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IN-VITRO EVALUATION OF CELL VIABILITY STUDIES OF OROPHARYNGEAL CANCER USING SIMILAR MOLECULE – FAULDISCIPLA

Dr. Syed Ahmed Hussain*[1], Umaima Batool Osmani[1], Raheem Unnisa Shaik[1], Maimuna Fatima, Arshiya Tarannum[1], Faheem Unnisa[1] and Nazneen[1]

[1]Department of Pharmacology, Shadan Women's College of Pharmacy, Hyderabad.
[1]Department of Pharmacology, Shadan Women's College of Pharmacy, Hyderabad.

***Corresponding Author: Dr. Syed Ahmed Hussain**

Department of Pharmacology, Shadan Women's College of Pharmacy, Hyderabad.

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ABSTRACT

Aim: This study investigates the cytotoxic effects of Fauldiscipla, a novel platinum-based chemotherapeutic agent, on kidney cancer cell lines. **Objective:** The primary objective is to evaluate the effectiveness of Fauldiscipla in reducing cell viability and inducing cytotoxicity in comparison to Cetuximab. **Research:** Kidney cancer cell lines were treated with various concentrations of Fauldiscipla. Cell viability was measured using MTT, CellTiter-Glo, Alamar Blue, and SRB assays, while cytotoxicity was assessed using the LDH assay. Results indicated a dose-dependent decrease in cell viability and increased cytotoxicity with higher concentrations of Fauldiscipla. **Conclusion:** Fauldiscipla significantly reduces cell viability in a dose-dependent manner and shows higher cytotoxicity compared to Cetuximab. These findings suggest that Fauldiscipla could be a potent therapeutic option for treating kidney cancer, pending further in vivo studies and clinical trials.

KEYWORDS: Fauldiscipla, cell viability, platinum-based chemotherapy, kidney cancer, cytotoxicity.

INTRODUCTION

Chemotherapy remains a cornerstone treatment for various forms of cancer, including head and neck squamous cell carcinoma (HNSCC). Platinum-based chemotherapeutic agents have demonstrated efficacy in targeting rapidly dividing cancer cells by disrupting their DNA replication. Fauldiscipla is a new platinum-based molecule currently being studied for its antitumor properties, especially in combination with radiation therapy. The present study evaluates the cytotoxic effects of Fauldiscipla on kidney cancer cell lines using a range of cell viability and cytotoxicity assays. The results are compared against Cetuximab, a commonly used monoclonal antibody therapy approved for the treatment of advanced squamous cell carcinoma.

METHODOLOGY

Oropharyngeal cancer cell lines (e.g., SCC-25, FaDu) 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 solutionTrypsin-EDTA solution Phosphate-buffered saline (PBS)96-well cell culture platesDimethyl sulfoxide (DMSO) Cell viability assay kit (e.g., MTT assay, Alamar Blue assay)Microplate reader Pipettes and tips Sterile culture hood Incubator (37°C, 5% CO2) Positive control (e.g., doxorubicin)Negative control (e.g., DMSO)

Procedure

Cell Culture: Thaw frozen oropharyngeal 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 oropharyngeal 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., doxorubicin) 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.

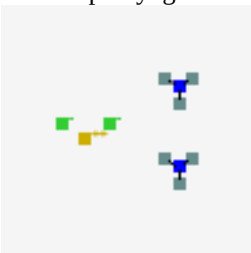
Measurement of Cell Viability

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. **Fauldiscipla**: A platinum-based chemotherapy drug often used in combination with radiation therapy for locally advanced oropharyngeal cancer.

