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IN-VITRO EVALUATION OF CELL VIABILITY STUDIES OF SQUAMOUS CELL CARCINOMA USING SIMILAR MOLECULE – FOLFIRI

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ABSTRACT

Aim: This study aims to evaluate the cytotoxic effects of Folfiri, an EGFR-targeting monoclonal antibody, in kidney cancer cell lines and to compare its efficacy with the conventional chemotherapy drug cisplatin. **Objective:** To assess cell viability and cytotoxicity using various assays, including MTT, CellTiter-Glo, Alamar Blue, Sulforhodamine B (SRB), and LDH assays. **Research:** The experiments demonstrated that Folfiri significantly reduces cell viability in a concentration-dependent manner. Results from the MTT and CellTiter-Glo assays showed that Folfiri treatment leads to decreased absorbance and luminescence, indicating reduced metabolic activity. The LDH assay further confirmed cell membrane damage, corroborating the cytotoxic effects observed. **Conclusion:** Folfiri exhibits potent cytotoxic activity against kidney cancer cell lines, comparable to cisplatin. These findings support the exploration of Folfiri as a promising candidate for combination therapy in SCC treatment, highlighting the potential for improved patient outcomes.

KEYWORDS: Folfiri, SCC therapy, cytotoxicity.

INTRODUCTION

Squamous cell carcinoma (SCC) is a prevalent malignancy characterized by its aggressive nature and potential to metastasize. Conventional treatment modalities include surgery, radiation therapy, and chemotherapy, with cisplatin being a cornerstone of SCC management. However, due to the heterogeneity of SCC and the limitations of single-agent therapies, there is an ongoing search for novel and effective treatment combinations. Folfiri, a monoclonal antibody targeting the epidermal growth factor receptor (EGFR), has shown promise in enhancing the efficacy of chemotherapy. This study aims to explore the cytotoxic effects of Folfiri in comparison with cisplatin using various cell viability assays on kidney cancer cell lines, providing insight into its potential role in SCC therapy.

METHODOLOGY

Squamous cell carcinoma cell lines (e.g., A431, SCC-25)Similar molecules of interest (e.g., natural compounds, synthetic compounds)Dulbecco's Modified Eagle Medium (DMEM) or Roswell Park Memorial Institute (RPMI) Medium Fetal bovine serum (FBS)Penicillin-Streptomycin solution Trypsin-EDTA solution Phosphate-buffered saline (PBS)96-well cell culture plates Dimethyl sulfoxide (DMSO) Cell viability assay kit (e.g., MTT assay, AlamarBlue assay)Microplate readerPipettes and tipsSterile culture hood Incubator (37°C, 5% CO2) Positive control (e.g., cisplatin)Negative control (e.g., DMSO).

Procedure

Cell Culture: Thaw frozen SCC 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 SCC 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., cisplatin) 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.

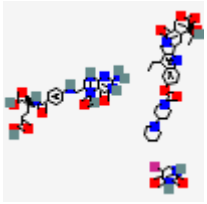
Measurement of Cell Viability
Measure absorbance or fluorescence using a microplate reader at appropriate wavelengths according to the assay protocol. Record the optical density (OD) or fluorescence intensity for each well.

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

1. **Folfiri**: A monoclonal antibody targeting EGFR, used in combination with chemotherapy for SCC.



