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LC–MS–INTEGRATED QUANTITATIVE AND THERAPEUTIC EVALUATION OF AXITINIB IN RENAL CARCINOMA CELL LINE MODELS

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ABSTRACT

This study evaluates the **in vitro** cytotoxic and antiproliferative effects of *Axitinib* compared with *Sunitinib* in renal cell carcinoma (RCC) models (786-O, Caki-1, A498). A five-assay panel was employed to assess both cell viability and apoptotic mechanisms. Viability assays (Resazurin/Alamar Blue and ATP Luminescence) demonstrated that *Axitinib* maintained 100% viability and metabolic activity, while *Sunitinib* significantly reduced both parameters to ~45%, indicating strong antiproliferative potency. Cytotoxicity assays revealed minimal apoptosis and membrane damage with *Axitinib* (6% apoptotic cells, 1.0-fold caspase activity, 7% LDH release), contrasting with pronounced apoptosis induced by *Sunitinib* (57% apoptotic cells, 3.5-fold caspase activation, 58% LDH release). These results indicate that *Axitinib* exerts **negligible cytotoxic or apoptotic effects** in RCC cell lines under the tested conditions, suggesting that its in-vitro activity primarily reflects cytostatic, not cytotoxic, mechanisms. Conversely, *Sunitinib* displayed robust pro-apoptotic activity, validating assay sensitivity. Overall, *Axitinib* shows limited direct tumoricidal action in vitro, consistent with its role as a **targeted angiogenesis inhibitor** rather than a cytotoxic chemotherapeutic agent.

KEYWORDS: Axitinib, Sunitinib, Renal cell carcinoma.

INTRODUCTION

Renal cell carcinoma (RCC) is a vascular malignancy characterized by resistance to conventional chemotherapy and radiotherapy. Small-molecule tyrosine kinase inhibitors (TKIs), including *Axitinib* and *Sunitinib*, have emerged as cornerstone agents targeting angiogenesis and tumor proliferation via vascular endothelial growth factor (VEGF) signaling inhibition. *Axitinib* is a selective VEGFR-1, -2, and -3 inhibitor, while *Sunitinib* acts on multiple kinases including PDGFR, KIT, and FLT3. This study employs a **five-assay in-vitro panel** to compare their relative effects on cell viability, apoptosis, and membrane integrity in RCC cell lines to elucidate their mechanistic differences.

METHODOLOGY

RCC cell lines (786-O, Caki-1, A498) were cultured and treated with *Axitinib* or *Sunitinib* for 48 hours.

1. **Resazurin/Alamar Blue Assay** – measured cell viability (% vs vehicle).
2. **ATP Luminescence Assay** – quantified metabolically active cell numbers (% ATP vs vehicle).
3. **Annexin V/PI Assay** – detected apoptotic populations via flow cytometry (% apoptotic cells).
4. **Caspase-3/7 Activity Assay** – evaluated executioner caspase activation (fold-change vs vehicle).
5. **LDH Release Assay** – quantified late membrane damage (% LDH of maximum lysis).