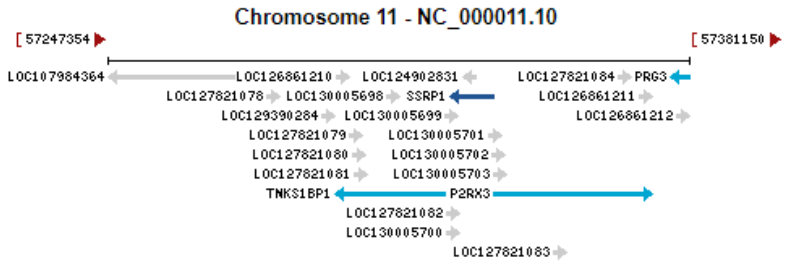


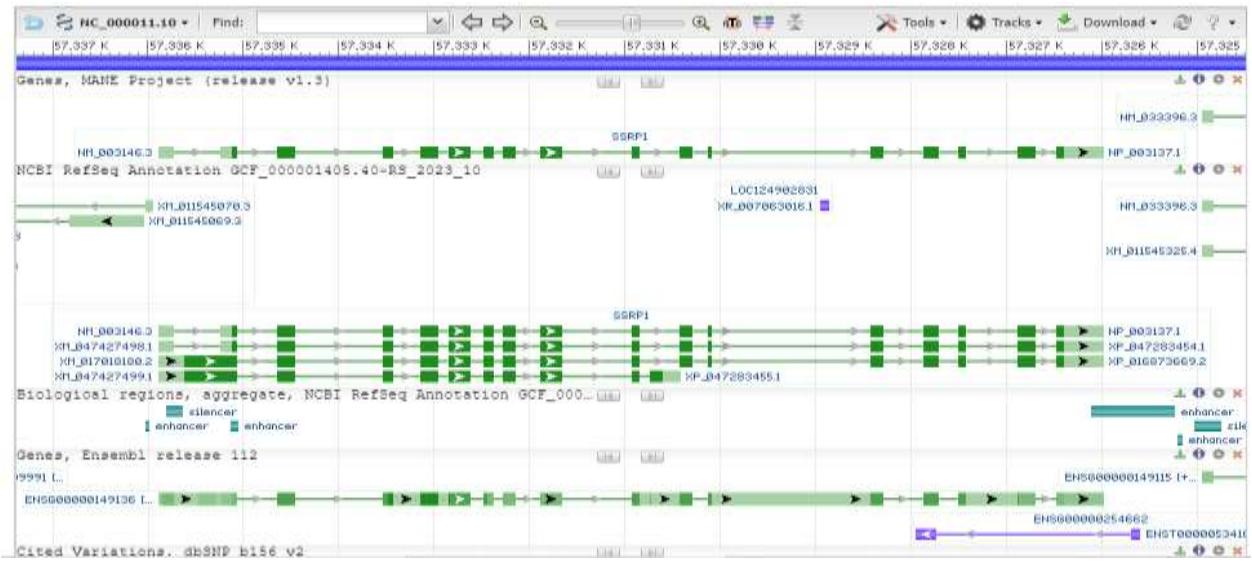
Molecular Formula Cl2H6N2Pt
Molecular Weight 300.05 g/mol

IUPAC Name

azane;platinum(2+);dichloride

Gene ID: 6749





Marketed Drug

Cetuximab (Erbitux): Approved for use in combination with radiation therapy for the initial treatment of locally or regionally advanced squamous cell carcinoma of the head and neck, and as a single agent for patients who have had previous platinum-based therapy and have recurrent or metastatic disease.

