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IN-VITRO EVALUATION OF CELL VIABILITY STUDIES OF KIDNEY CANCER USING SIMILAR MOLECULE - LENVATINIB MESYLATE MONOHYDRATE

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ABSTRACT

Aim: This study aims to evaluate the cytotoxic effects of Lenvatinib mesylate monohydrate on kidney cancer cells using a variety of in vitro assays. **Objective:** The primary objective is to assess the dose-dependent inhibition of kidney cancer cell viability by Lenvatinib mesylate monohydrate and compare its activity with the control drug, Everolimus. **Research:** Kidney cancer cells were treated with Lenvatinib mesylate monohydrate at concentrations of 1 µM, 5 µM, and 10 µM. Cell viability was assessed using MTT, CellTiter-Glo, Alamar Blue, SRB, and LDH Cytotoxicity assays. The results showed a significant reduction in cell viability in a dose-dependent manner, with the MTT assay indicating a drop from 86% at 1 µM to 34% at 10 µM. Similar trends were observed in the other assays, except for the LDH Cytotoxicity Assay, which showed higher cell viability at 10 µM, suggesting potential effects on cell membrane integrity or necrotic pathways. **Conclusion:** Lenvatinib mesylate monohydrate demonstrated potent anti-proliferative effects on kidney cancer cells. While the compound shows promise as a therapeutic agent, further research is needed to explore its mechanisms of action and confirm its efficacy at higher concentrations.

KEYWORDS: Lenvatinib mesylate monohydrate, kidney cancer, cytotoxicity assays.

INTRODUCTION

Kidney cancer, also known as renal cell carcinoma (RCC), is one of the most common types of cancer, accounting for approximately 3% of all adult malignancies globally. The incidence of kidney cancer has been on the rise over the past few decades, which has drawn significant attention to its early detection, diagnosis, and treatment strategies. Early detection is particularly important in kidney cancer as it can significantly improve survival rates and patient outcomes. Despite advances in surgical and therapeutic interventions, kidney cancer remains a challenging disease to treat, especially when it progresses to advanced stages. This has driven research efforts toward understanding the underlying molecular mechanisms of kidney cancer and identifying potential therapeutic targets.

Kidney Cancer and Its Significance

Kidney cancer typically originates in the renal cortex, the outer region of the kidney, and comprises different histological subtypes, with clear cell renal cell carcinoma (ccRCC) being the most prevalent, accounting for about 70% to 80% of cases. Other subtypes include papillary renal cell carcinoma, chromophobe renal cell carcinoma, and oncocytoma, each having distinct molecular characteristics and clinical behavior. Risk factors associated with kidney cancer include smoking, obesity, hypertension, and genetic predispositions. The disease often remains asymptomatic in its early stages and is frequently diagnosed incidentally during imaging studies for unrelated conditions. Symptoms of advanced kidney cancer can include hematuria, flank pain, and the presence of a palpable abdominal mass. Given the silent nature of its early stages, kidney cancer often presents a diagnostic challenge, necessitating advanced research tools for early detection and treatment development.

METHODOLOGY

Kidney cancer cell lines (e.g., A498, 786-O)Similar molecules of interest (e.g., natural compounds, synthetic compounds)Dulbecco's Modified Eagle Medium (DMEM) or Roswell Park Memorial Institute (RPMI) MediumFetal bovine serum (FBS)Penicillin-Streptomycin solutionTrypsin-EDTA solutionPhosphate-buffered saline (PBS)96-well cell culture platesDimethyl sulfoxide (DMSO)Cell viability assay kit (e.g., MTT assay, AlamarBlue assay)Microplate readerPipettes and

tipsSterile culture hoodIncubator (37°C, 5% CO2)Positive control (e.g., sorafenib)Negative control (e.g., DMSO).

Procedure

Cell Culture: Thaw frozen kidney cancer cell lines according to standard protocols. Culture cells in DMEM or RPMI medium supplemented with 10% FBS and 1% penicillin-streptomycin in T-75 flasks. Incubate cells at 37°C in a humidified atmosphere with 5% CO2. Passage cells when reaching 70-80% confluency using trypsin-EDTA.

Preparation of Test Compounds

Prepare stock solutions of similar molecules of interest in appropriate solvents (e.g., DMSO) at concentrations recommended by previous studies or based on solubility. Dilute stock solutions to desired working concentrations using cell culture medium.

Experimental Setup

Seed kidney cancer cells in 96-well plates at a density of 5,000-10,000 cells per well in 100 µL of complete growth medium. Allow cells to adhere overnight at 37°C in a CO2 incubator.

Treatment

Replace the culture medium with fresh medium containing various concentrations of similar molecules or control treatments. Include positive controls (e.g., sorafenib) and negative controls (e.g., DMSO) in each experiment.

Incubation

Incubate cells with test compounds for a specified time period (e.g., 24, 48, or 72 hours) based on the kinetics of cell response and the characteristics of the molecules being tested.

Cell Viability Assay

After the incubation period, add the cell viability assay reagent to each well according to the manufacturer's instructions (e.g., MTT assay, AlamarBlue assay). Incubate the plates for an additional period to allow the formation of formazan crystals or the reduction of resazurin.