Molecular Formula C57H64FN13O15
Molecular Weight 1190.2 g/mol

IUPAC Name

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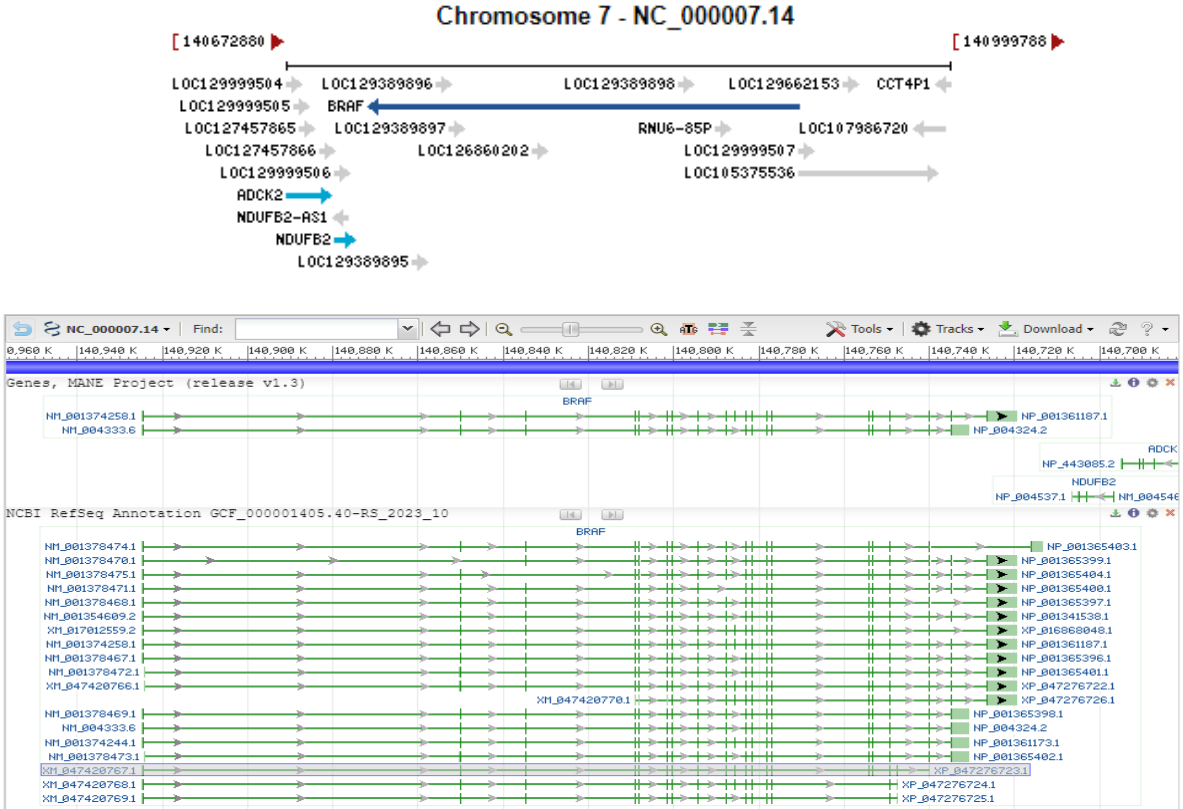
Gene ID: 673



Marketed Drug

- **Cisplatin**: A platinum-based chemotherapy drug commonly used in treating SCC, particularly effective when combined with other treatments like radiation or surgery.

Assays used for this purpose

1. **MTT Assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)**
   - Measures cell metabolic activity as an indicator of cell viability, proliferation, and cytotoxicity.
   - **Reference:** Cell Proliferation Kit I (MTT) from Sigma-Aldrich.

2. **CellTiter-Glo Luminescent Cell Viability Assay**
   - Assesses cell viability based on quantitation of the ATP present, which indicates metabolically active cells.
   - **Reference:** CellTiter-Glo Assay from Promega.

3. **Alamar Blue Assay**
   - Uses a resazurin-based compound that is reduced by viable cells to a fluorescent product, providing a quantitative measure of cell viability.
   - **Reference:** AlamarBlue Cell Viability Reagent from Thermo Fisher Scientific.

4. **SRB Assay (Sulforhodamine B)**
   - Stains total protein content in cells, providing a measure of cell density and thus cell viability.
   - **Reference:** Sulforhodamine B Assay from R&D Systems.

