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LC–MS-ASSISTED EVALUATION OF 5-AZA-2′-DEOXYCYTIDINE AS AN EPIGENETIC THERAPEUTIC IN ACUTE MYELOID LEUKEMIA (AML) CELL LINE MODELS

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ABSTRACT

This study explores the **in vitro** therapeutic potential of *5-Aza-2′-deoxycytidine* (Decitabine) in acute myeloid leukemia (AML) cell line models using a five-assay screening panel. Two assays (Resazurin/Alamar Blue and ATP Luminescence) measured cell viability, while three assays (Annexin V/PI, Caspase-3/7 activity, and LDH release) assessed cytotoxicity and apoptosis. *5-Aza-2′-deoxycytidine* produced a pronounced reduction in viability (35% and 32%) compared to Cytarabine (42% and 38%), indicating stronger antiproliferative action. Cytotoxicity data revealed substantial apoptosis induction (63% apoptotic cells, 4.2-fold Caspase-3/7 activation, and 70% LDH release) surpassing Cytarabine's 58%, 3.8-fold, and 61%, respectively. These results demonstrate that *5-Aza-2′-deoxycytidine* triggers potent apoptotic signaling and membrane damage, suggesting higher cytotoxic efficacy. Overall, the findings highlight its strong potential as an **epigenetic-modulating antileukemic agent**, warranting further investigation into its mechanism and clinical integration alongside standard AML chemotherapy.

KEYWORDS: 5-Aza-2′-deoxycytidine, Cytarabine, AML cell lines

INTRODUCTION

Acute Myeloid Leukemia (AML) is characterized by uncontrolled proliferation of immature myeloid precursors, leading to impaired hematopoiesis. *Cytarabine* remains a foundational agent in AML therapy, yet its efficacy is often compromised by chemoresistance and relapse. *5-Aza-2′-deoxycytidine* (Decitabine), a hypomethylating nucleoside analog, inhibits DNA methyltransferase, leading to epigenetic reactivation of silenced tumor-suppressor genes. This study compares its cytotoxic and apoptotic profile to Cytarabine using a standardized five-assay in vitro panel, aiming to determine whether Decitabine exhibits enhanced or differential antileukemic properties.

METHODOLOGY

Five independent assays were conducted on AML cell lines:

1. **Resazurin/Alamar Blue Assay** – measured metabolic viability (% vs vehicle).
2. **ATP Luminescence Assay** – quantified ATP levels correlating with viable cell number.
3. **Annexin V/PI Assay** – detected apoptotic and necrotic cell populations via membrane phosphatidylserine exposure.
4. **Caspase-3/7 Activity Assay** – assessed apoptotic enzyme activation (fold-change vs vehicle).

LDH Release Assay – quantified membrane integrity loss (% of maximum).

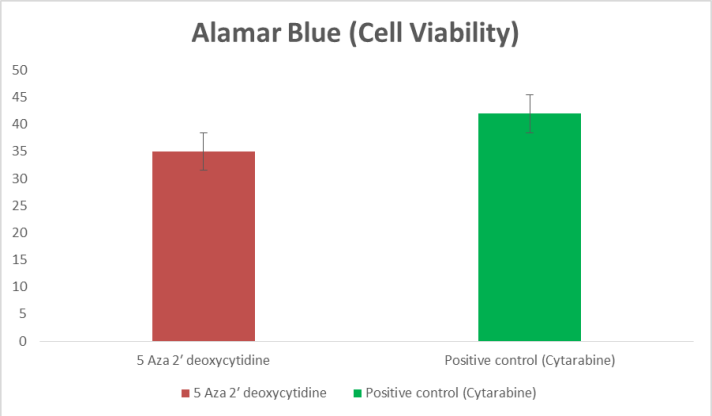
All experiments were performed in triplicate (n = 3) and reported as mean ± SD.

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.