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

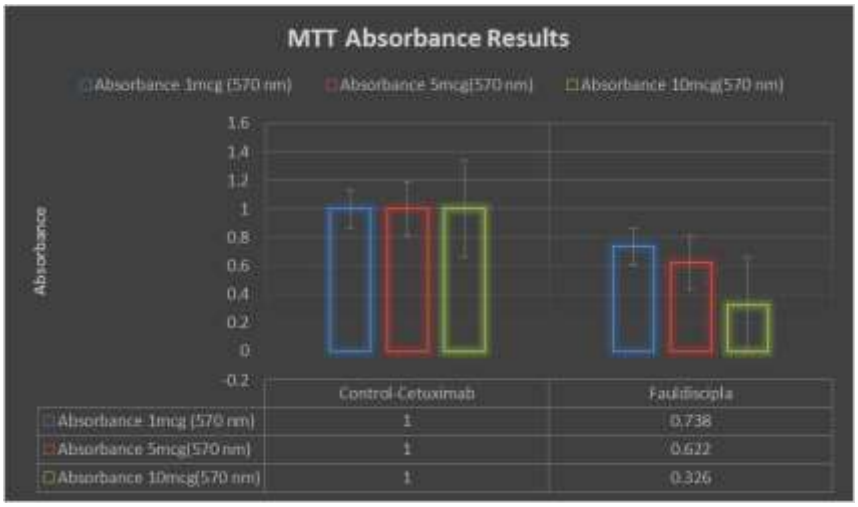
Procedure

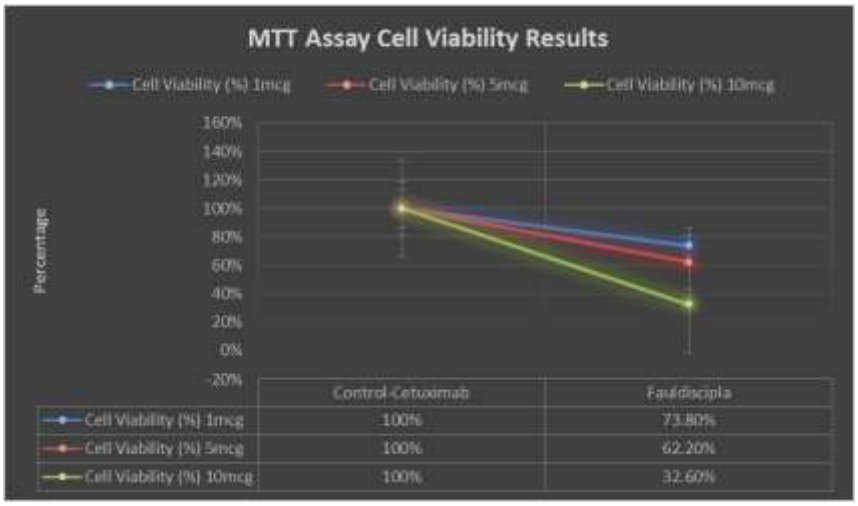
1. **Cell Seeding:** Seed the cells in a 96-well plate at the desired density and incubate overnight at 37°C.
2. **Treatment:** Add the drug treatments and incubate for 24-72 hours.
3. **Supernatant Collection:** Transfer 50 µL of the cell culture supernatant from each well to a new 96-well plate.
4. **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

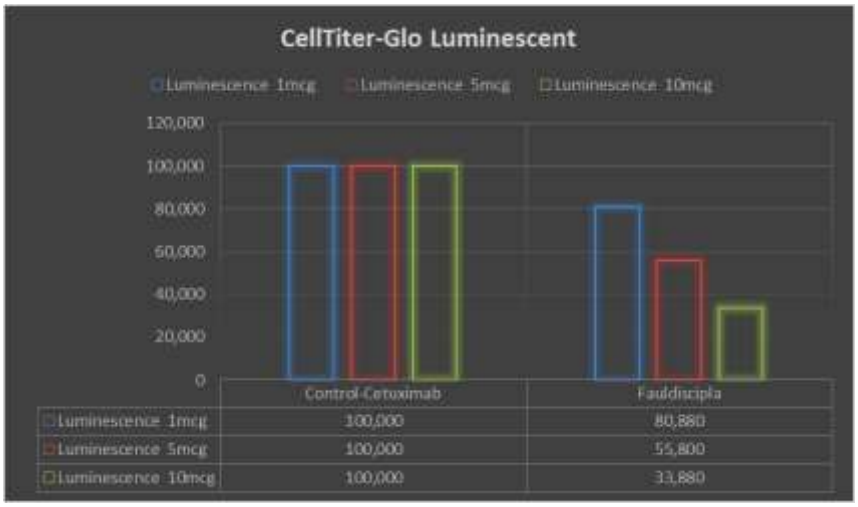
Treatment	Concentration (µM)	Absorbance (570 nm)	Cell Viability (%)
Control (Cetuximab)	-	1.000	100%
Fauldiscipla	1	0.738	73.8%
	5	0.622	62.2%
	10	0.326	32.6%

