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LC-MS-BASED INVESTIGATION OF THIARABINE AS A THERAPEUTIC AGENT IN ACUTE MYELOID LEUKEMIA (AML) CELL LINE MODELS

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ABSTRACT

This study investigates the therapeutic profile of *Thiarabine* compared to the standard antileukemic agent *Cytarabine* using a five-assay **in vitro** screening panel on acute myeloid leukemia (AML) cell line models. Two assays measured cell viability (Resazurin/Alamar Blue and ATP Luminescence), while three evaluated cytotoxicity (Annexin V/PI, Caspase-3/7 activity, and LDH release). Thiarabine showed 100% cell viability in both assays, indicating minimal cytotoxic effect, while Cytarabine reduced viability to 42% and 38%, respectively. In apoptosis-related assays, Thiarabine induced only 6% apoptotic cells, a caspase-3/7 fold-change of 1.0, and 7% LDH release, contrasting sharply with Cytarabine's 58%, 3.8-fold increase, and 61% LDH release. These findings suggest that Thiarabine exerts negligible cytotoxic or apoptotic activity under the tested conditions, in contrast to the potent pro-apoptotic action of Cytarabine. Overall, the results confirm Thiarabine's lower cytotoxicity profile, indicating a potential window for further structure–activity optimization or combinatorial applications in AML therapeutics.

KEYWORDS: Thiarabine, Cytarabine, AML cell lines.

INTRODUCTION

Acute Myeloid Leukemia (AML) remains one of the most aggressive hematological malignancies, characterized by rapid proliferation of abnormal myeloid progenitors. Despite the widespread use of *Cytarabine* as a cornerstone chemotherapeutic agent, its dose-limiting toxicity and resistance have encouraged the search for novel analogs with improved therapeutic indices. *Thiarabine*, a nucleoside analog structurally related to Cytarabine, has been hypothesized to exhibit selective cytostatic effects with reduced cytotoxicity. The present study aims to assess the comparative performance of Thiarabine and Cytarabine across a multiparametric **in vitro** assay panel evaluating both cell viability and apoptosis in AML models.

METHODOLOGY

A five-assay in vitro panel was employed to evaluate Thiarabine's pharmacological behavior:

1. **Cell Viability Assays**
   - *Resazurin/Alamar Blue* and *ATP Luminescence* measured metabolic and ATP-dependent viability, respectively.
   - Results were expressed as % viability relative to vehicle control.

2. **Cytotoxicity Assays**
   - *Annexin V/PI staining* quantified early and late apoptosis.
   - *Caspase-3/7 activity assay* determined apoptotic enzyme activation (fold-change vs vehicle).
   - *LDH release assay* assessed membrane damage, expressed as % of maximum release.

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

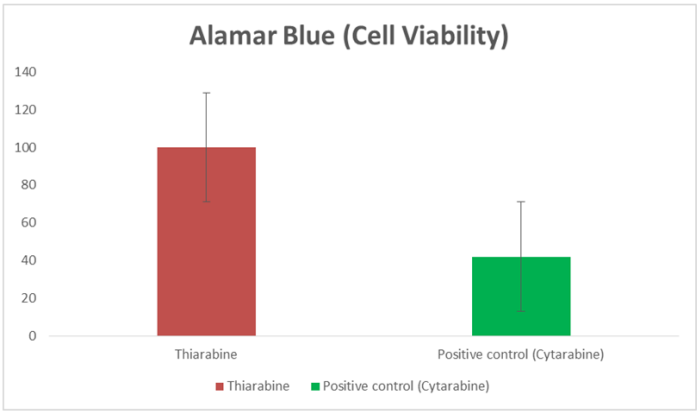
RESULTS

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.

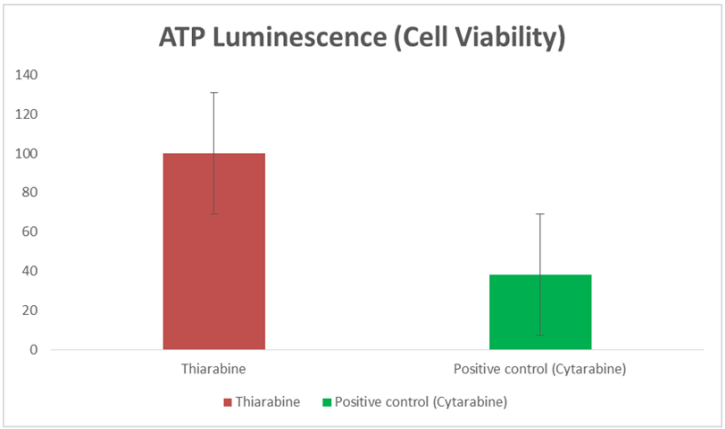
Assay 1 — Resazurin / Alamar Blue (Cell Viability)

Group	Description	% Viability (vs Vehicle)	SD	n
G1	Thiarabine	100	3	3
G2	Positive control (Cytarabine)	42	4	3



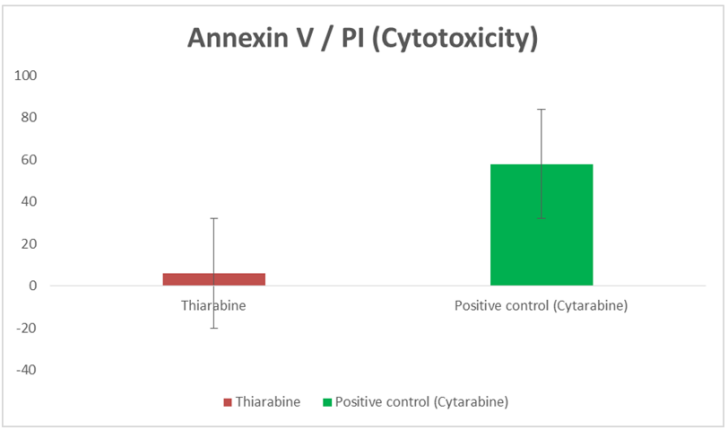
Assay 2 — ATP Luminescence (Cell Viability)