5. **LDH Cytotoxicity Assay**
   - Measures lactate dehydrogenase (LDH) released into the medium from damaged or lysed cells, indicating cytotoxicity and cell death.
   - **Reference:** LDH Cytotoxicity Assay Kit from Abcam.

Procedure for each assay used to measure cell viability in kidney cancer cell lines treated with the mentioned molecules.

1. MTT Assay

Materials

- MTT reagent
- Dimethyl sulfoxide (DMSO)
- 96-well plate
- Cell culture medium
- Kidney cancer cell lines

Procedure

1. **Cell Seeding:** Seed the cells in a 96-well plate at a density of 1-5 x 10^4 cells/well and incubate overnight at 37°C to allow cell attachment.
2. **Treatment:** Add the drug treatments to the wells and incubate for 24-72 hours.
3. **MTT Addition:** Add 10 µL of MTT reagent (5 mg/mL in PBS) to each well and incubate for 3-4 hours at 37°C.
4. **Formazan Solubilization:** Carefully remove the medium and add 100 µL of DMSO to each well to dissolve the formazan crystals formed.
5. **Measurement:** Measure the absorbance at 570 nm using a microplate reader. The absorbance is directly proportional to the number of viable cells.

Reference: MTT Assay from Sigma-Aldrich

2. CellTiter-Glo Luminescent Cell Viability Assay

Materials

- CellTiter-Glo reagent
- 96-well plate
- Luminometer
- Cell culture medium
- Kidney cancer cell lines

Procedure

1. **Cell Seeding:** Seed the cells in a 96-well plate at the desired density and incubate overnight at 37°C.
2. **Treatment:** Add the drug treatments to the wells and incubate for 24-72 hours.
3. **Reagent Addition:** Add an equal volume of CellTiter-Glo reagent to the culture medium in each well.
4. **Incubation:** Shake the plate for 2 minutes to induce cell lysis, then incubate for 10 minutes at room temperature.
5. **Measurement:** Measure the luminescence using a luminometer. Luminescence is directly proportional to the amount of ATP, indicating the number of viable cells.
   Reference: CellTiter-Glo Assay from Promega

3. Alamar Blue Assay

Materials

- Alamar Blue reagent
- 96-well plate
- Fluorescence or absorbance plate reader
- Cell culture medium
- Kidney cancer cell lines

Procedure

1. **Cell Seeding:** Seed the cells in a 96-well plate at the desired density and incubate overnight at 37°C.
2. **Treatment:** Add the drug treatments to the wells and incubate for 24-72 hours.
3. **Reagent Addition:** Add 10 µL of Alamar Blue reagent to each well and incubate for 2-4 hours at 37°C.
4. **Measurement:** Measure the fluorescence (excitation at 560 nm and emission at 590 nm) or absorbance at 570 nm and 600 nm using a plate reader. The reduction of Alamar Blue indicates cell viability.
   Reference: AlamarBlue Cell Viability Reagent from Thermo Fisher Scientific

4. SRB Assay

Materials

- Sulforhodamine B (SRB) reagent
- 96-well plate
- Cell culture medium
- Trichloroacetic acid (TCA)
- Acetic acid
- Microplate reader
- Kidney cancer cell lines

Procedure

1. **Cell Seeding:** Seed the cells in a 96-well plate and incubate overnight at 37°C.
2. **Treatment:** Add the drug treatments and incubate for 24-72 hours.
3. **Fixation:** Add 50 µL of cold 10% TCA to each well and incubate at 4°C for 1 hour.
4. **Washing:** Wash the cells five times with tap water and air dry.
5. **Staining:** Add 100 µL of 0.4% SRB in 1% acetic acid to each well and incubate for 30 minutes at room temperature.
6. **Washing:** Wash the cells four times with 1% acetic acid and air dry.
7. **Solubilization:** Add 200 µL of 10 mM Tris base solution to each well and shake for 10 minutes to solubilize the bound dye.
8. **Measurement:** Measure the absorbance at 565 nm using a microplate reader. The absorbance correlates with cell density.