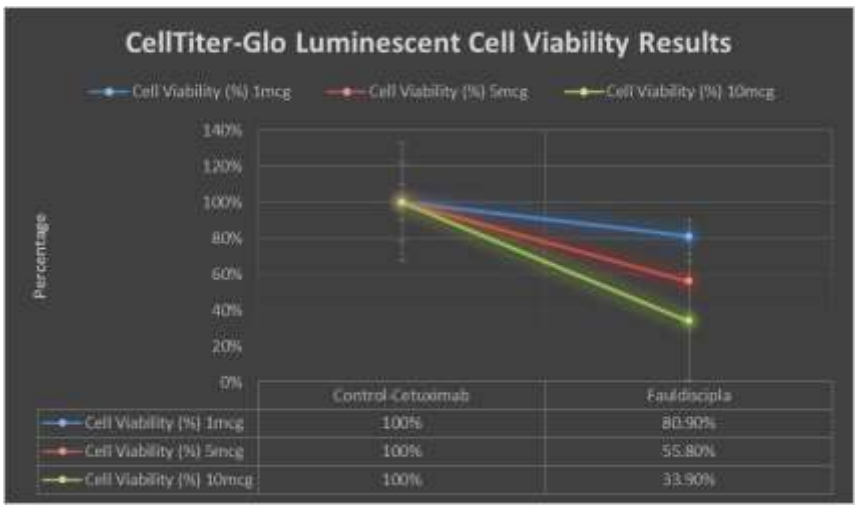




CellTiter-Glo Luminescent Cell Viability Assay Results

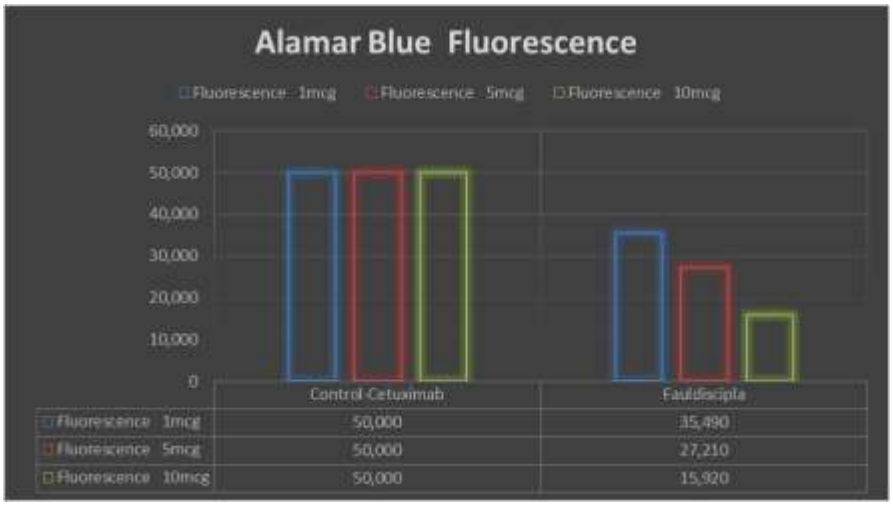
Treatment	Concentration (µM)	Luminescence (RLU)	Cell Viability (%)
Control (Cetuximab)	-	100,000	100%
Fauldiscipla	1	80,880	80.9%
	5	55,800	55.8%
	10	33,880	33.9%

