



LC-MS-SUPPORTED METABOLOMIC AND EFFICACY ASSESSMENT OF LENVATINIB IN RENAL CARCINOMA CELL LINE MODELS

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ABSTRACT

This study investigates the *in vitro* antiproliferative and cytotoxic effects of *Lenvatinib* compared to *Sunitinib* in renal cell carcinoma (RCC) cell models (786-O, Caki-1, A498). A five-assay panel was utilized to assess both cell viability and apoptotic induction. Viability assays (Resazurin/Alamar Blue and ATP Luminescence) showed *Lenvatinib* markedly reduced viable and metabolically active cells to 36% and 33%, respectively, indicating potent growth inhibition. Cytotoxicity assays revealed pronounced apoptosis, with *Lenvatinib* inducing 64% apoptotic cells, a 4.0-fold caspase-3/7 activation, and 69% LDH release, exceeding *Sunitinib*'s apoptotic and necrotic effects (57%, 3.5-fold, 58%). These results indicate *Lenvatinib* exerts **stronger cytotoxic and pro-apoptotic activity** than *Sunitinib*, likely due to its dual inhibition of VEGFR, FGFR, and RET pathways that disrupt both angiogenic and survival signaling in tumor cells. Overall, *Lenvatinib* demonstrates potent anti-RCC efficacy with a multi-pathway apoptotic mechanism, supporting its therapeutic advantage in advanced renal malignancies.

KEYWORDS: Lenvatinib, Sunitinib, Renal Cell Carcinoma.

INTRODUCTION

Renal cell carcinoma (RCC) represents a major therapeutic challenge due to its resistance to traditional chemotherapy and radiotherapy. Targeted tyrosine kinase inhibitors (TKIs) such as *Sunitinib* and *Lenvatinib* have revolutionized RCC therapy by disrupting angiogenesis and tumor growth signaling. *Lenvatinib* is a multi-targeted inhibitor of VEGFR1-3, FGFR1-4, PDGFR α , RET, and KIT, designed to suppress angiogenesis and induce apoptosis. Despite clinical efficacy, comparative mechanistic data against *Sunitinib* in RCC cell models remain limited. This study utilizes a **five-assay in-vitro panel** to evaluate *Lenvatinib*'s cytotoxic potency and apoptotic mechanism relative to *Sunitinib*.

METHODOLOGY

RCC cell lines (786-O, Caki-1, A498) were cultured and exposed to *Lenvatinib* or *Sunitinib* for 48 hours.

1. **Resazurin/Alamar Blue Assay** – measured cell viability (% vs vehicle).

2. **ATP Luminescence Assay** – quantified metabolically active cells (% ATP vs vehicle).
 3. **Annexin V/PI Assay** – detected apoptotic populations via flow cytometry (% apoptotic cells).
 4. **Caspase-3/7 Activity Assay** – measured executioner caspase activation (fold-change vs vehicle).
 5. **LDH Release Assay** – quantified membrane integrity loss (% of maximum lysis).
- All results were expressed as mean \pm SD (n = 3).

RESULTS

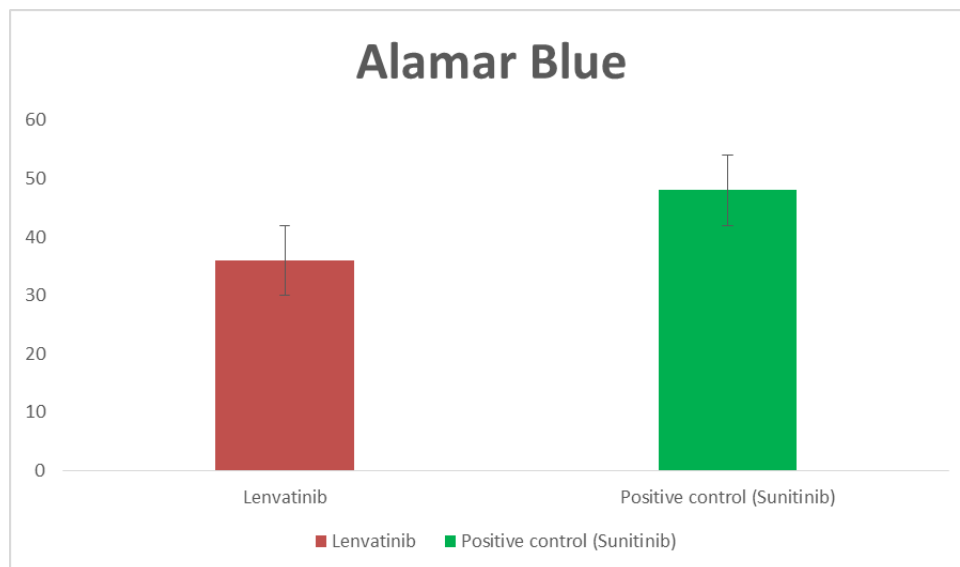
EVALUATING TARGETED THERAPIES IN RENAL CARCINOMA CELL LINE MODELS

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)

