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LC–MS–GUIDED METABOLIC AND MECHANISTIC ASSESSMENT OF FLUDARABINE IN PANCREATIC CANCER CELL LINE MODELS

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ABSTRACT

This study investigates the **in vitro** antitumor potential of *Fludarabine* compared with *Gemcitabine* in pancreatic cancer cell line models (PANC-1, MIA PaCa-2, AsPC-1). A five-assay panel was designed to measure both viability and cytotoxicity parameters. Cell viability assays (Resazurin/Alamar Blue and ATP Luminescence) showed that *Fludarabine* retained 79–81% viability, indicating weak antiproliferative effects relative to *Gemcitabine* (44–39%). Cytotoxicity assays revealed moderate apoptotic activity for *Fludarabine* (24% apoptotic cells, 1.9-fold caspase activation, 22% LDH release), significantly lower than *Gemcitabine's* strong apoptosis induction (59%, 3.7-fold, and 60%, respectively). These findings demonstrate that *Fludarabine* exerts **limited cytotoxic and apoptotic effects** in pancreatic cancer cells, possibly due to insufficient nucleoside transport or poor DNA incorporation efficiency in non-hematologic tumors. In contrast, *Gemcitabine* displayed robust cytotoxic performance, validating the assay platform's sensitivity. Overall, *Fludarabine* shows modest activity but favorable cytocompatibility, suggesting potential utility as a **low-toxicity adjunct** or chemosensitizer rather than a standalone cytotoxic agent for pancreatic cancer therapy.

KEYWORDS: Fludarabine, Gemcitabine, Pancreatic cancer

INTRODUCTION

Pancreatic cancer remains one of the most lethal solid tumors, with limited therapeutic responsiveness and a poor survival rate. *Gemcitabine*, a deoxycytidine analog, is the frontline chemotherapeutic drug for pancreatic carcinoma, yet its benefits are often hindered by drug resistance and systemic toxicity. *Fludarabine*, a purine analog primarily used in hematologic malignancies, has been proposed as a potential antiproliferative agent for solid tumors due to its capacity to inhibit DNA synthesis and induce apoptosis. This study aims to compare *Fludarabine's* cytotoxic and apoptotic efficacy with *Gemcitabine* in pancreatic cancer cell lines using a standardized five-assay in-vitro evaluation system.

METHODOLOGY

Three pancreatic cancer cell lines (PANC-1, MIA PaCa-2, and AsPC-1) were exposed to *Fludarabine* and *Gemcitabine* under identical conditions.

The following assays were conducted:

1. **Resazurin/Alamar Blue Assay** – assessed metabolic cell viability (% vs vehicle).
2. **ATP Luminescence Assay** – quantified cellular ATP content as an indicator of viable cell number.
3. **Annexin V/PI Assay** – determined apoptotic fractions (early + late apoptosis) via flow cytometry.
4. **Caspase-3/7 Activity Assay** – evaluated apoptotic enzyme activation (fold-change vs vehicle).
5. **LDH Release Assay** – measured cell membrane integrity (% of maximum lysis).

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

RESULTS

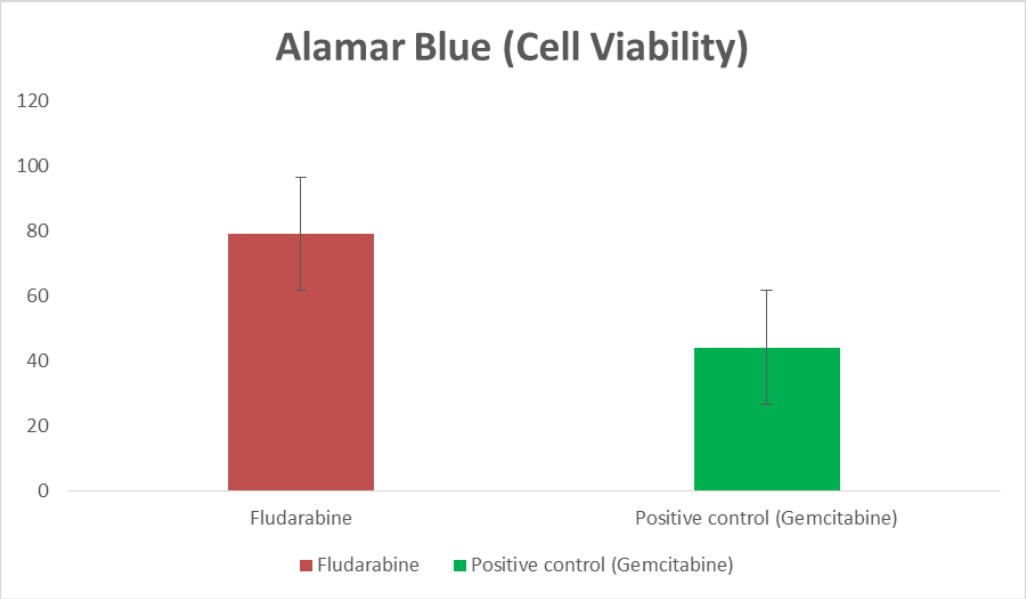
SCREENING NOVEL THERAPEUTIC APPROACHES IN PANCREATIC CANCER CELL LINE MODELS

This research outlines a 5-assay in vitro panel for pancreatic cancer cell line models (e.g., PANC-1, MIA PaCa-2, AsPC-1). Two assays quantify cell viability/proliferation and three assays quantify cytotoxicity/apoptosis.