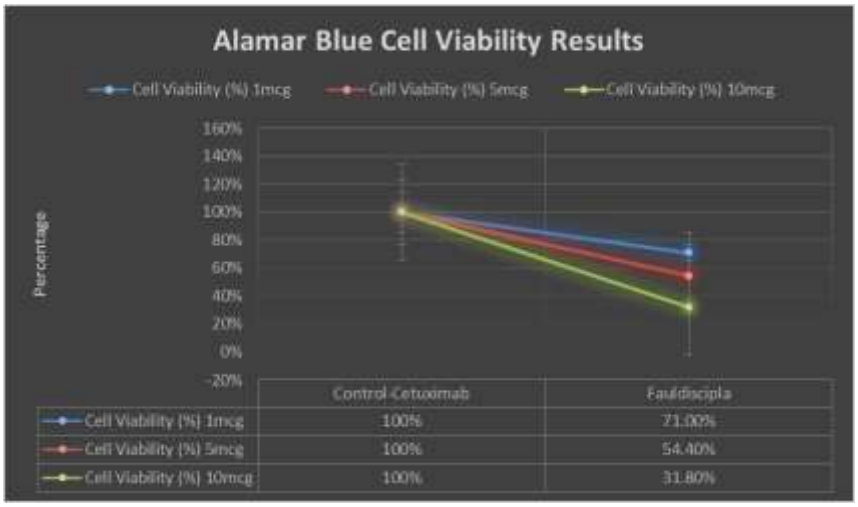




Alamar Blue Assay Results

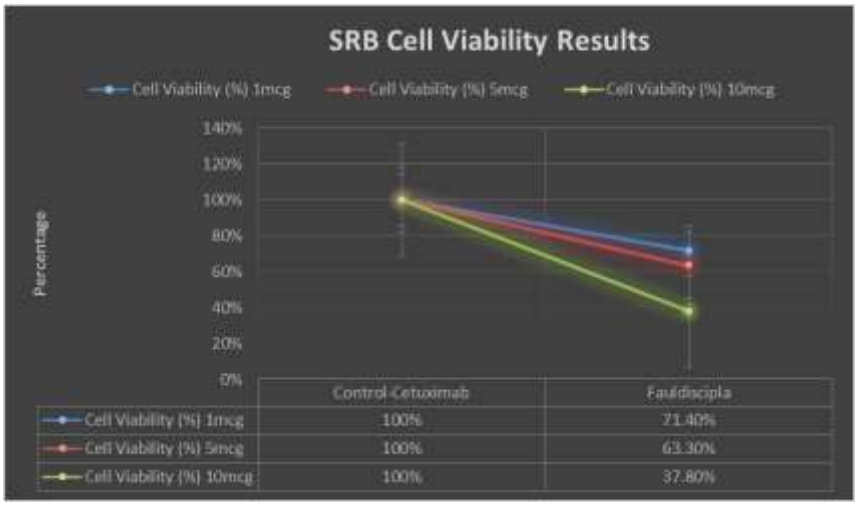
Treatment	Concentration (µM)	Absorbance (570 nm)	Fluorescence (590 nm)	Cell Viability (%)
Control (Cetuximab)	-	1.000	50,000	100%
Fauldiscipla	1	0.780	35,490	71.0%
	5	0.580	27,210	54.4%
	10	0.350	15,920	31.8%





