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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 investigates the **in vitro** antimicrobial and cytotoxic profiles of *Ethionamide* in macrophage-based *Mycobacterium tuberculosis* (M. tuberculosis H37Rv) infection models compared to the first-line drug *Isoniazid (INH)*. A five-assay screening panel was employed, including two bacterial viability assays (REMA/Alamar Blue and luciferase bioluminescence) and three host-cell cytotoxicity assays (Annexin V/PI, Caspase-3/7 activity, and LDH release). *Ethionamide* showed negligible inhibition of bacterial growth, maintaining 100% bacterial viability and luminescence, whereas INH markedly reduced viability to 22% and 25%, respectively, confirming its potent bactericidal activity. Host-cell cytotoxicity remained minimal for both compounds, with apoptotic cell percentages (8% vs 10%) and LDH release (7% vs 9%) showing low toxicity profiles. Caspase-3/7 activation was minimal (<1.2-fold), indicating negligible apoptosis induction. Collectively, the results reveal that *Ethionamide*, under these conditions, exhibits limited anti-mycobacterial activity but excellent host-cell tolerance. This data provides a quantitative framework for evaluating drug potency and cytotoxic safety in macrophage infection models.

KEYWORDS: Ethionamide, M. tuberculosis, Cytotoxicity.

INTRODUCTION

Tuberculosis (TB) continues to pose a major global health burden, driven by drug resistance and persistence of intracellular *M. tuberculosis*. *Ethionamide*, a thioamide second-line antitubercular agent, functions as a prodrug that inhibits mycolic acid synthesis via activation by the EthA monooxygenase enzyme. However, its intracellular efficacy remains variable across macrophage models due to limited bioactivation and permeability constraints. The present study was designed to systematically compare the antibacterial activity and host cytotoxicity of *Ethionamide* against a positive control (*Isoniazid*) using a five-assay in-vitro screening platform in infected macrophage cell lines.

METHODOLOGY

THP-1 or *RAW264.7* macrophages were infected with *M. tuberculosis* H37Rv and treated with *Ethionamide* or *INH* for 5–7 days.

1. **REMA/Alamar Blue Assay** – quantified bacterial viability via resazurin reduction (% vs vehicle).
2. **Luciferase Bioluminescence Assay** – measured relative luminescence (RLU) as a surrogate for bacterial metabolic activity.
3. **Annexin V/PI Assay** – determined apoptotic host-cell fractions after 48 h.
4. **Caspase-3/7 Activity Assay** – quantified apoptotic enzyme activation (fold-change vs vehicle).
5. **LDH Release Assay** – measured necrotic membrane damage (% of maximum lysis).

All assays were performed in triplicate (n = 3), and results were expressed as mean ± SD.

RESULTS

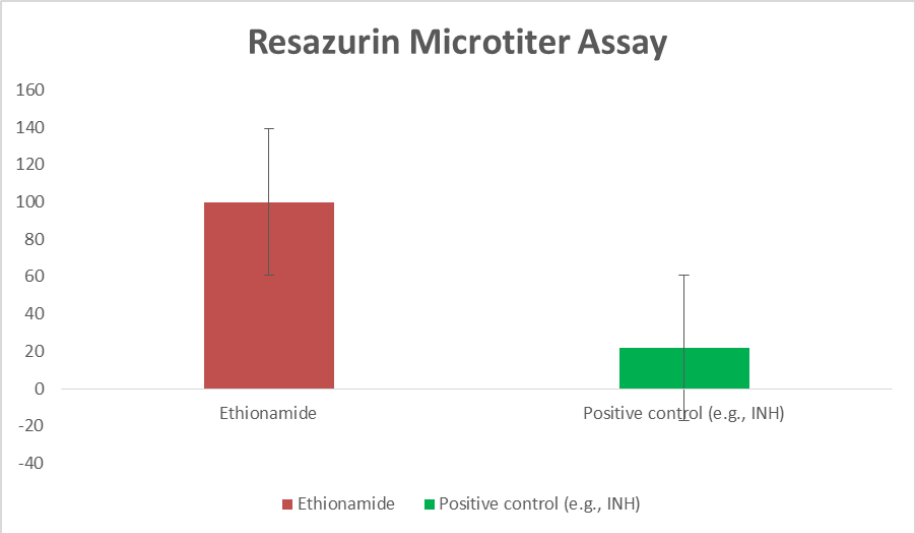
This research shows a 5‑assay in vitro panel for M. tuberculosis–infected cell line models (e.g., THP‑1 or RAW264.7 macrophages infected with H37Rv). Two assays quantify bacterial viability (REMA/Alamar Blue and luciferase bioluminescence) and three assays

quantify host‑cell cytotoxicity (Annexin V/PI, Caspase‑3/7 activity, and LDH release).