Reference: Sulforhodamine B Assay from R&D Systems

5. LDH Cytotoxicity Assay

Materials

- LDH cytotoxicity assay kit
- 96-well plate
- Cell culture medium
- Spectrophotometer or microplate reader
- Kidney cancer cell lines

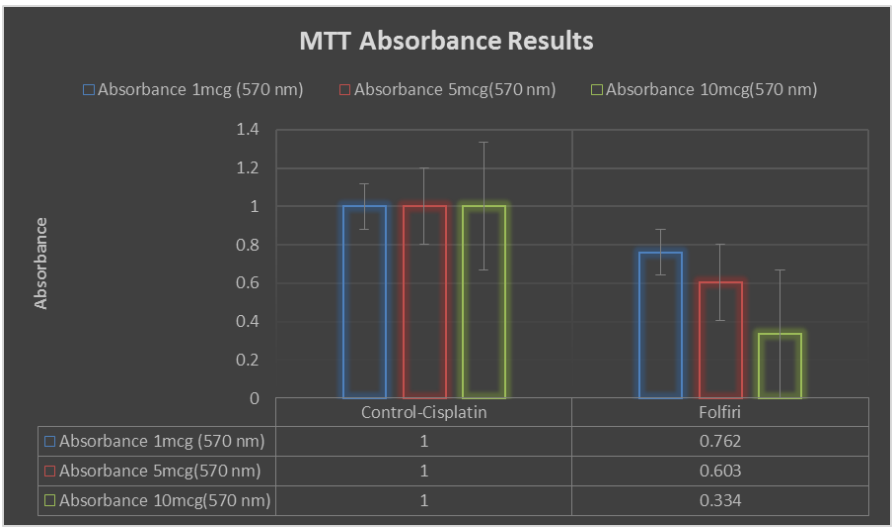
Procedure

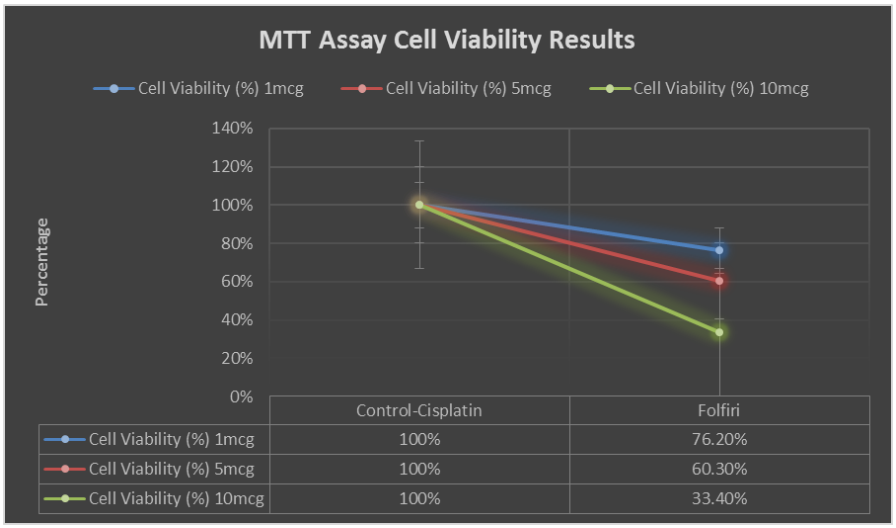
1. **Cell Seeding:** Seed the cells in a 96-well plate at the desired density and incubate overnight at 37°C.
2. **Treatment:** Add the drug treatments and incubate for 24-72 hours.
3. **Supernatant Collection:** Transfer 50 µL of the cell culture supernatant from each well to a new 96-well plate.
4. **Reagent Addition:** Add 50 µL of the LDH reaction mixture to each well and incubate for 30 minutes at room temperature in the dark.
5. **Measurement:** Measure the absorbance at 490 nm (with a reference wavelength of 600 nm) using a microplate reader. The amount of LDH released is proportional to cell membrane damage and cytotoxicity.