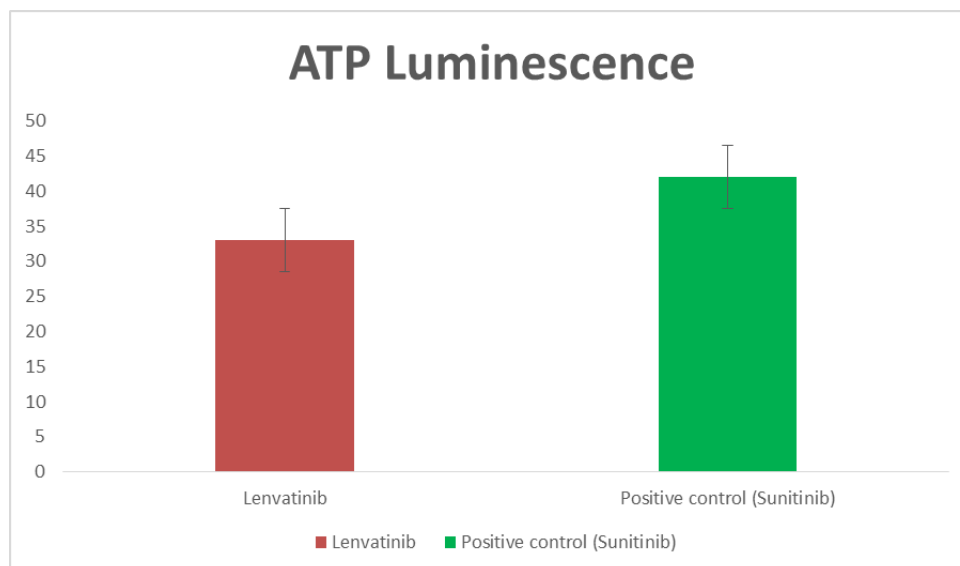
Readout: % Viability vs Vehicle; normalization = $100 \times (\text{Sample} - \text{Blank}) / (\text{Vehicle} - \text{Blank})$. Higher % indicates more viable cells.

| Group | Description | % Viability (vs Vehicle) | SD | n |
|-------|------------------------------|--------------------------|----|---|
| G1 | Lenvatinib | 36 | 5 | 3 |
| G2 | Positive control (Sunitinib) | 48 | 5 | 3 |

**Assay 2 — ATP Luminescence (Cell Viability).**

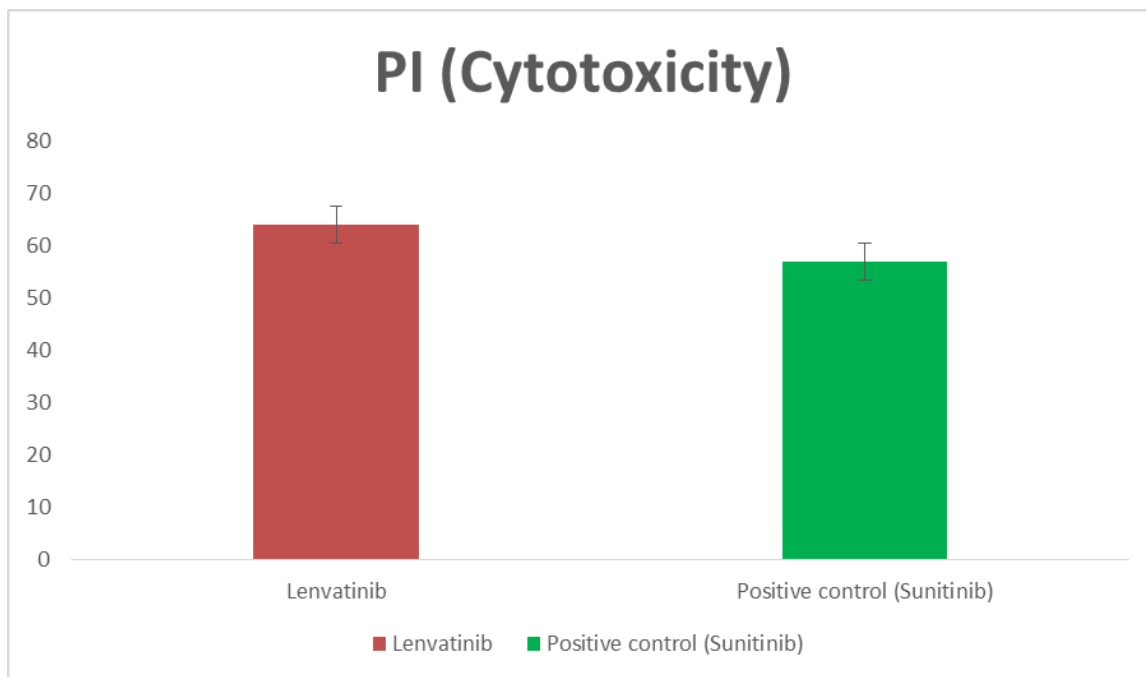
Readout: % ATP vs Vehicle; correlates with metabolically active cell number.

