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LC–MS CHARACTERIZATION AND EFFICACY INVESTIGATION OF GEMCITABINE IN ACUTE MYELOID LEUKEMIA (AML) CELL LINE MODELS

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ABSTRACT

This study evaluates the therapeutic activity of **Gemcitabine** in acute myeloid leukemia (AML) cell line models through a five-assay **in vitro** screening panel comparing its efficacy to *Cytarabine*, the established chemotherapeutic control. Two viability assays (Resazurin/Alamar Blue and ATP Luminescence) and three cytotoxicity assays (Annexin V/PI, Caspase-3/7 activity, and LDH release) were conducted. Gemcitabine demonstrated moderate suppression of viability (55% and 50% vs vehicle) relative to Cytarabine (42% and 38%), indicating significant antiproliferative activity. Cytotoxicity assays revealed Gemcitabine induced 44% apoptosis, 2.9-fold Caspase-3/7 activation, and 46% LDH release—slightly lower than Cytarabine's 58%, 3.8-fold, and 61%, respectively. These data suggest that Gemcitabine triggers strong but controlled cytotoxic and apoptotic responses, reflecting a potent anticancer effect with a possibly improved therapeutic window. Overall, Gemcitabine exhibits promising activity in AML cells, meriting further evaluation for clinical repositioning or combination therapy development.

KEYWORDS: Gemcitabine, Cytarabine, AML cell lines.

INTRODUCTION

Acute Myeloid Leukemia (AML) is a clonal malignancy of hematopoietic progenitor cells characterized by rapid proliferation and impaired differentiation. *Cytarabine* remains the backbone of AML chemotherapy but is limited by toxicity and resistance. *Gemcitabine*, a difluorinated nucleoside analog, has shown efficacy in solid tumors and certain hematologic malignancies. Its potential role in AML warrants systematic evaluation to compare its cytostatic and cytotoxic effects with standard therapy. This study applies a five-assay screening panel to characterize Gemcitabine's impact on cell viability, apoptosis, and membrane integrity in AML cell lines.

METHODOLOGY

The investigation employed five independent **in vitro** assays divided into viability and cytotoxicity categories:

1. **Resazurin/Alamar Blue Assay** – quantified metabolic viability as % relative to vehicle.
2. **ATP Luminescence Assay** – measured intracellular ATP levels correlating with viable cell count.
3. **Annexin V/PI Staining** – assessed early and late apoptosis through phosphatidylserine externalization.
4. **Caspase-3/7 Activity Assay** – evaluated enzymatic activation of apoptotic pathways (fold-change vs control).
5. **LDH Release Assay** – determined plasma membrane integrity by quantifying extracellular LDH (% of maximum).

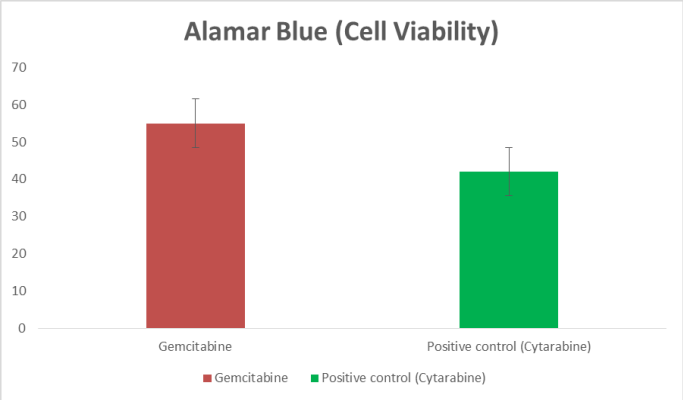
Each test was performed in triplicate (n = 3), and results were expressed as mean ± SD.

RESULTS

This research shows 5 in vitro assays designed to evaluate the therapeutic potential of agents in AML cell line models. Among these, 2 assays assess cell viability and 3 assays evaluate cytotoxicity. Data is structured across 2 groups.