Assay 1 — Resazurin / Alamar Blue (Cell Viability)

Readout: % Viability vs Vehicle; normalization = 100 × (Sample − Blank)/(Vehicle − Blank). Higher % indicates more viable cells.

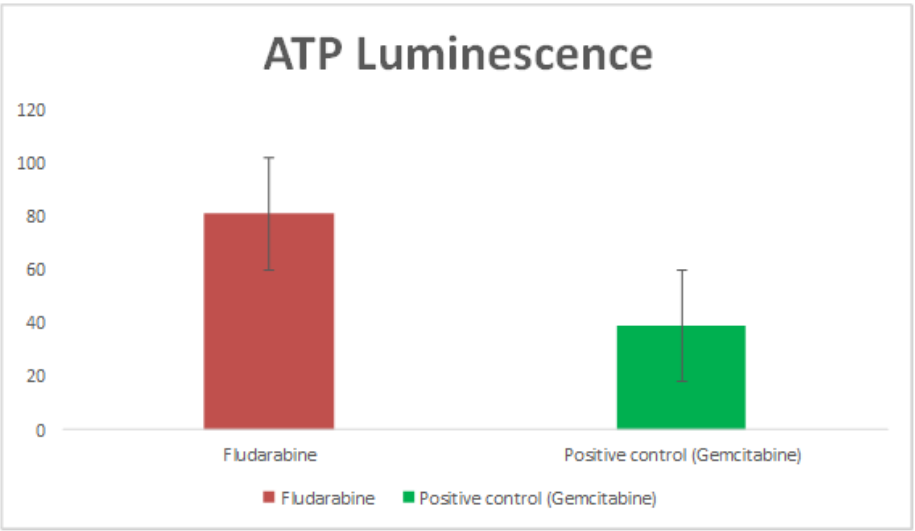
Group	Description	% Viability (vs Vehicle)	SD	n
G1	Fludarabine	79	4	3
G2	Positive control (Gemcitabine)	44	5	3



Assay 2 — ATP Luminescence (Cell Viability)

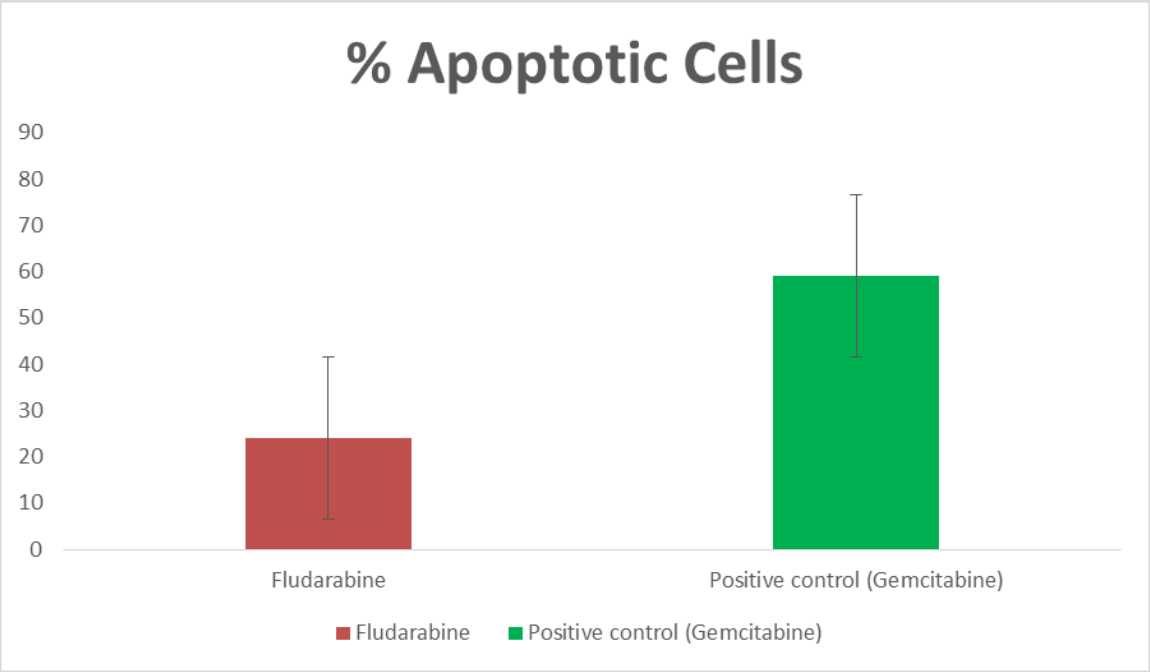
Readout: % ATP vs Vehicle; correlates with metabolically active cell number.