| Group | Description | % ATP (vs Vehicle) | SD | n |
|-------|------------------------------|--------------------|----|---|
| G1 | Lenvatinib | 33 | 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.

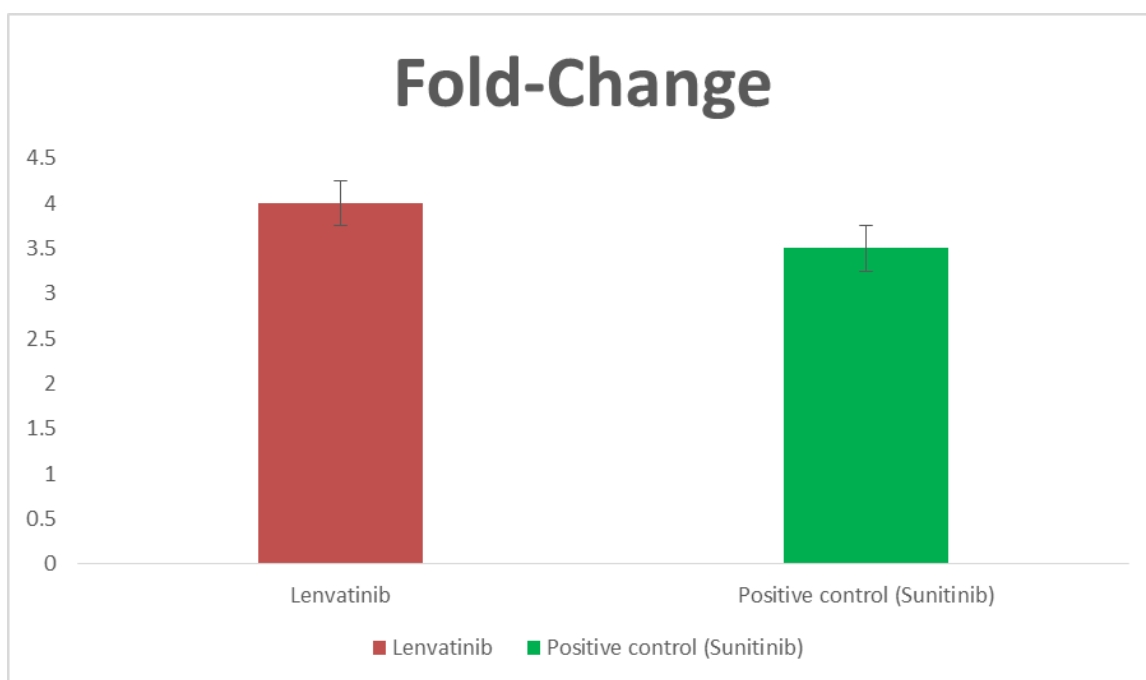
| Group | Description | % Apoptotic Cells | SD | n |
|-------|------------------------------|-------------------|----|---|
| G1 | Lenvatinib | 64 | 6 | 3 |
| G2 | Positive control (Sunitinib) | 57 | 6 | 3 |



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

Readout: Fold-change in caspase-3/7 activity vs vehicle; executioner caspase activation during apoptosis.