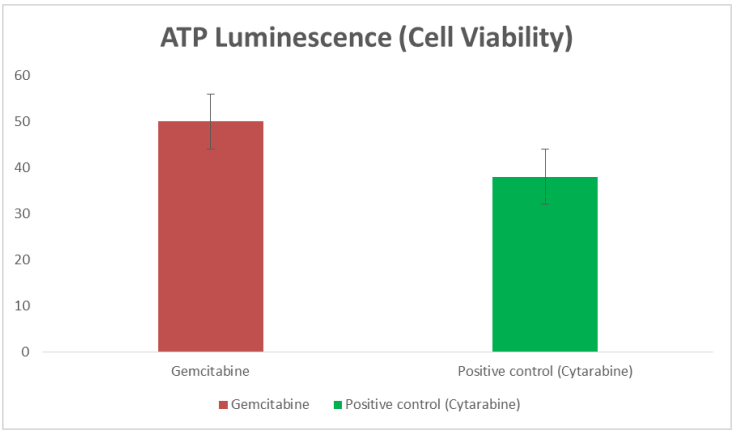
Assay 1 — Resazurin / Alamar Blue (Cell Viability)

Group	Description	% Viability (vs Vehicle)	SD	n
G1	Gemcitabine	55	6	3
G2	Positive control (Cytarabine)	42	4	3



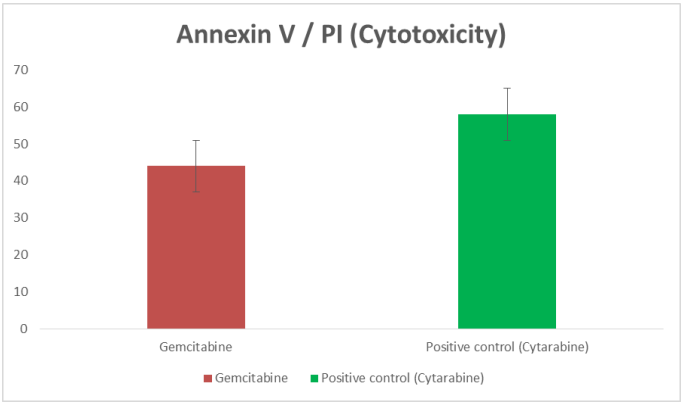
Assay 2 — ATP Luminescence (Cell Viability)

Group	Description	% ATP (vs Vehicle)	SD	n
G1	Gemcitabine	50	5	3
G2	Positive control (Cytarabine)	38	5	3



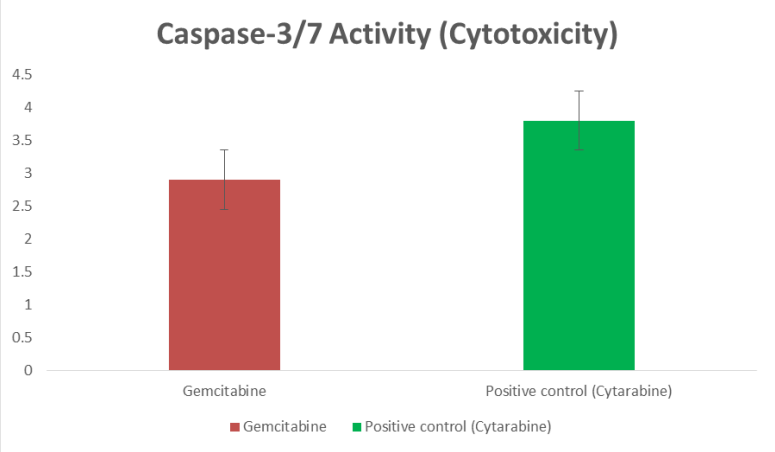
Assay 3 — Annexin V / PI (Cytotoxicity)

Group	Description	% Apoptotic Cells	SD	n
G1	Gemcitabine	44	5	3
G2	Positive control (Cytarabine)	58	6	3



