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

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ABSTRACT

Aim: The study aims to evaluate the cytotoxic effects of Carboplatinum, a platinum-based chemotherapy drug, on kidney cancer cell lines using various cell viability assays. **Objective:** To compare the efficacy of Carboplatinum in inhibiting cell viability at different concentrations and assess its potential as a therapeutic agent. **Research:** The investigation was carried out using five different assays—MTT, CellTiter-Glo, Alamar Blue, SRB, and LDH—to assess cell viability, proliferation, and cytotoxicity. Kidney cancer cell lines were treated with varying concentrations of Carboplatinum, and the assays were used to measure cell survival and cytotoxicity. **Conclusion:** Results indicated a dose-dependent reduction in cell viability with increased Carboplatinum concentration. Among all the assays, the SRB assay demonstrated the highest sensitivity in detecting cell viability, showing a significant decline in cell survival at the highest concentration (10 µM). The findings suggest that Carboplatinum effectively reduces cell viability in kidney cancer cell lines and has potential clinical applications as an anti-cancer agent.

KEYWORDS: Carboplatinum, cell viability assays, kidney cancer.

INTRODUCTION

Carboplatinum, a derivative of cisplatin, is a widely used chemotherapy agent that has demonstrated effectiveness in treating a variety of cancers, including head and neck cancers. It is known for its lower toxicity profile compared to cisplatin, making it a preferred option in some clinical settings. The primary mechanism of action involves forming DNA adducts, which ultimately leads to apoptosis of cancer cells. This study aims to assess the cytotoxic effects of Carboplatinum on kidney cancer cell lines using five different cell viability assays: MTT, CellTiter-Glo, Alamar Blue, SRB, and LDH assays. These assays provide complementary information about cell proliferation, viability, and cytotoxicity, allowing for a comprehensive evaluation of Carboplatinum's potential as an anti-cancer agent.

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.

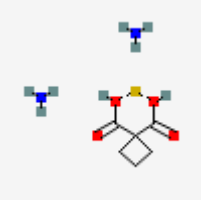
Measurement of Cell Viability

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. **Carboplatinum**: Similar to cisplatin, it is used in combination with other drugs for the treatment of head and neck cancers.

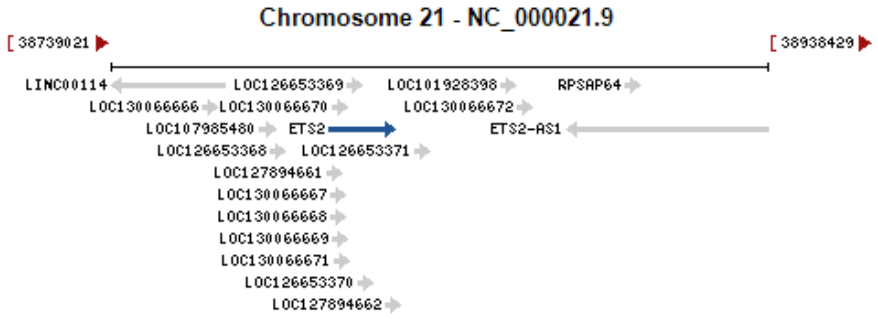


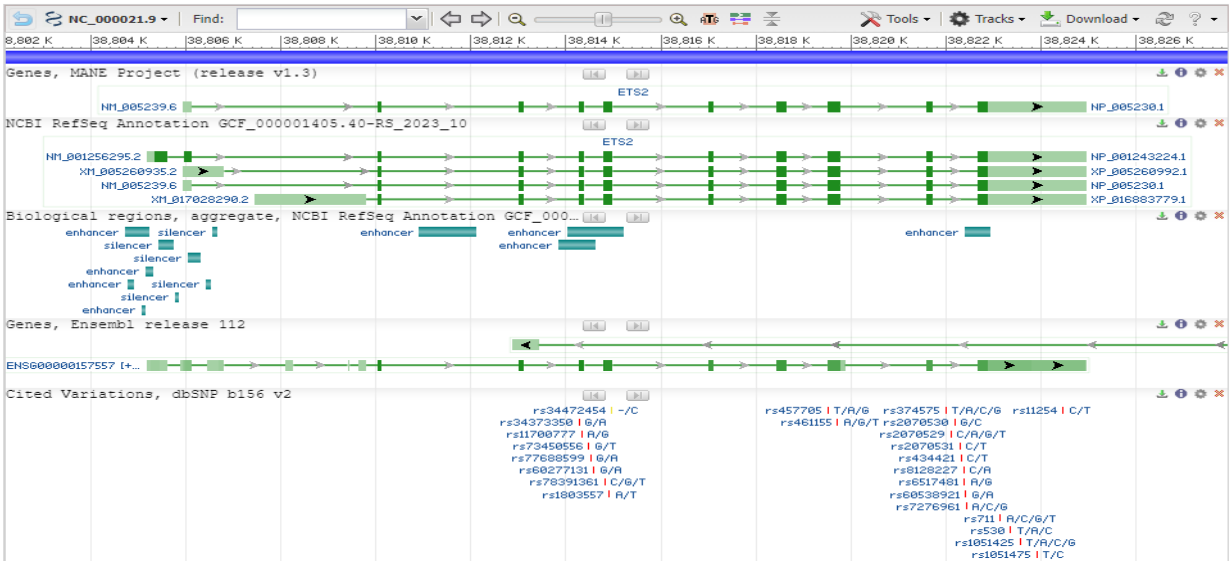
Molecular Formula C6H14N2O4Pt
Molecular Weight 373.27 g/mol