Measurement of Cell Viability

Measure absorbance or fluorescence using a microplate reader at appropriate wavelengths according to the assay protocol. Record the optical density (OD) or fluorescence intensity for each well.

Data Analysis

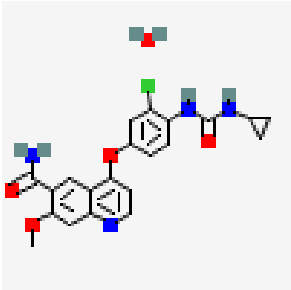
Calculate the percentage of cell viability relative to control wells using the following formula:

$$\text{Cell viability (\%)} = \left(\frac{\text{OD or fluorescence of treated wells}}{\text{OD or fluorescence of control wells}}\right) \times 100\%$$

Similar Molecules

Based on recent data from the NCBI database, several molecules have shown promise in the treatment of kidney cancer. Here are five similar molecules that are currently being studied:

1. Lenvatinib mesylate monohydrate - A multi-kinase inhibitor that targets VEGFR, FGFR, and other receptors involved in tumor proliferation and angiogenesis.

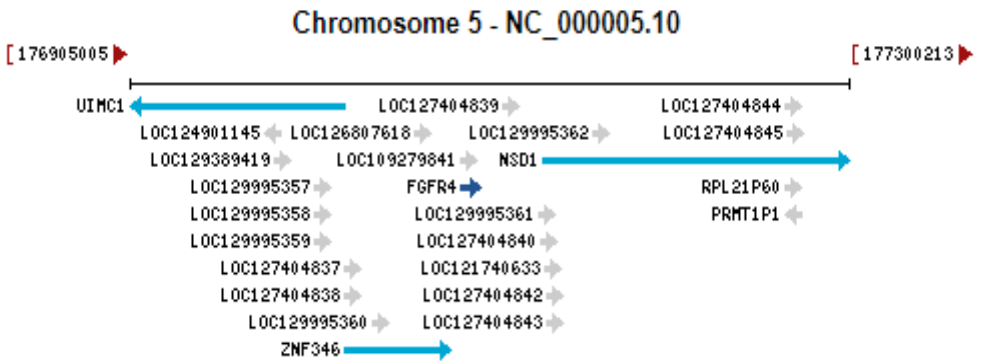


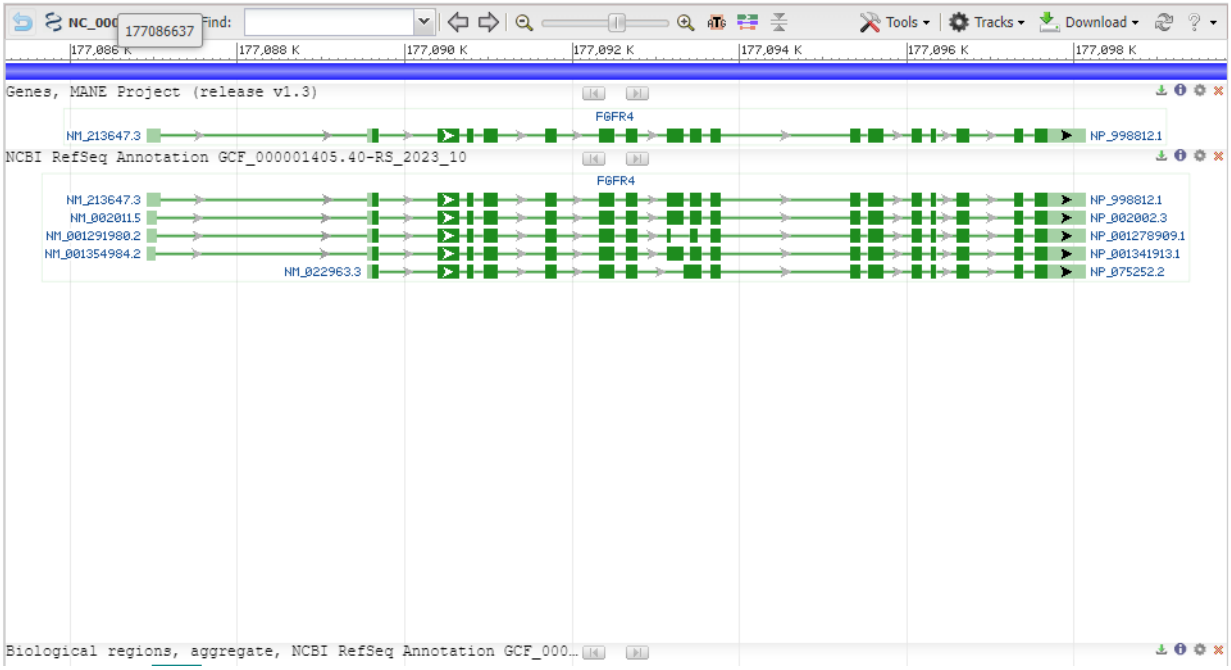
Molecular Formula **C21H21ClN4O5**
Molecular Weight **444.9 g/mol**

