022;13:842376.
37. Goldar S, Khaniani MS, Derakhshan SM, Baradaran B. Molecular mechanisms of apoptosis and roles in cancer development and treatment. *Asian Pac J Cancer Prev.* 2015;16:2129-44.
38. Jan R, Chaudhry GS. Understanding apoptosis and apoptotic pathways targeted cancer therapeutics. *Adv Pharm Bull.* 2019;9:205-18.
39. Lee SH, Park SM, Park JH, Shin DY, Kim GY, Ryu CH, *et al.* Induction of apoptosis in human leukemia U937 cells by anthocyanins through down-regulation of Bcl-2 and activation of caspases. *Int J Oncol.* 2009;34(4):1077-83.
40. Reis-Silva CSM, Branco PC, Lima K, Silva FL, Moreno PRH, Guallar V, *et al.* Embelin potentiates venetoclax-induced apoptosis in acute myeloid leukemia cells. *Toxicol in vitro.* 2021;76:105207.
41. Liu F, Knight T, Su Y, Edwards H, Wang G, Wang Y, *et al.* Venetoclax synergistically enhances the anti-leukemic activity of vosaroxin against acute myeloid leukemia cells *ex vivo*. *Target Oncol.* 2019;14(3):351-64.
42. Akers LJ, Fang W, Levy AG, Franklin AR, Huang P, Zweidler-McKay PA. Targeting glycolysis in leukemia: a novel inhibitor 3-BrOP in combination with rapamycin. *Leuk Res.* 2011;35(6):814-20.
43. Urbańska K, Orzechowski A. Unappreciated role of LDHA and LDHB to control apoptosis and autophagy in tumor cells. *Int J Mol Sci.* 2019;20(9):2085.
44. Zhang R, Jin W, Wang K. Glycolysis-driven prognostic model for acute myeloid leukemia: insights into the immune landscape and drug sensitivity. *Biomedicines.* 2025;13(4):834.
45. Pandey P, Lakhanpal S, Mahmood D, Kang HN, Kim B, Kang S, *et al.* Bergenin, a bioactive flavonoid: advancements in the prospects of anticancer mechanism, pharmacokinetics and nanoformulations. *Front Pharmacol.* 2025;15:1481587.

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