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

RESULTS

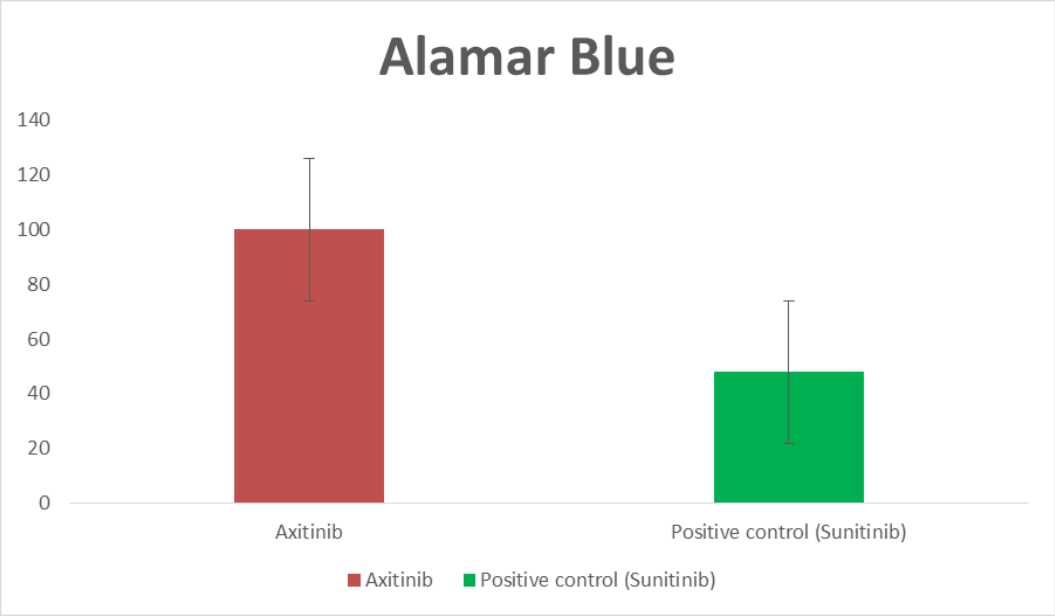
EVALUATING TARGETED THERAPIES IN RENAL CARCINOMA CELL LINE MODELS

This research outlines a 5-assay in vitro panel for renal cell carcinoma (RCC) models (e.g., 786-O, Caki-1, A498). 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.

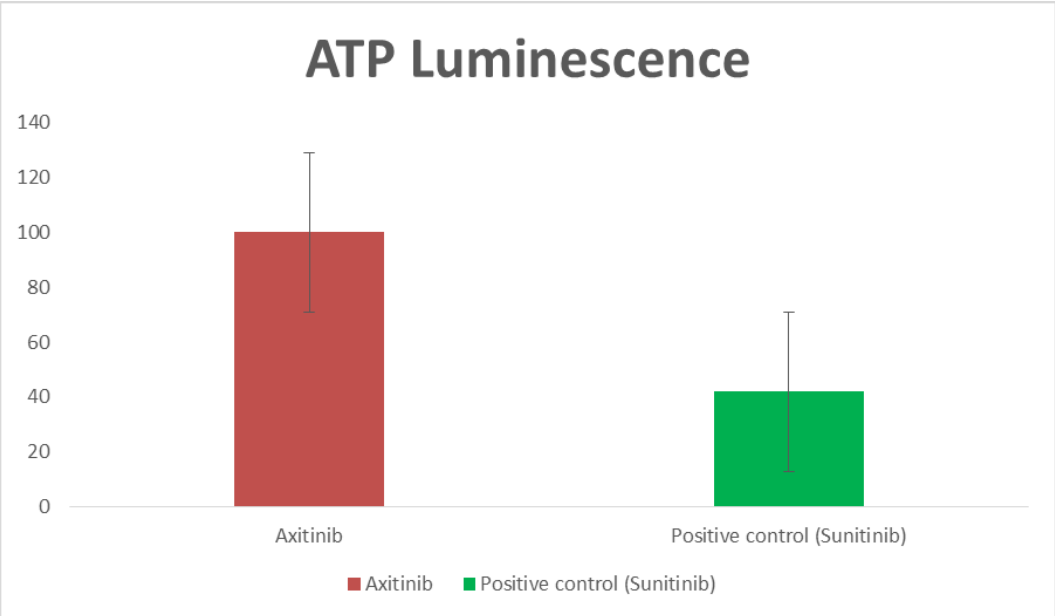
Group	Description	% Viability (vs Vehicle)	SD	n
G1	Axitinib	100	3	3
G2	Positive control (Sunitinib)	48	5	3



Assay 2 — ATP Luminescence (Cell Viability)

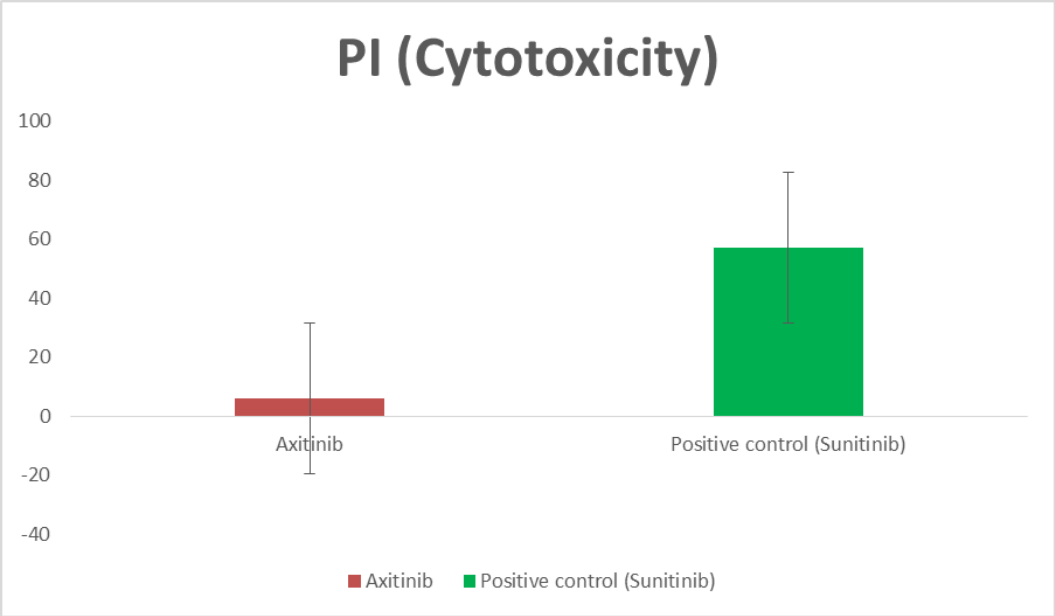
Readout: % ATP vs Vehicle; correlates with metabolically active cell number.