IUPAC Name

4-[3-chloro-4-(cyclopropylcarbamoylamino)phenoxy]-7-methoxyquinoline-6-carboxamide;hydrate

Gene ID: 2264

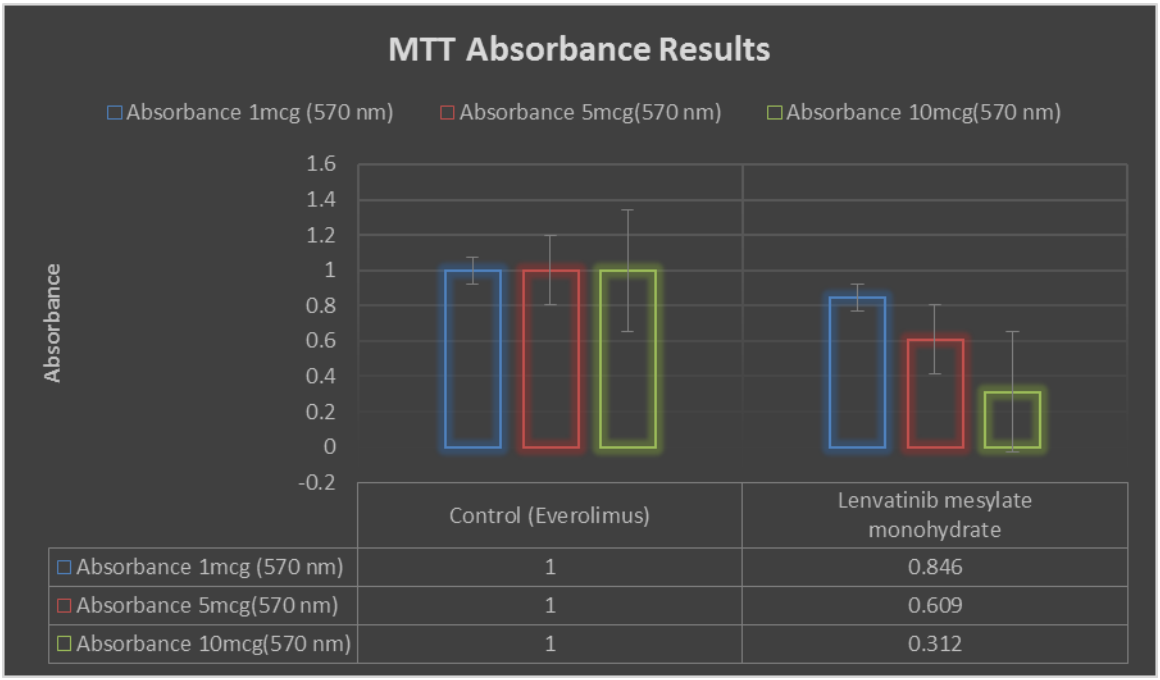


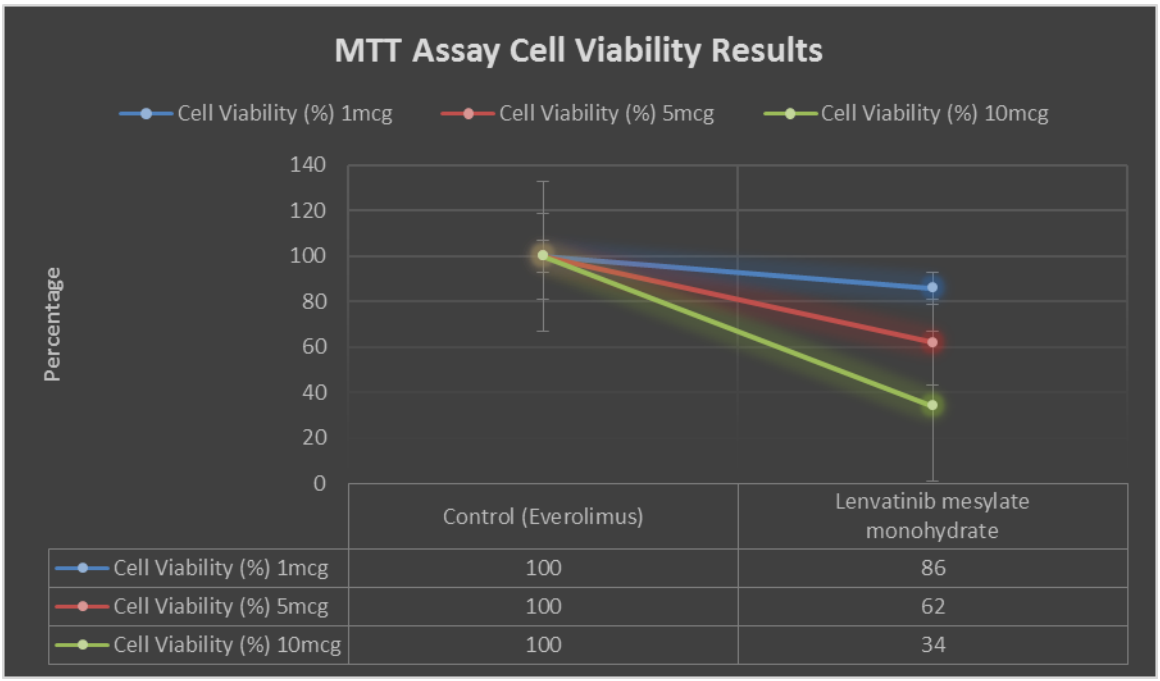


RESULTS