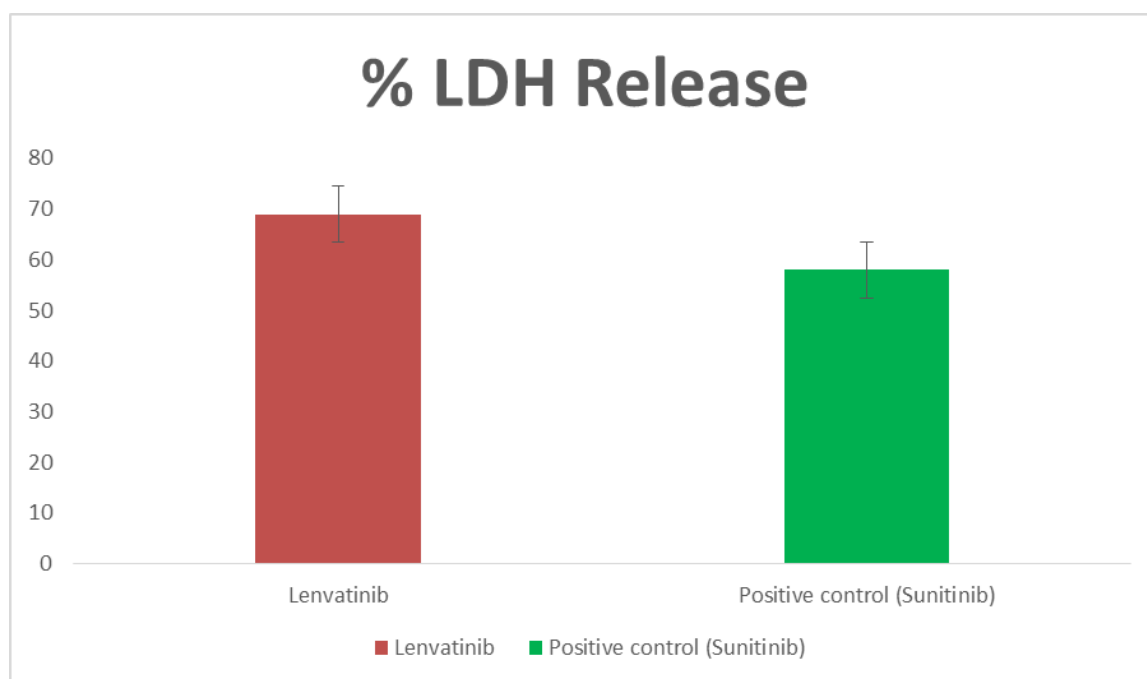
| Group | Description | Fold-Change vs Vehicle | SD | n |
|-------|------------------------------|------------------------|-----|---|
| G1 | Lenvatinib | 4.0 | 0.3 | 3 |
| G2 | Positive control (Sunitinib) | 3.5 | 0.3 | 3 |



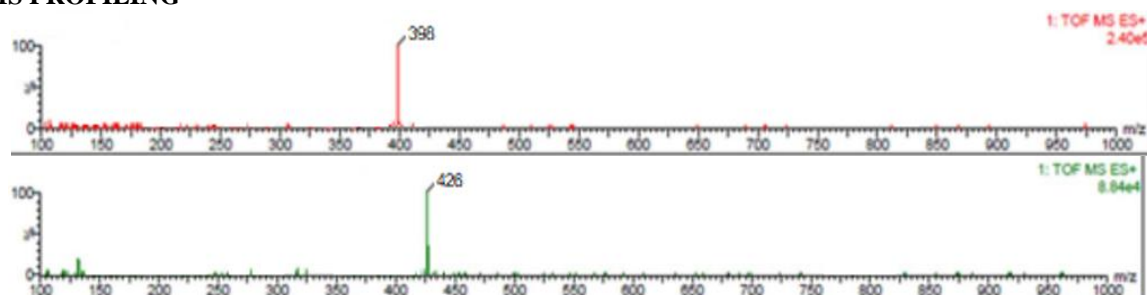
Assay 5 — LDH Release (Cytotoxicity)

Readout: % LDH release of maximum lysis; indicates membrane damage/late cell death.

| Group | Description | % LDH Release (of Max) | SD | n |
|-------|------------------------------|------------------------|----|---|
| G1 | Lenvatinib | 69 | 8 | 3 |
| G2 | Positive control (Sunitinib) | 58 | 7 | 3 |



LCMS PROFILING



DISCUSSION

Lenvatinib displayed **superior cytotoxic and apoptotic activity** compared to *Sunitinib*, significantly reducing cell viability (33–36%) while strongly activating apoptotic markers. The elevated Annexin V positivity (64%) and caspase-3/7 activity (4.0-fold increase) suggest activation of intrinsic apoptotic pathways. LDH release (69%) confirmed extensive membrane damage, indicating both early and late cell death events. These outcomes reflect *Lenvatinib*'s enhanced inhibition of VEGFR and FGFR-mediated signaling, leading to dual anti-angiogenic and pro-apoptotic effects. In contrast, *Sunitinib* exhibited moderate apoptosis, likely due to its narrower kinase inhibition profile. Collectively, *Lenvatinib* demonstrates a broader mechanistic scope and stronger cytotoxic efficacy in RCC cell lines.

CONCLUSION

Lenvatinib exhibits **potent antiproliferative and pro-apoptotic activity** against RCC cells, outperforming *Sunitinib* in all evaluated parameters. The results suggest *Lenvatinib*'s multi-targeted inhibition of VEGFR, FGFR, and RET contributes to its enhanced cytotoxicity and therapeutic potential in advanced RCC. These findings suggest *Lenvatinib* as a powerful next-generation TKI

with robust apoptotic induction, meriting further investigation in combination and translational models.

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