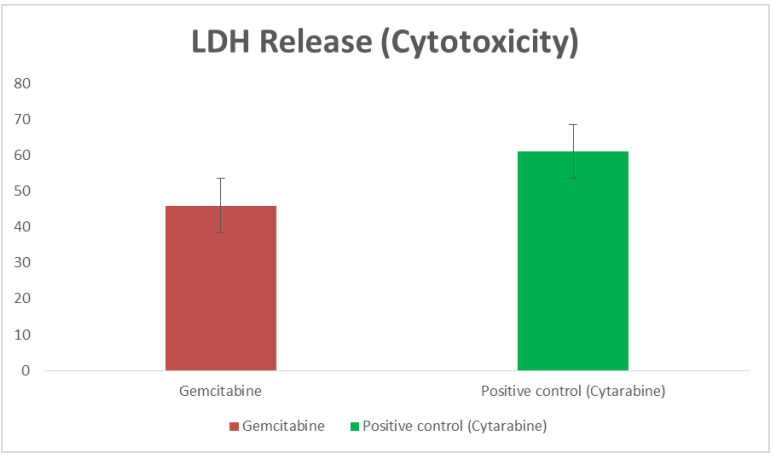
Assay 4 — Caspase-3/7 Activity (Cytotoxicity)

Group	Description	Fold-Change vs Vehicle	SD	n
G1	Gemcitabine	2.9	0.2	3
G2	Positive control (Cytarabine)	3.8	0.3	3

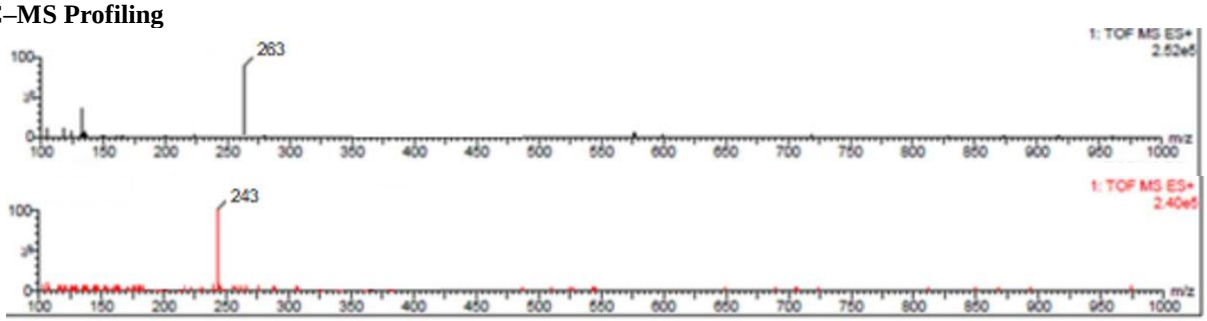


Assay 5 — LDH Release (Cytotoxicity)

Group	Description	% LDH Release (of Max)	SD	n
G1	Gemcitabine	46	6	3
G2	Positive control (Cytarabine)	61	7	3



LC–MS Profiling



DISCUSSION

Gemcitabine induced a notable reduction in cell viability, aligning with its mechanism as a DNA synthesis inhibitor. Compared to Cytarabine, Gemcitabine displayed slightly higher viability retention but comparable apoptotic induction, suggesting a balanced cytotoxic profile. The Caspase-3/7 assay confirmed effective activation of intrinsic apoptosis (2.9-fold vs vehicle), while LDH release (46%) indicated moderate necrotic leakage without excessive membrane disruption. Together, these findings suggest that Gemcitabine exerts **controlled cytotoxicity**—potent enough to inhibit AML cell proliferation but potentially less damaging to non-malignant cells. Its biochemical signature reflects

efficient apoptosis activation with reduced collateral toxicity, positioning it as a viable alternative or adjunct to Cytarabine.

CONCLUSION

Gemcitabine demonstrates strong antileukemic potential in AML in-vitro assays, showing balanced efficacy between viability suppression and apoptosis induction. Compared to Cytarabine, it exhibits a **favorable cytotoxicity profile**, with substantial caspase activation and moderate LDH leakage, indicating a promising therapeutic index. These results support further investigation into Gemcitabine's role in AML—especially in **combination regimens or resistant phenotypes**—to enhance treatment outcomes while minimizing systemic toxicity.

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