MTT Assay Results

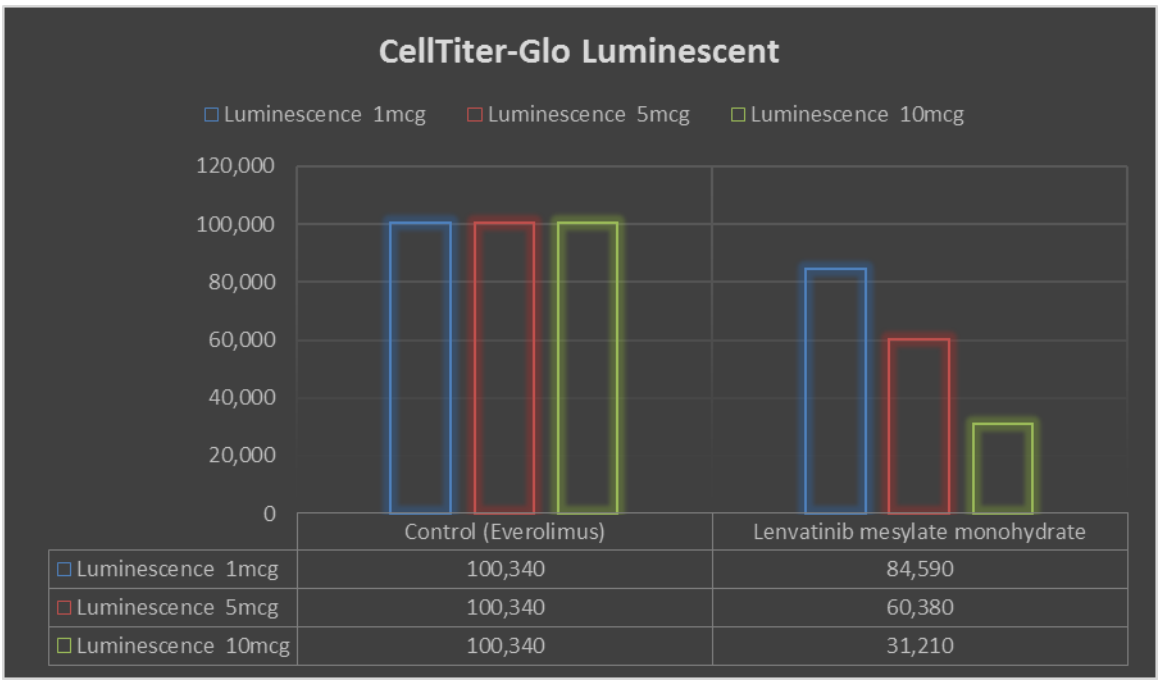
Treatment	Concentration (µM)	Absorbance (570 nm)	Cell Viability (%)
Control (Everolimus)	-	1.000	100
Lenvatinib mesylate monohydrate	1	0.846	86
	5	0.609	62
	10	0.312	34