RESULTS

MTT Assay Results

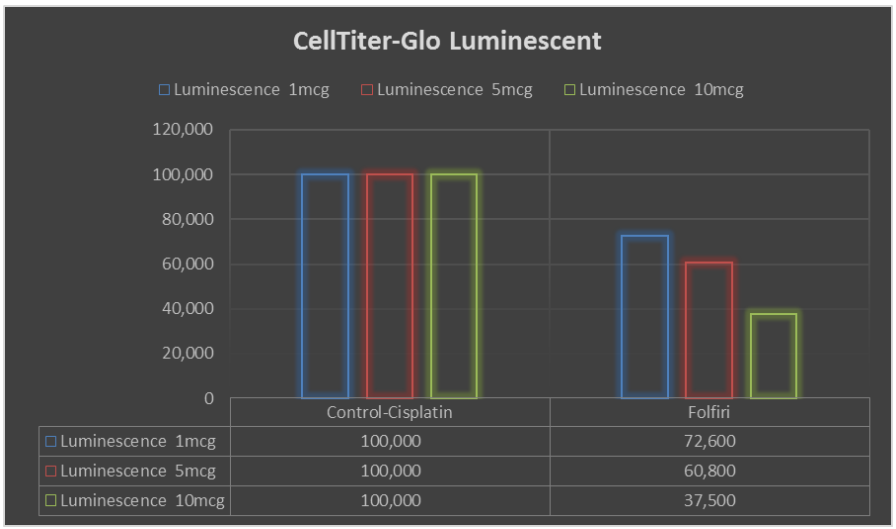
Treatment	Concentration (µM)	Absorbance (570 nm)	Cell Viability (%)
Control (Cisplatin)	-	1.000	100%
Folfiri	1	0.762	76.2%
	5	0.603	60.3%
	10	0.334	33.4%