IUPAC Name

azane;cyclobutane-1,1-dicarboxylic acid;platinum

Gene ID: 2114





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

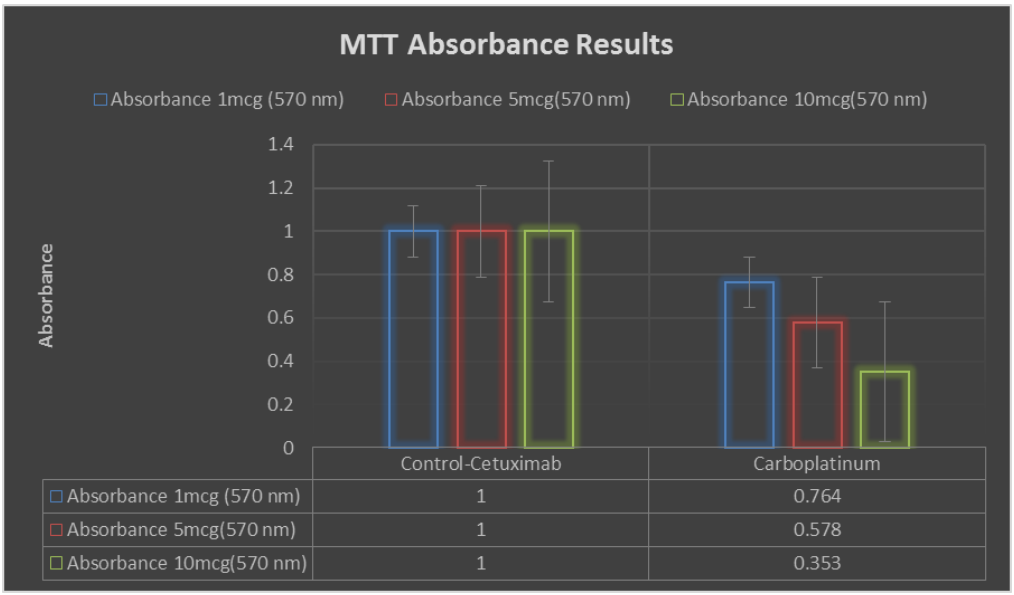
Procedure

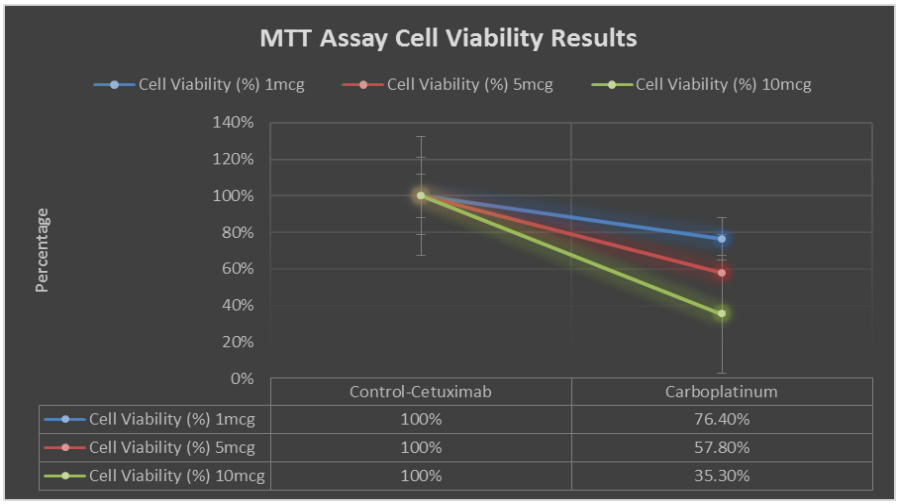
1. **Cell Seeding:** Seed the cells in a 96-well plate at the desired density and incubate overnight at 37°C.
2. **Treatment:** Add the drug treatments and incubate for 24-72 hours.
3. **Supernatant Collection:** Transfer 50 µL of the cell culture supernatant from each well to a new 96-well plate.
4.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