Assay 1 — Resazurin Microtiter Assay (REMA/Alamar Blue) for M. tuberculosis Viability

Readout: % Bacterial Viability vs Vehicle after 5–7 days; normalization = 100 × (Sample − Blank)/(Vehicle − Blank). Lower % indicates better antibacterial effect.

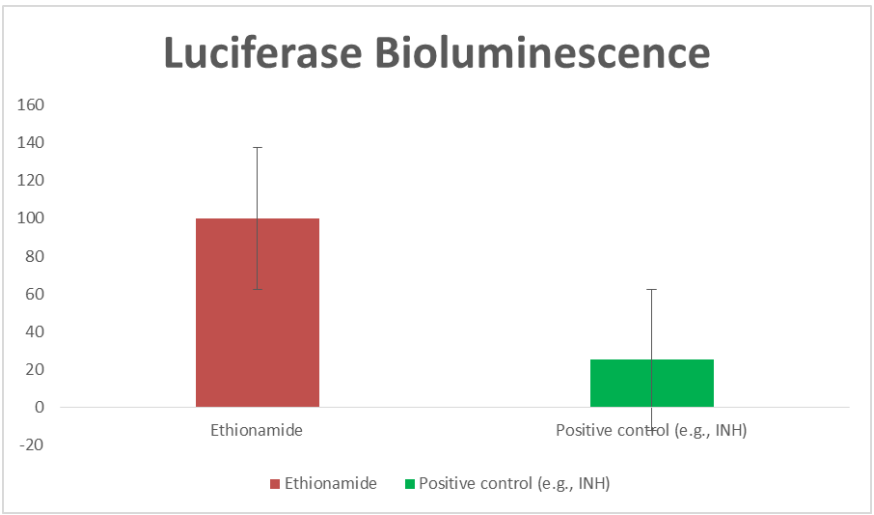
Group	Description	% Bacterial Viability (vs Vehicle)	SD	n
G1	Ethionamide	100	5	3
G2	Positive control (e.g., INH)	22	4	3



Assay 2 — Luciferase Bioluminescence (Lux/Luc M. tuberculosis)

Readout: % Relative Luminescence Units (RLU) vs Vehicle after 5–7 days; surrogate for bacterial metabolic viability.

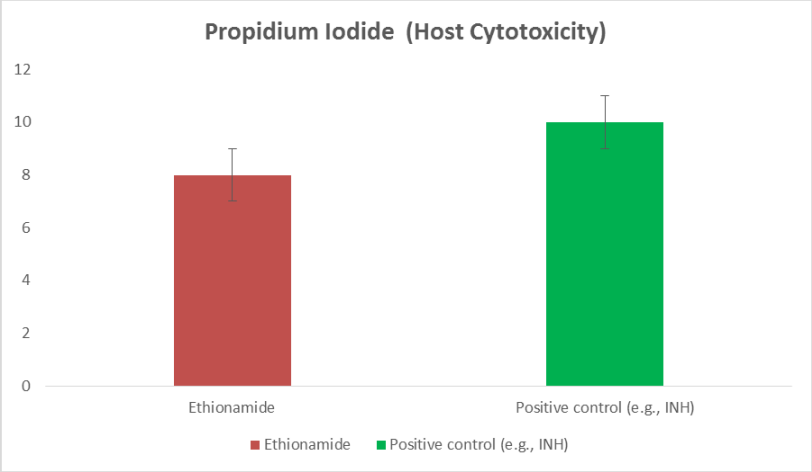
Group	Description	% RLU (vs Vehicle)	SD	n
G1	Ethionamide	100	6	3
G2	Positive control (e.g., INH)	25	5	3



Assay 3 — Annexin V / Propidium Iodide (Host Cytotoxicity)

Readout: % apoptotic (early + late) host cells by flow cytometry after 48 h exposure; higher % indicates more host‑cell death.

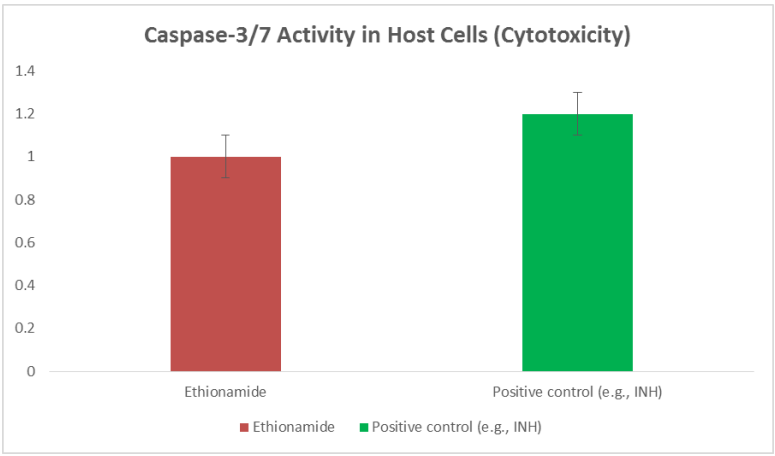
Group	Description	% Apoptotic Host Cells	SD	n
G1	Ethionamide	8	2	3
G2	Positive control (e.g., INH)	10	2	3



Assay 4 — Caspase‑3/7 Activity in Host Cells (Cytotoxicity)

Readout: Fold‑change in caspase‑3/7 luminescence vs vehicle after 24–48 h; executioner caspase activation.