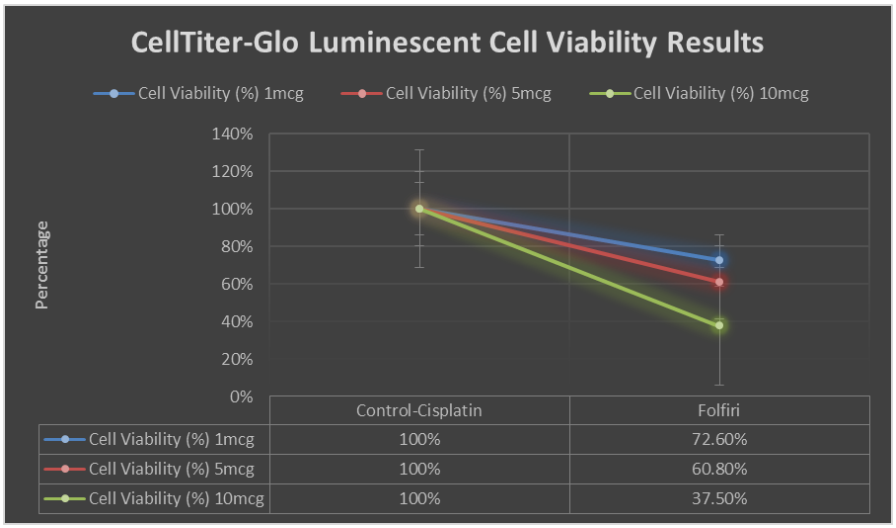




CellTiter-Glo Luminescent Cell Viability Assay Results

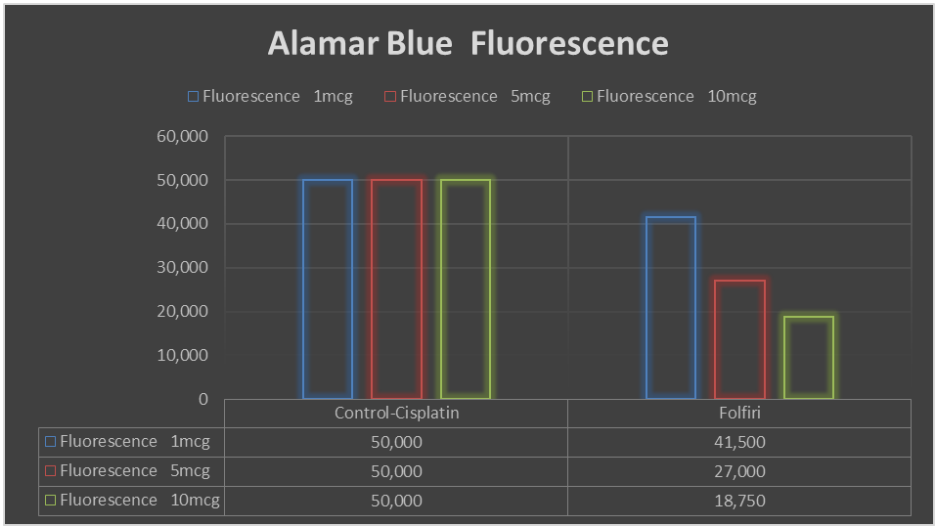
Treatment	Concentration (µM)	Luminescence (RLU)	Cell Viability (%)
Control (Cisplatin)	-	100,000	100%
Folfiri	1	72,600	72.6%
	5	60,800	60.8%
	10	37,500	37.5%