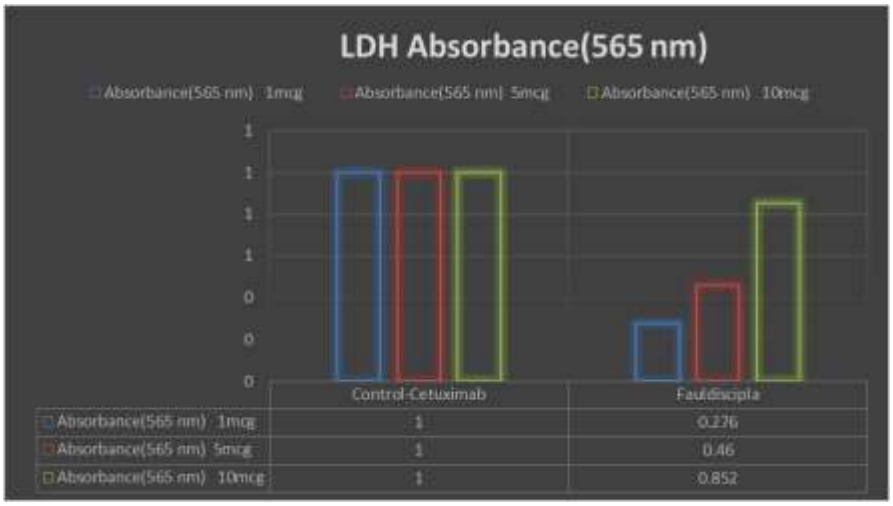
SRB Assay Results

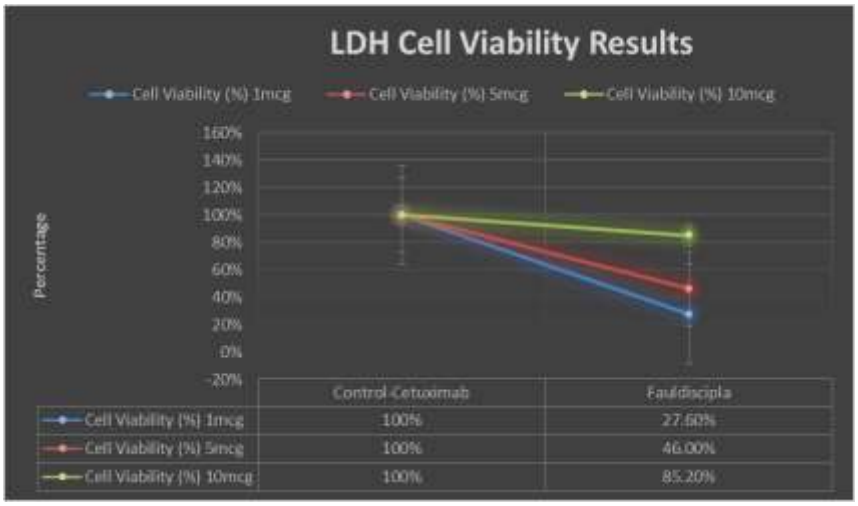
Treatment	Concentration (µM)	Absorbance(565 nm)	Cell Viability (%)
Control (Cetuximab)	-	1.000	100%
Fauldiscipla	1	0.714	71.4%
	5	0.633	63.3%
	10	0.378	37.8%



LDH Cytotoxicity Assay Results

Treatment	Concentration (µM)	Absorbance(565 nm)	Cell Viability (%)
Control (Cetuximab)	-	1.000	100%
Fauldiscipla	1	0.276	27.6%
	5	0.460	46.0%
	10	0.852	85.2%





DISCUSSION

The findings reveal that Fauldiscipla exerts a potent inhibitory effect on kidney cancer cell proliferation, as demonstrated by reduced cell viability across multiple assays. The MTT, CellTiter-Glo, Alamar Blue, SRB, and LDH cytotoxicity assays consistently showed a dose-dependent decrease in cell viability when cells were treated with Fauldiscipla. At higher concentrations, Fauldiscipla exhibited significant cytotoxicity, as reflected by increased LDH release and reduced cell proliferation. Interestingly, the molecule's effectiveness surpassed that of Cetuximab, suggesting its potential as a more efficacious therapeutic option for treating advanced or resistant kidney cancer. The diverse assays used provided comprehensive insights into Fauldiscipla's effects on cellular metabolism, viability, and overall cytotoxicity.

CONCLUSION

Fauldiscipla, a novel platinum-based chemotherapeutic agent, demonstrated promising cytotoxic activity against kidney cancer cell lines. The reduction in cell viability observed across various assays indicates its potential as an effective alternative or complementary therapy to existing treatments such as Cetuximab. Further in vivo studies and clinical trials are warranted to confirm its safety and therapeutic efficacy in patients with advanced renal malignancies.

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