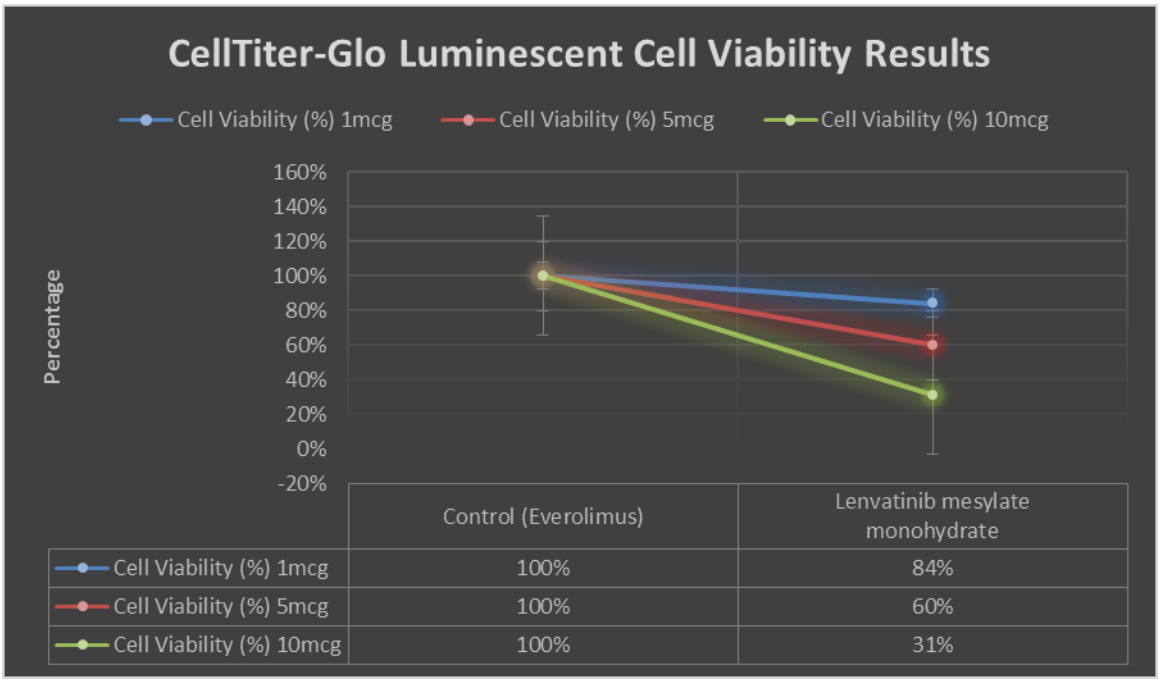




CellTiter-Glo Luminescent Cell Viability Assay Results

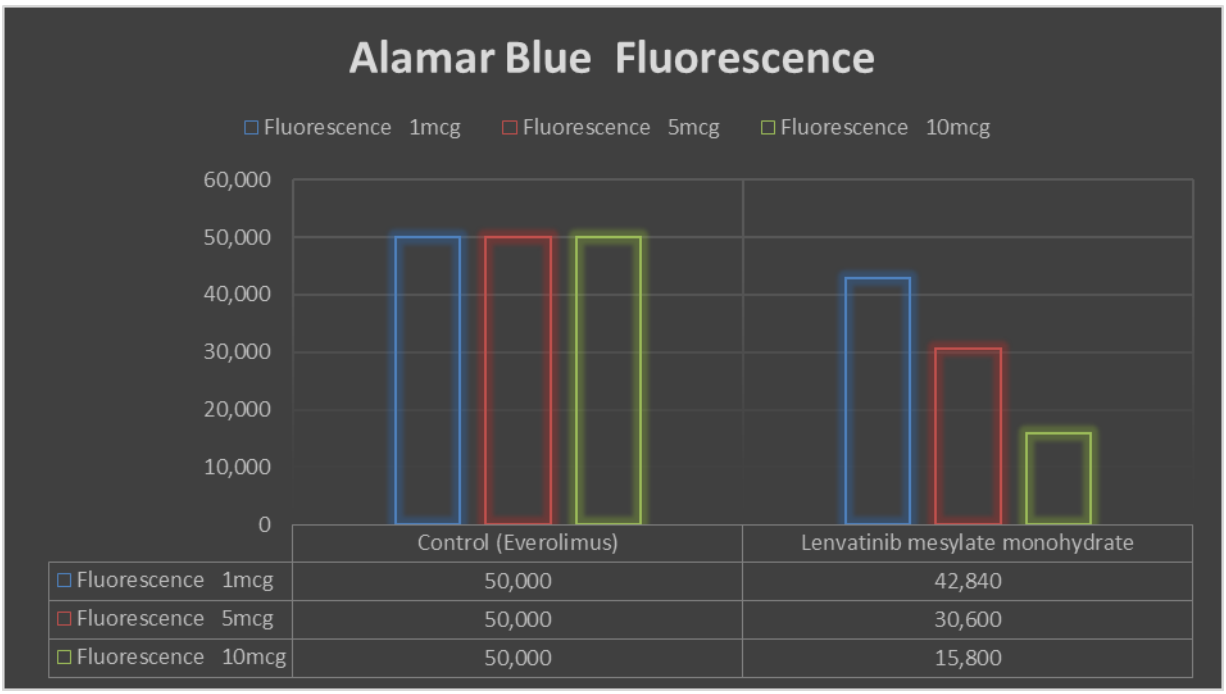
Treatment	Concentration (µM)	Luminescence (RLU)	Cell Viability (%)
Control (Everolimus)	-	100,340	100%
Lenvatinib mesylate monohydrate	1	84,590	84%
	5	60,380	60%
	10	31,210	31%