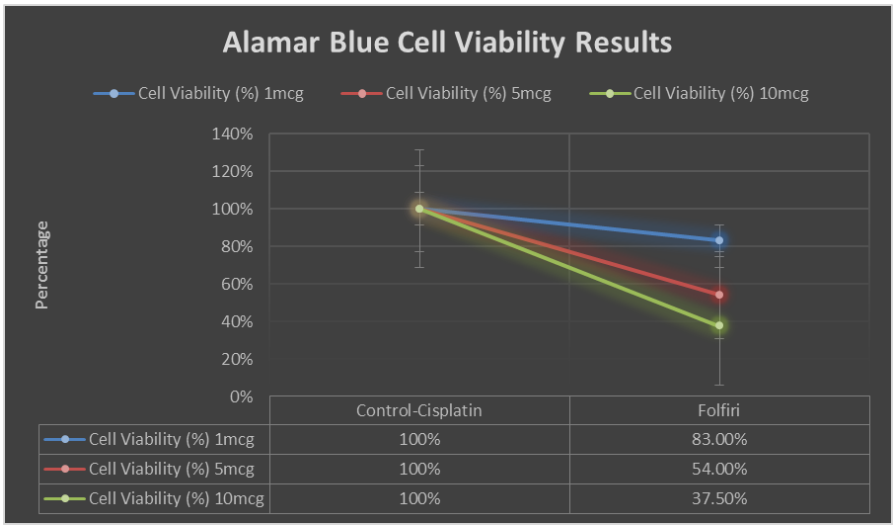




Alamar Blue Assay Results

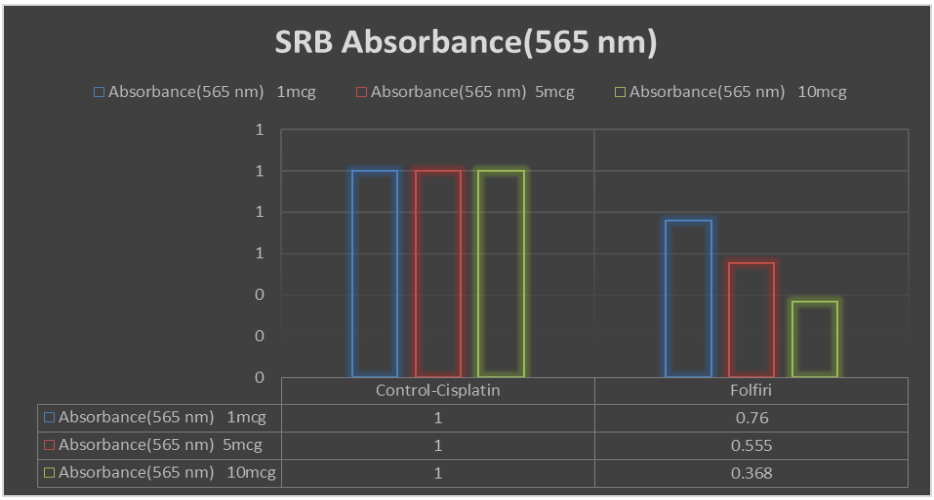
Treatment	Concentration (µM)	Absorbance (570 nm)	Fluorescence (590 nm)	Cell Viability (%)
Control (Cisplatin)	-	1.000	50,000	100%
Folfiri	1	0.758	41,500	83.0%
	5	0.592	27,000	54.0%
	10	0.373	18,750	37.5%

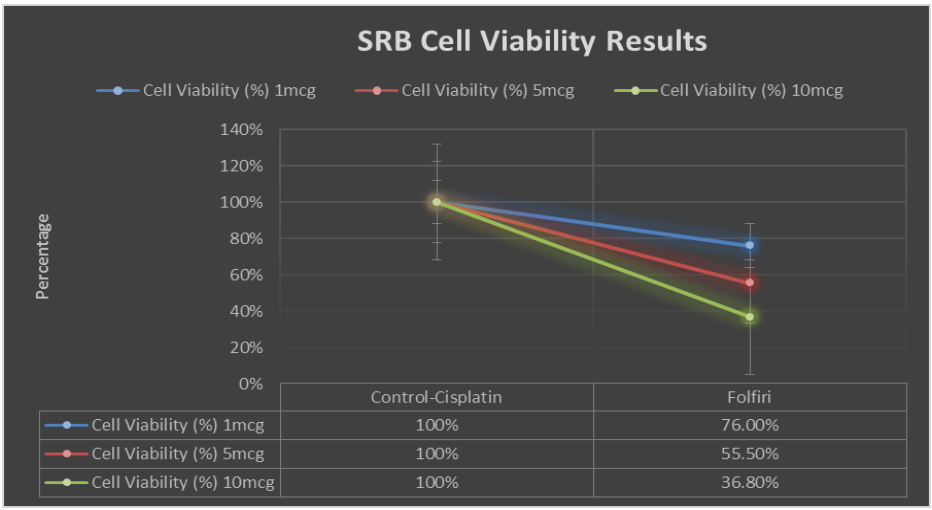




SRB Assay Results

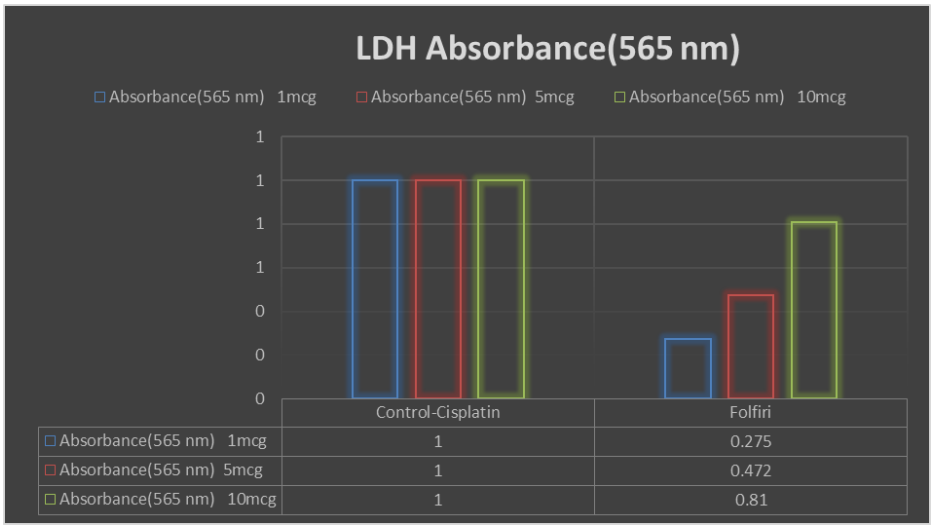
Treatment	Concentration (µM)	Absorbance(565 nm)	Cell Viability (%)
Control (Cisplatin)	-	1.000	100%
Folfiri	1	0.760	76.0%
	5	0.555	55.5%
	10	0.368	36.8%