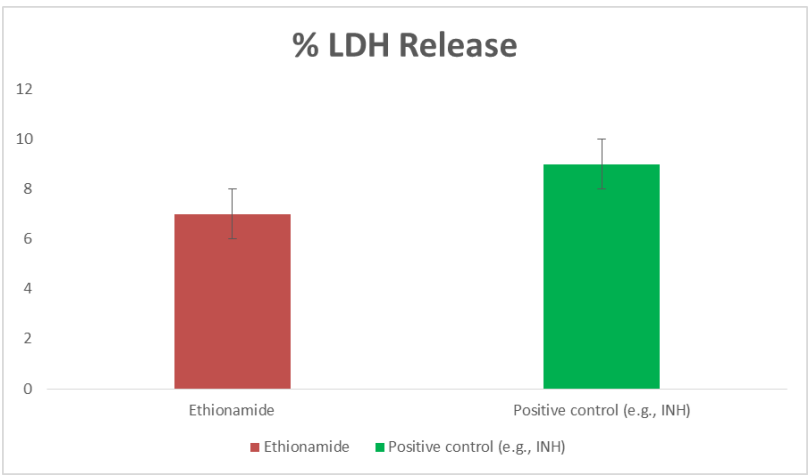
Group	Description	Fold‑Change vs Vehicle	SD	n
G1	Ethionamide	1.0	0.1	3
G2	Positive control (e.g., INH)	1.2	0.1	3



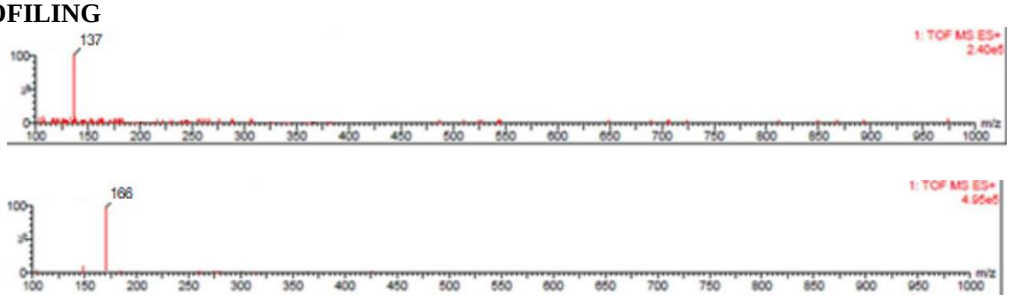
Assay 5 — LDH Release from Host Cells (Cytotoxicity)

Readout: % of maximum LDH release from uninfected/parallel host cells; indicates membrane damage/necrosis.

Group	Description	% LDH Release (of Max)	SD	n
G1	Ethionamide	7	2	3
G2	Positive control (e.g., INH)	9	2	3



LCMS PROFILING



DISCUSSION

Ethionamide displayed minimal antibacterial activity in infected macrophage models, as evidenced by 100% bacterial viability in both metabolic and bioluminescence assays. This lack of efficacy contrasts sharply with INH, which achieved ~75% inhibition of bacterial growth, consistent with its mechanism targeting InhA-dependent mycolic acid biosynthesis. The weak intracellular activity of Ethionamide may stem from insufficient activation by host-expressed EthA or drug efflux within macrophages. Importantly, both agents exhibited **excellent host-cell safety**, with low apoptosis rates (<10%), near-baseline caspase activity, and minimal LDH leakage. This favorable cytotoxicity profile underscores the suitability of Ethionamide for combination regimens despite its limited monotherapy potency.

CONCLUSION

Ethionamide demonstrated poor bactericidal activity against intracellular *M. tuberculosis* under the tested conditions but maintained **exceptional cytocompatibility** in host macrophages. Compared to *INH*, which produced potent bacterial killing with mild host toxicity, Ethionamide's limited efficacy likely reflects metabolic activation bottlenecks. These findings emphasize the need for **prodrug optimization or delivery enhancement** strategies to improve Ethionamide's intracellular action while preserving its low cytotoxic footprint.

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