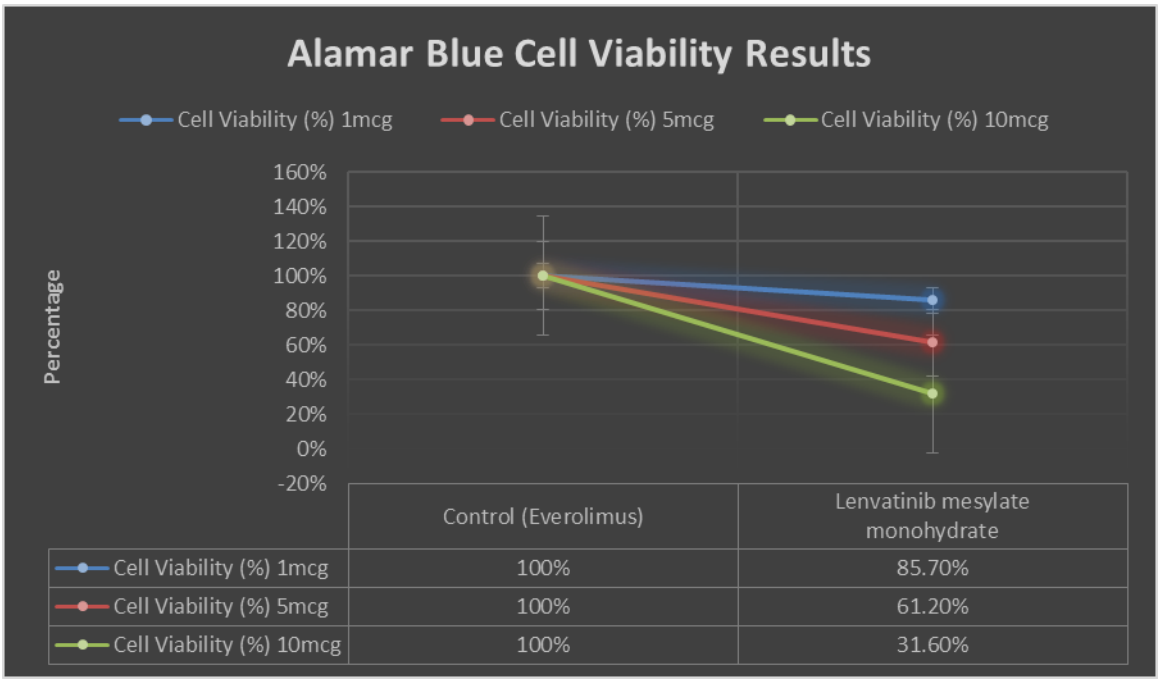




Alamar Blue Assay Results

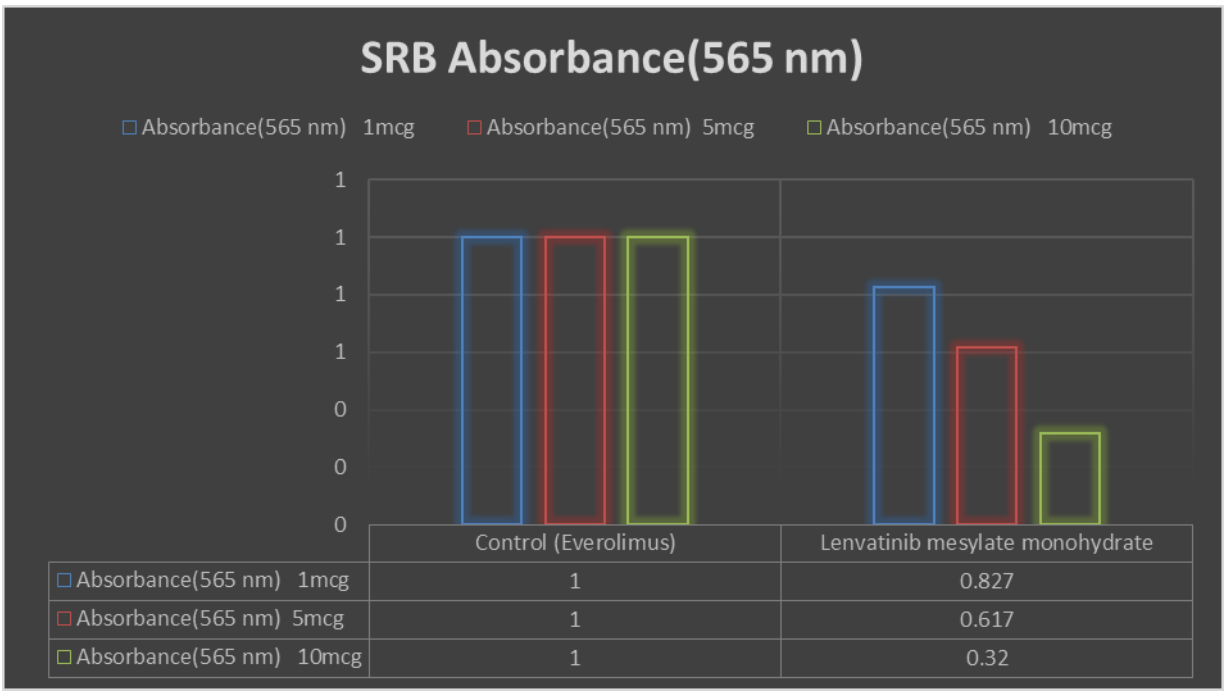
Treatment	Concentration (µM)	Absorbance (570 nm)	Fluorescence (590 nm)	Cell Viability (%)
Control (Everolimus)	-	1.000	50,000	100%
Lenvatinib mesylate monohydrate	1	0.840	42,840	85.7%
	5	0.600	30,600	61.2%
	10	0.310	15,800	31.6%