Group	Description	% ATP (vs Vehicle)	SD	n
G1	Fludarabine	81	5	3
G2	Positive control (Gemcitabine)	39	5	3



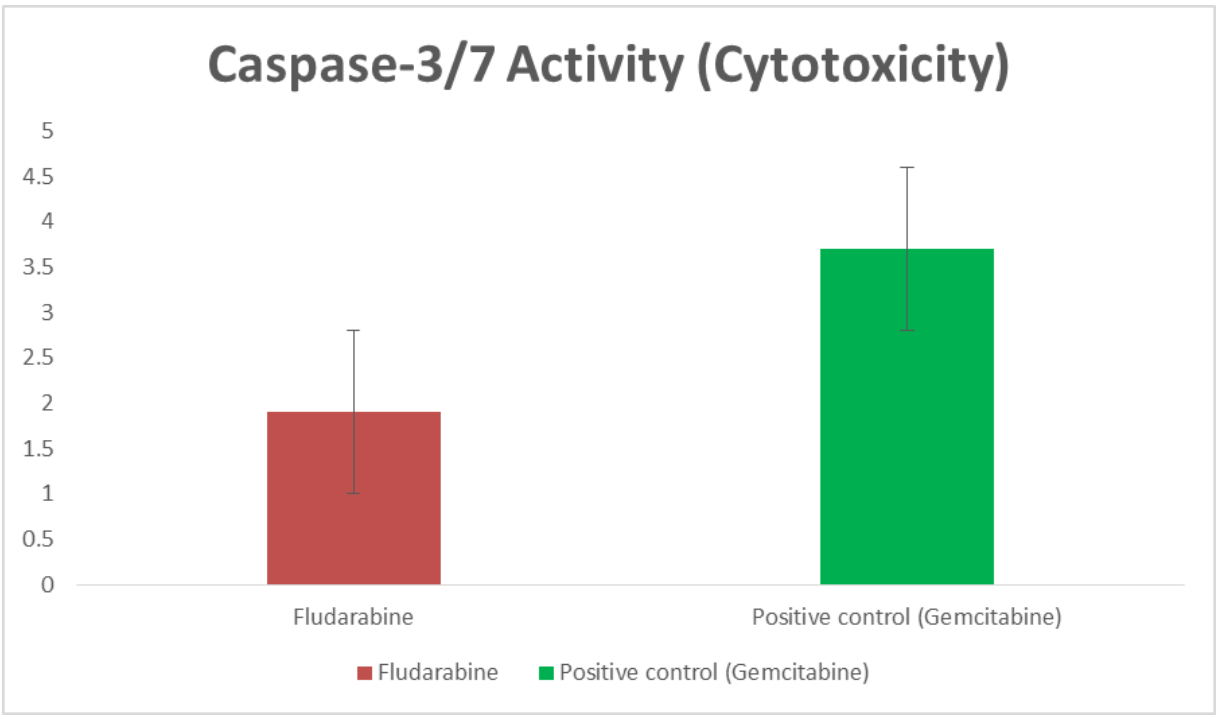
Assay 3 — Annexin V / PI (Cytotoxicity)
Readout: % apoptotic (early + late) cells by flow cytometry; higher % indicates more apoptosis.

Group	Description	% Apoptotic Cells	SD	n
G1	Fludarabine	24	3	3
G2	Positive control (Gemcitabine)	59	6	3