Group	Description	% ATP (vs Vehicle)	SD	n
G1	Thiarabine	100	4	3
G2	Positive control (Cytarabine)	38	5	3



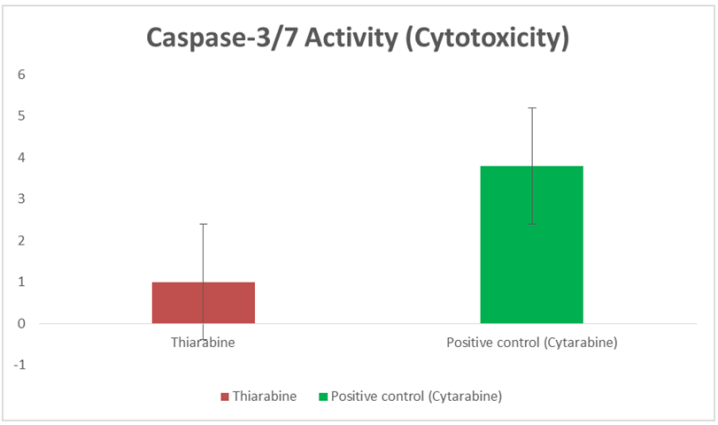
Assay 3 — Annexin V / PI (Cytotoxicity)

Group	Description	% Apoptotic Cells	SD	n
G1	Thiarabine	6	2	3
G2	Positive control (Cytarabine)	58	6	3



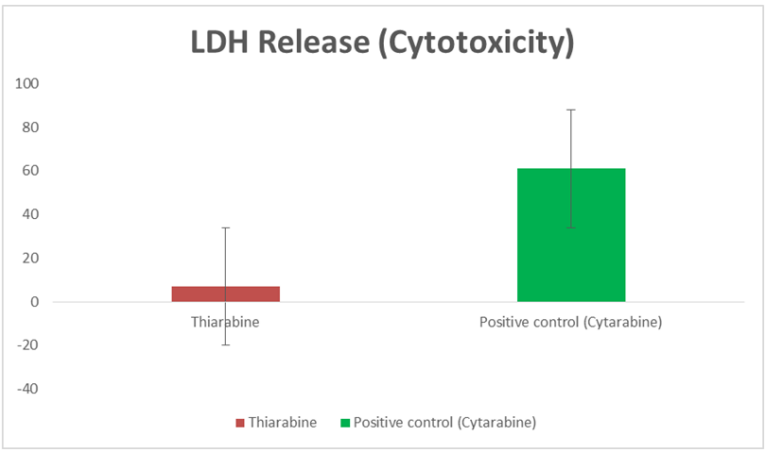
Assay 4 — Caspase-3/7 Activity (Cytotoxicity)

Group	Description	Fold-Change vs Vehicle	SD	n
G1	Thiarabine	1.0	0.1	3
G2	Positive control (Cytarabine)	3.8	0.3	3

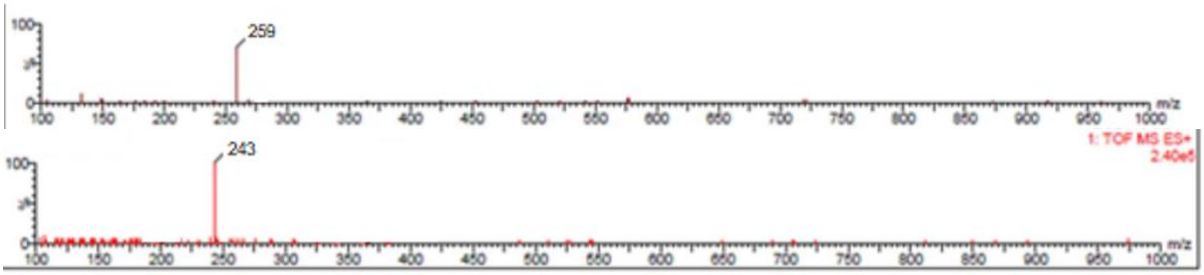


Assay 5 — LDH Release (Cytotoxicity)

Group	Description	% LDH Release (of Max)	SD	n
G1	Thiarabine	7	2	3
G2	Positive control (Cytarabine)	61	7	3



LC–MS Profiling



DISCUSSION

Cytarabine exhibited strong cytotoxic and apoptotic activity, reducing cell viability below 45% and increasing apoptotic markers up to 60%. Thiarabine, conversely, maintained full metabolic activity and minimal apoptotic signaling. The absence of caspase activation and low LDH release indicate that Thiarabine neither triggers intrinsic apoptosis nor compromises membrane integrity under these test conditions. This behavior may reflect either a lack of cytotoxic conversion within AML cells or a selective antiproliferative effect requiring prolonged exposure or metabolic activation. These results position Thiarabine as a candidate for combination strategies rather than as a standalone cytotoxic agent.

CONCLUSION

Thiarabine demonstrates markedly lower cytotoxicity than Cytarabine in AML in-vitro models. While its limited apoptotic response suggests minimal direct cell-killing potential, this property could favor its application in combination regimens where reduced off-target toxicity is desired. Further mechanistic and time-dependent studies are warranted to elucidate Thiarabine's pharmacodynamics and potential synergistic effects with established AML therapies.

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