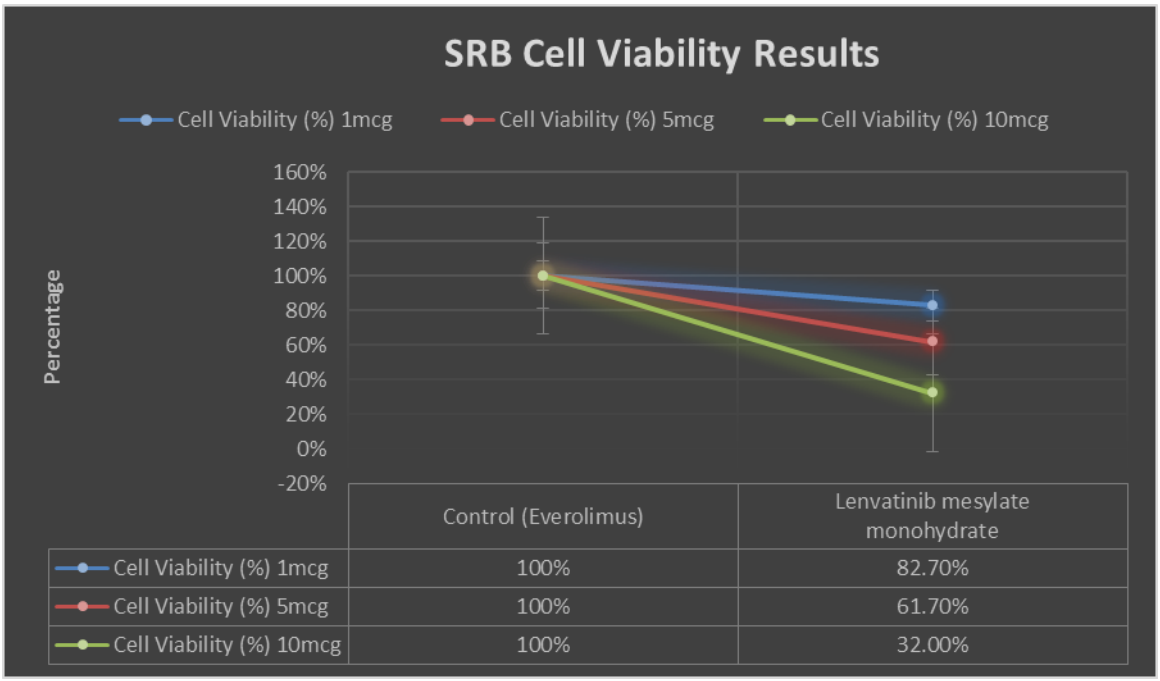




SRB Assay Results

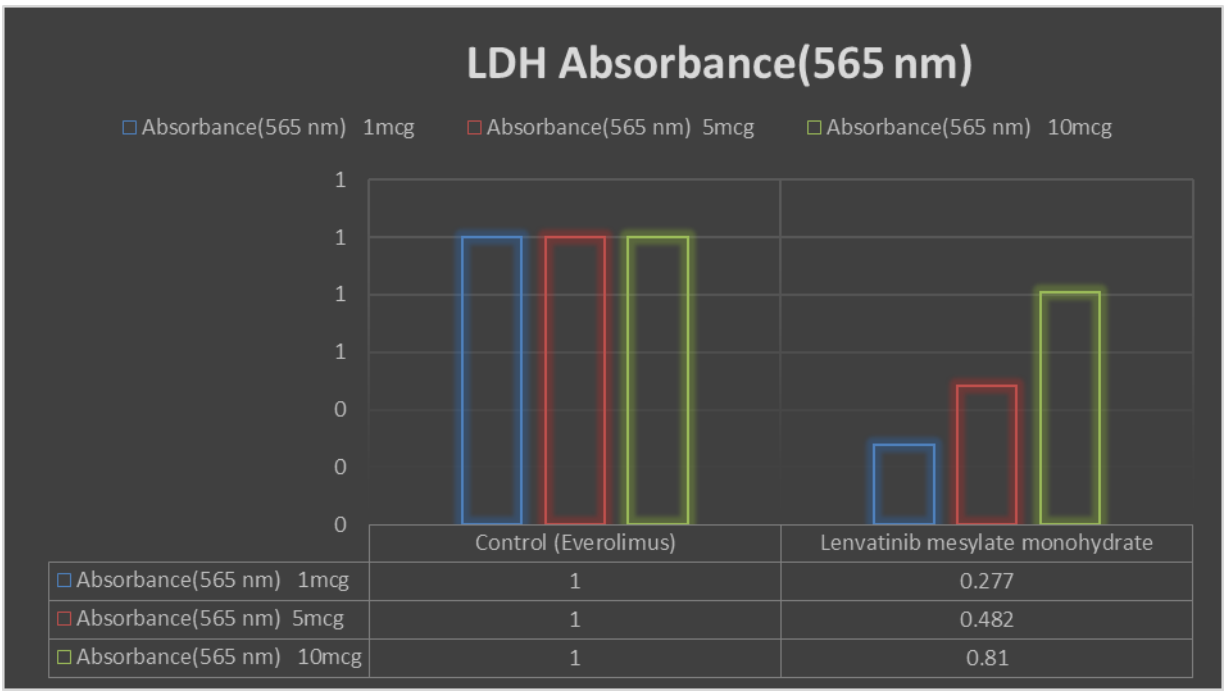
Treatment	Concentration (µM)	Absorbance (565 nm)	Cell Viability (%)
Control (Everolimus)	-	1.000	100%
Lenvatinib mesylate monohydrate	1	0.827	82.7%
	5	0.617	61.7%
	10	0.320	32.0%