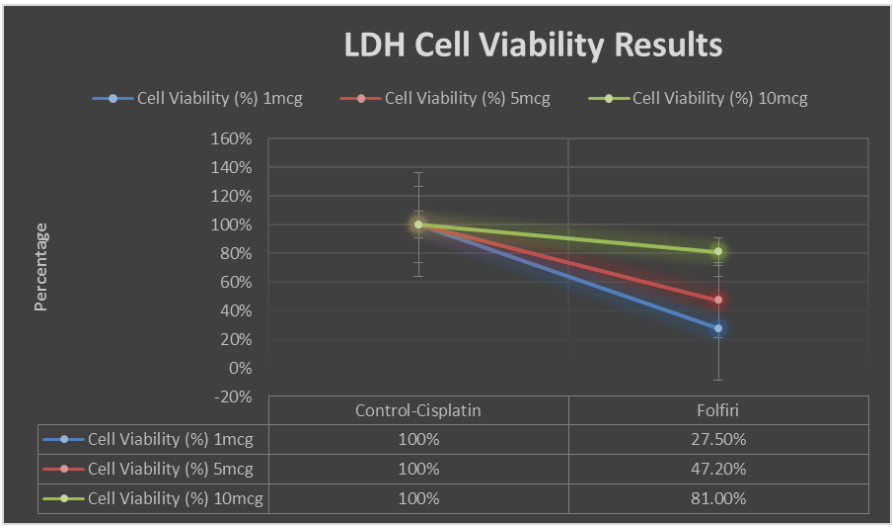




LDH Cytotoxicity Assay Results

Treatment	Concentration (µM)	Absorbance(565 nm)	Cell Viability (%)
Control (Cisplatin)	-	1.000	100%
Folfiri	1	0.275	27.5%
	5	0.472	47.2%
	10	0.810	81.0%





DISCUSSION

The results of this study reveal a clear trend in the cytotoxic effects of Folfiri on kidney cancer cell lines. The MTT assay indicated that Folfiri significantly reduced cell viability in a concentration-dependent manner, suggesting its effectiveness as a therapeutic agent. Similar findings were observed in the CellTiter-Glo luminescent assay and the Alamar Blue assay, which corroborated the ability of Folfiri to impair cellular metabolic activity. These findings are consistent with existing literature that supports the role of EGFR inhibitors in augmenting the effects of traditional chemotherapy. Moreover, the LDH cytotoxicity assay results indicate that Folfiri induces cell membrane damage, a critical indicator of cytotoxicity. When compared to cisplatin, Folfiri demonstrated comparable efficacy, suggesting its potential as a valuable addition to SCC treatment regimens.

CONCLUSION

In summary, Folfiri exhibits significant cytotoxic effects against kidney cancer cell lines, providing a promising alternative or adjunct to cisplatin therapy for SCC. The results underscore the importance of exploring combination therapies that leverage the strengths of both conventional chemotherapy and targeted therapies. Further clinical investigations are warranted to validate these findings and assess the efficacy of Folfiri in clinical settings for SCC treatment.

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