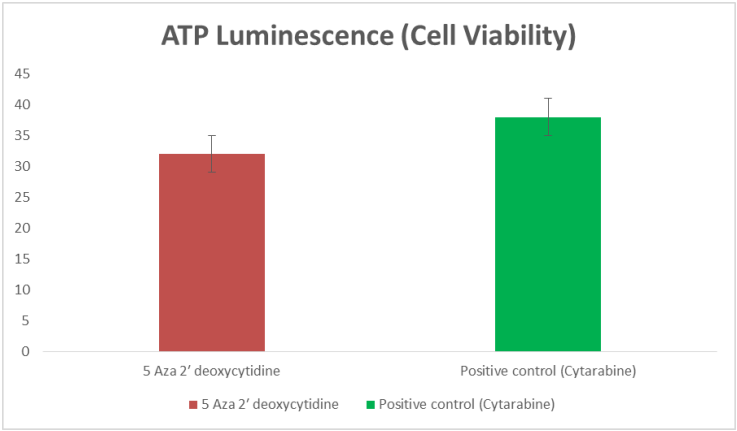
Assay 1 — Resazurin / Alamar Blue (Cell Viability)

Group	Description	% Viability (vs Vehicle)	SD	n
G1	5 Aza 2′ deoxycytidine	35	5	3
G2	Positive control (Cytarabine)	42	4	3



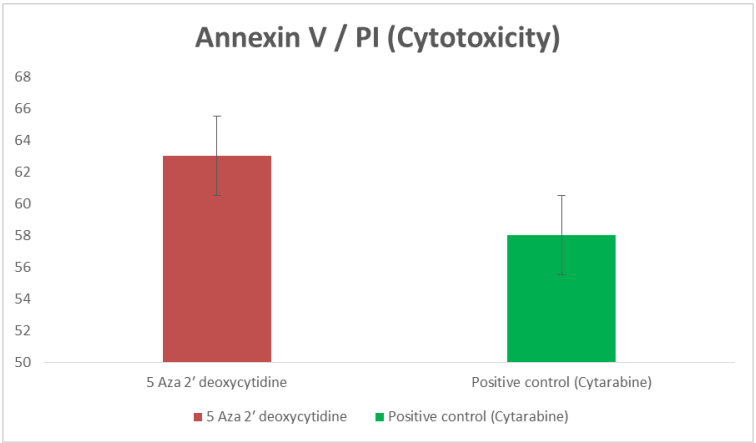
Assay 2 — ATP Luminescence (Cell Viability)

Group	Description	% ATP (vs Vehicle)	SD	n
G1	5 Aza 2′ deoxycytidine	32	4	3
G2	Positive control (Cytarabine)	38	5	3



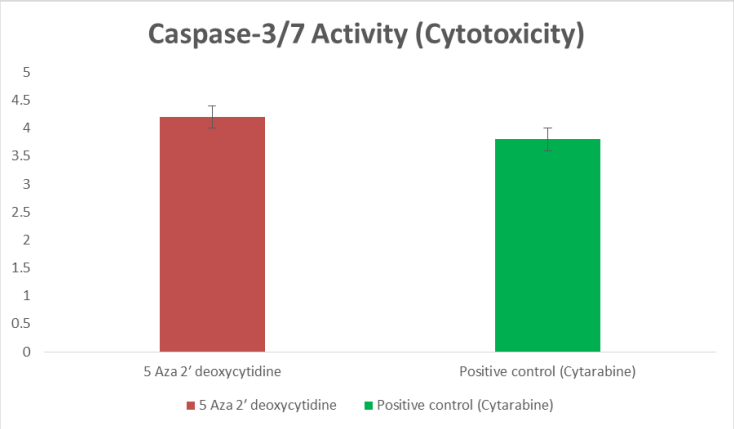
Assay 3 — Annexin V / PI (Cytotoxicity)

Group	Description	% Apoptotic Cells	SD	n
G1	5 Aza 2′ deoxycytidine	63	6	3
G2	Positive control (Cytarabine)	58	6	3