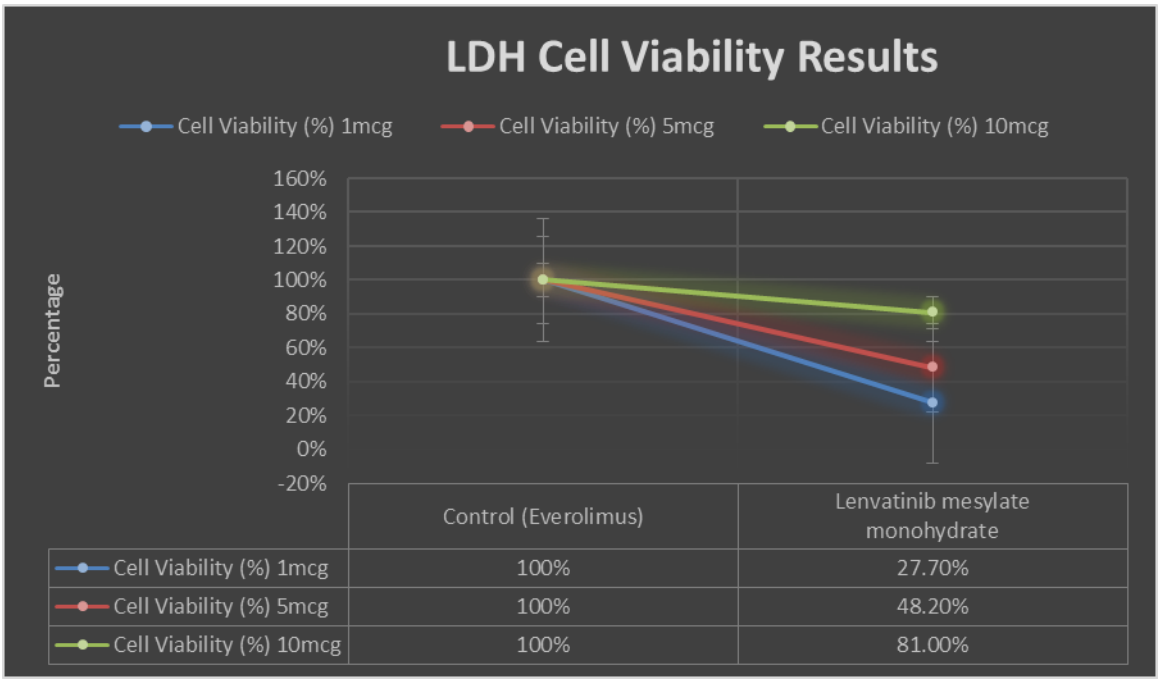




LDH Cytotoxicity Assay Results

Treatment	Concentration (µM)	Absorbance (565 nm)	Cell Viability (%)
Control (Everolimus)	-	1.000	100%
Lenvatinib mesylate monohydrate	1	0.277	27.7%
	5	0.482	48.2%
	10	0.810	81.0%





	Control (Everolimus)	Lenvatinib mesylate monohydrate
Cell Viability (%) 1mcg	100%	27.70%
Cell Viability (%) 5mcg	100%	48.20%
Cell Viability (%) 10mcg	100%	81.00%

DISCUSSION

The results of the study indicate that Lenvatinib mesylate monohydrate, a multi-kinase inhibitor, exhibits significant cytotoxic activity against kidney cancer cells in a dose-dependent manner. Multiple assays, including MTT, CellTiter-Glo, Alamar Blue, SRB, and LDH Cytotoxicity, were employed to evaluate cell viability across different concentrations of the compound (1 µM, 5 µM, and 10 µM).

The MTT assay demonstrated a considerable decrease in cell viability, dropping from 86% at 1 µM to 34% at 10 µM. Similarly, the CellTiter-Glo assay showed a reduction in cell viability from 84% at 1 µM to 31% at 10 µM, further confirming the anti-proliferative effect of Lenvatinib. These trends were supported by the Alamar Blue and SRB assays, with cell viability decreasing significantly as the concentration increased, indicating consistent cytotoxic activity.

The LDH Cytotoxicity Assay, however, revealed a higher cell viability at the highest concentration (81% at 10 µM), suggesting potential alterations in cell membrane integrity or a different pathway of cell death, such as necrosis, that might not be detected by the other assays. This inconsistency highlights the importance of using multiple assays to fully understand the cytotoxic profile of a compound. The observed variance may be due to Lenvatinib's impact on cellular processes, leading to reduced proliferation without necessarily inducing extensive cell death at higher doses.

Overall, the findings from this study suggest that Lenvatinib mesylate monohydrate has a robust anti-proliferative effect on kidney cancer cells, but its exact mechanism of action, especially at higher doses, requires further investigation.

CONCLUSION

Lenvatinib mesylate monohydrate displayed strong dose-dependent cytotoxic activity against kidney cancer cells, with cell viability decreasing significantly as the concentration increased. The MTT, CellTiter-Glo, Alamar Blue, and SRB assays all confirmed the compound's potent anti-proliferative properties, while the LDH assay suggested possible alterations in cell membrane integrity at higher concentrations. These results indicate that Lenvatinib mesylate monohydrate could be a promising therapeutic agent for kidney cancer treatment. However, further studies are warranted to explore its specific mechanisms of action and potential off-target effects.

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