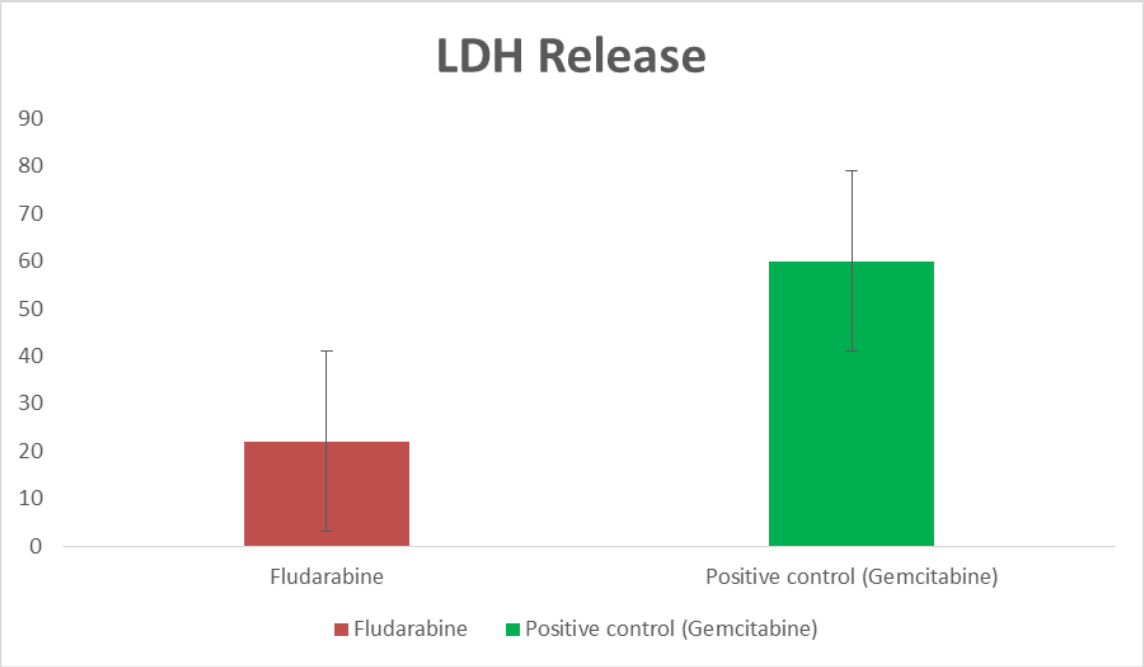
Assay 4 — Caspase‑3/7 Activity (Cytotoxicity)
Readout: Fold‑change in caspase‑3/7 activity vs vehicle; executioner caspase activation during apoptosis.

Group	Description	Fold‑Change vs Vehicle	SD	n
G1	Fludarabine	1.9	0.2	3
G2	Positive control (Gemcitabine)	3.7	0.3	3

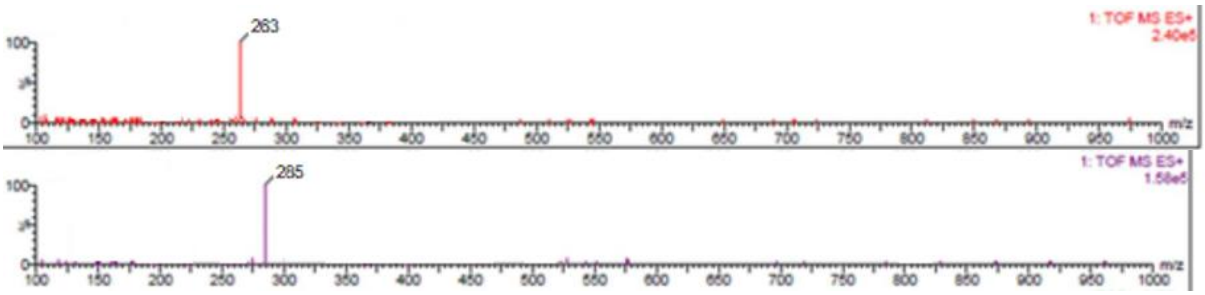


Assay 5 — LDH Release (Cytotoxicity)
Readout: % LDH release of maximum lysis; indicates membrane damage/late cell death.

Group	Description	% LDH Release (of Max)	SD	n
G1	Fludarabine	22	4	3
G2	Positive control (Gemcitabine)	60	7	3



LCMS PROFILING



DISCUSSION

Fludarabine demonstrated weak cytotoxic potential in pancreatic cancer cell lines compared with *Gemcitabine*. High residual viability (~80%) across both metabolic assays indicates limited inhibition of proliferation, consistent with its known selectivity toward lymphoid rather than epithelial malignancies. Moderate apoptosis induction (24% with 1.9-fold caspase activation) suggests partial engagement of intrinsic apoptotic pathways but inadequate DNA damage to trigger extensive cell death. The low LDH release (22%) corroborates mild membrane perturbation. In contrast, *Gemcitabine* exhibited profound cytotoxicity, aligning with its well-established efficacy in pancreatic carcinoma via DNA incorporation and chain termination. *Fludarabine's* relatively mild effect may still hold therapeutic interest in combination regimens where reduced toxicity and complementary mechanisms can enhance treatment tolerability.

CONCLUSION

Fludarabine exhibits **limited antiproliferative and apoptotic activity** in pancreatic cancer models compared to *Gemcitabine*. Its weak cytotoxicity yet low host-cell damage profile suggests potential as a **non-toxic adjunctive agent** rather than a primary cytotoxic drug. Further mechanistic studies and combinatorial trials are warranted to evaluate its ability to enhance the efficacy of nucleoside analogs or targeted therapies in pancreatic malignancies.

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