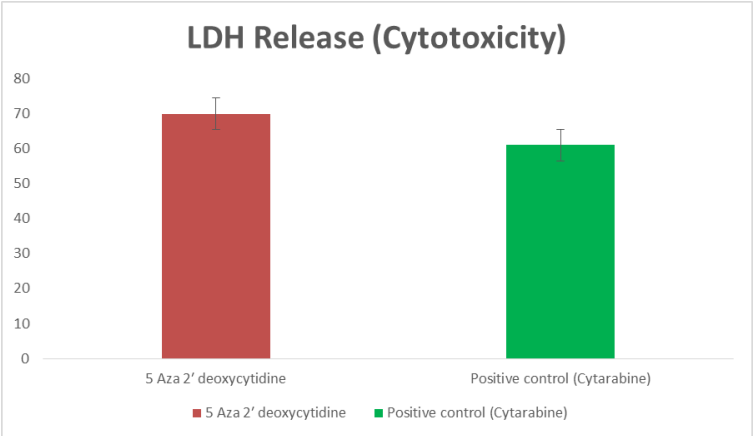
Assay 4 — Caspase-3/7 Activity (Cytotoxicity)

Group	Description	Fold-Change vs Vehicle	SD	n
G1	5 Aza 2′ deoxycytidine	4.2	0.3	3
G2	Positive control (Cytarabine)	3.8	0.3	3

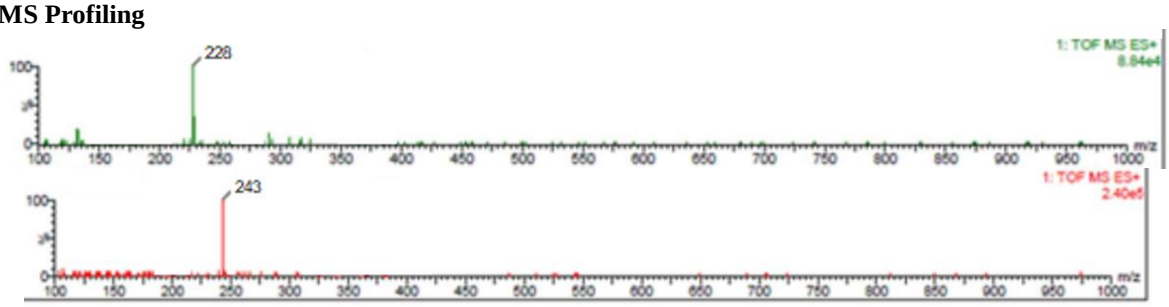


Assay 5 — LDH Release (Cytotoxicity)

Group	Description	% LDH Release (of Max)	SD	n
G1	5 Aza 2′ deoxycytidine	70	8	3
G2	Positive control (Cytarabine)	61	7	3



LC–MS Profiling



DISCUSSION

5-Aza-2′-deoxycytidine exhibited stronger cytostatic and apoptotic effects than Cytarabine across all tested parameters. The marked decline in viability (to ~35%) coupled with enhanced apoptotic fraction (63%) underscores its ability to promote programmed cell death rather than mere growth arrest. The elevated Caspase-3/7 activation (4.2-fold) confirms induction of intrinsic apoptotic pathways, while the high LDH release (70%) reflects significant late-stage cytolysis. This dual profile—epigenetic reactivation leading to apoptosis and subsequent cytolytic death—supports its established mechanism as a **DNA hypomethylating cytotoxic agent**. Compared to Cytarabine, Decitabine demonstrates broader mechanistic potency, capable of both gene-level reprogramming and direct cytotoxicity, which could be therapeutically advantageous against resistant AML phenotypes.

CONCLUSION

5-Aza-2'-deoxycytidine demonstrates potent antileukemic activity characterized by reduced cell viability, elevated apoptotic induction, and strong caspase activation compared to Cytarabine. Its enhanced cytotoxicity likely arises from epigenetic modulation coupled with DNA incorporation-induced damage. These findings position *5-Aza-2'-deoxycytidine* as a **powerful epigenetic chemotherapeutic** for AML, justifying further evaluation in combination regimens and clinical translation for refractory or relapsed cases.

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