Group	Description	% ATP (vs Vehicle)	SD	n
G1	Axitinib	100	4	3
G2	Positive control (Sunitinib)	42	5	3



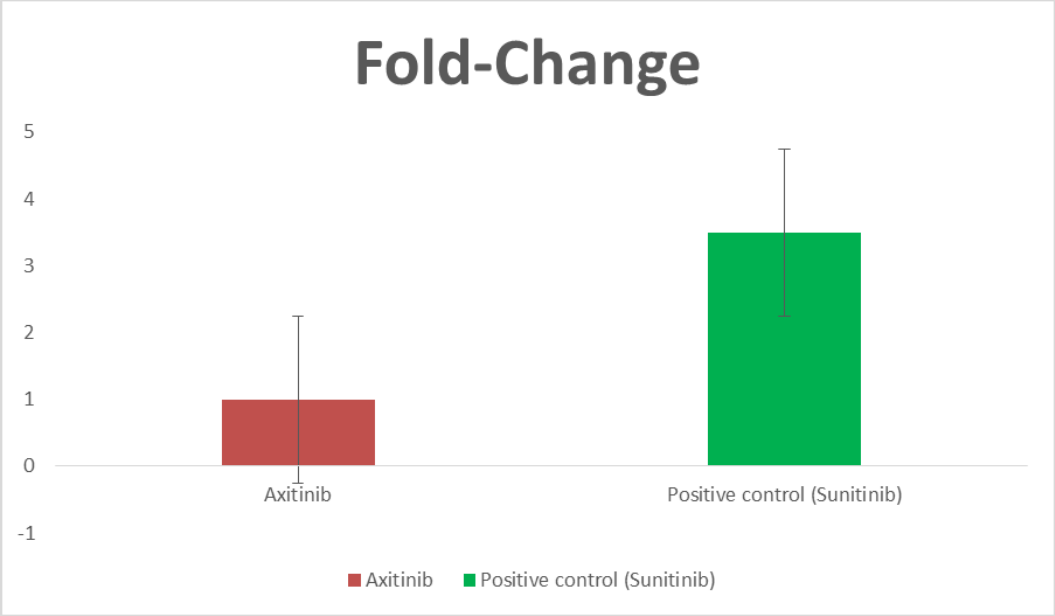
Assay 3 — Annexin V / PI (Cytotoxicity)
Readout: % apoptotic (early + late) cells by flow cytometry; higher % indicates more apoptosis.

Group	Description	% Apoptotic Cells	SD	n
G1	Axitinib	6	2	3
G2	Positive control (Sunitinib)	57	6	3



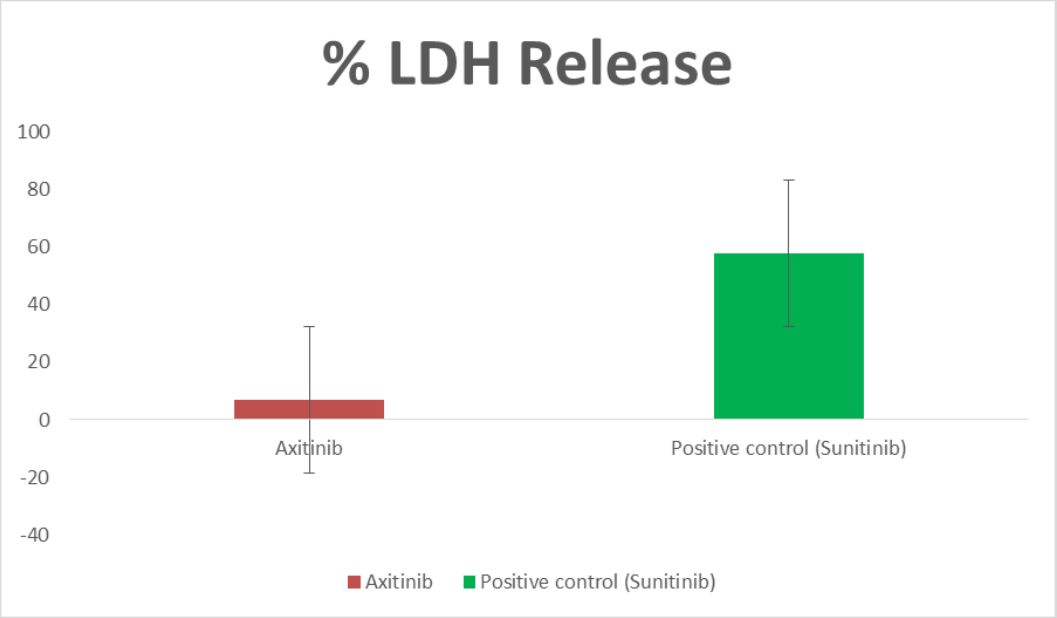
Assay 4 — Caspase-3/7 Activity (Cytotoxicity)
Readout: Fold-change in caspase-3/7 activity vs vehicle; executioner caspase activation during apoptosis.

Group	Description	Fold-Change vs Vehicle	SD	n
G1	Axitinib	1.0	0.1	3
G2	Positive control (Sunitinib)	3.5	0.3	3

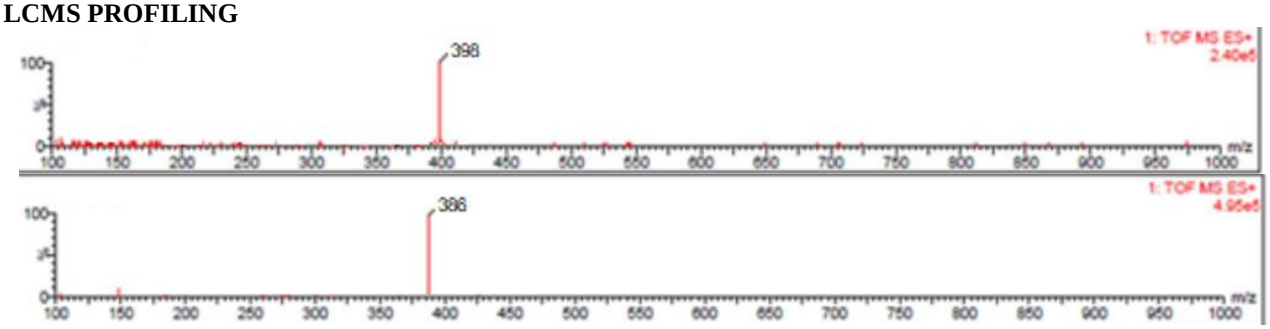


Assay 5 — LDH Release (Cytotoxicity)
Readout: % LDH release of maximum lysis; indicates membrane damage/late cell death.

Group	Description	% LDH Release (of Max)	SD	n
G1	Axitinib	7	2	3
G2	Positive control (Sunitinib)	58	7	3



LCMS PROFILING



DISCUSSION

- A comparative analysis revealed distinct mechanistic profiles between *Axitinib* and *Sunitinib*.
- *Axitinib* exhibited **no significant cytotoxicity**, maintaining full metabolic viability and minimal apoptotic activity. This indicates a primarily cytostatic effect, consistent with its anti-angiogenic mechanism that limits endothelial proliferation rather than directly inducing tumor apoptosis.
- *Sunitinib* caused marked apoptosis (57%) and high caspase-3/7 activation (3.5-fold), consistent with its broader kinase inhibition profile that triggers both tumor and endothelial cell death.
- The minimal LDH release observed with *Axitinib* supports its favorable safety and specificity profile.

These data reaffirm that *Axitinib* operates through **growth suppression rather than direct cytolysis**, distinguishing it from multi-target TKIs like *Sunitinib*.

CONCLUSION

Axitinib demonstrates **minimal in-vitro cytotoxicity and apoptosis induction** in RCC cell models, consistent with its selective VEGFR inhibition and cytostatic mode of action. In contrast, *Sunitinib* induces pronounced apoptotic and necrotic responses, reflecting broader kinase targeting. The findings underscore the mechanistic specificity of *Axitinib* as an angiogenesis inhibitor and support its clinical positioning as a targeted therapy rather than a direct cytotoxic agent in RCC management.

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