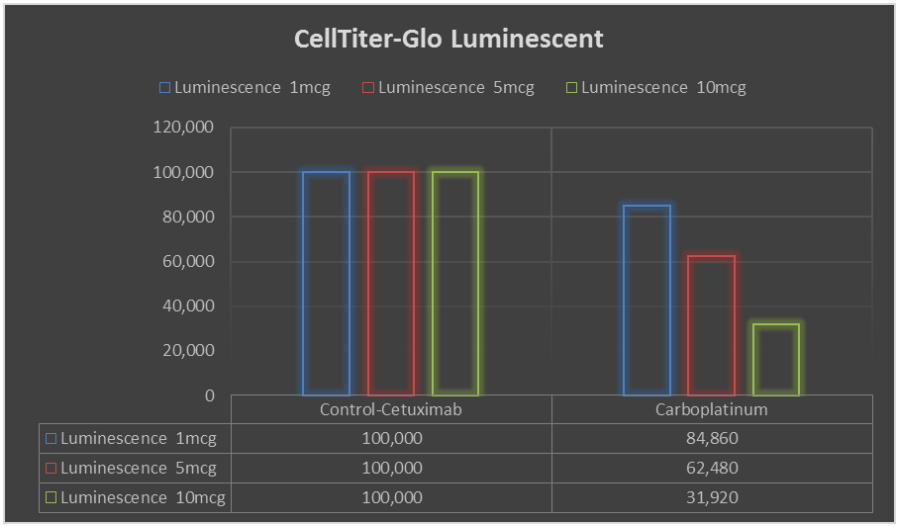
Treatment	Concentration (µM)	Absorbance (570 nm)	Cell Viability (%)
Control (Cetuximab)	-	1.000	100%
Carboplatinum	1	0.764	76.4%
	5	0.578	57.8%
	10	0.353	35.3%

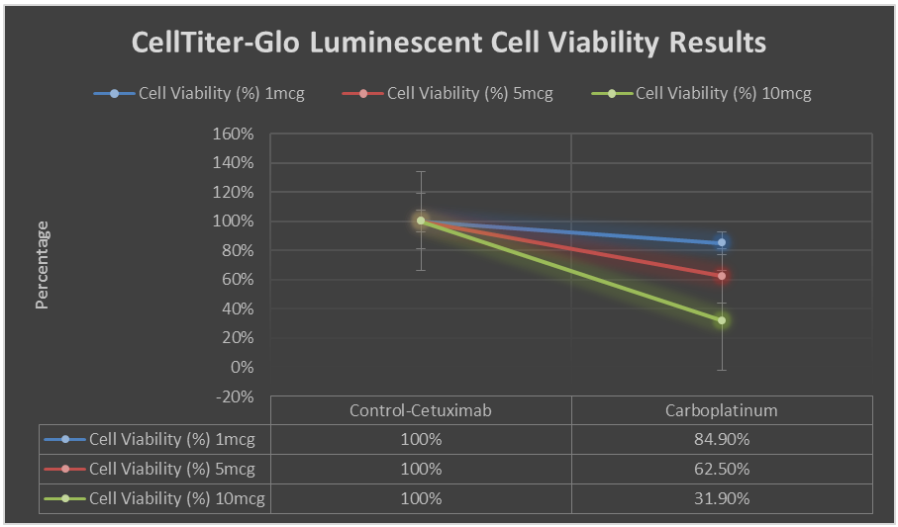




CellTiter-Glo Luminescent Cell Viability Assay Results

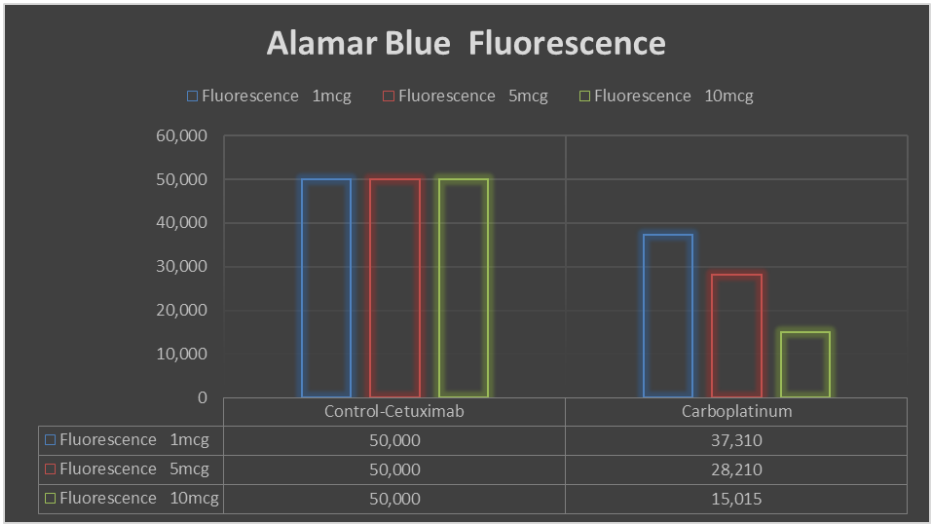
Treatment	Concentration (µM)	Luminescence (RLU)	Cell Viability (%)
Control (Cetuximab)	-	100,000	100%
Carboplatinum	1	84,860	84.9%
	5	62,480	62.5%
	10	31,920	31.9%

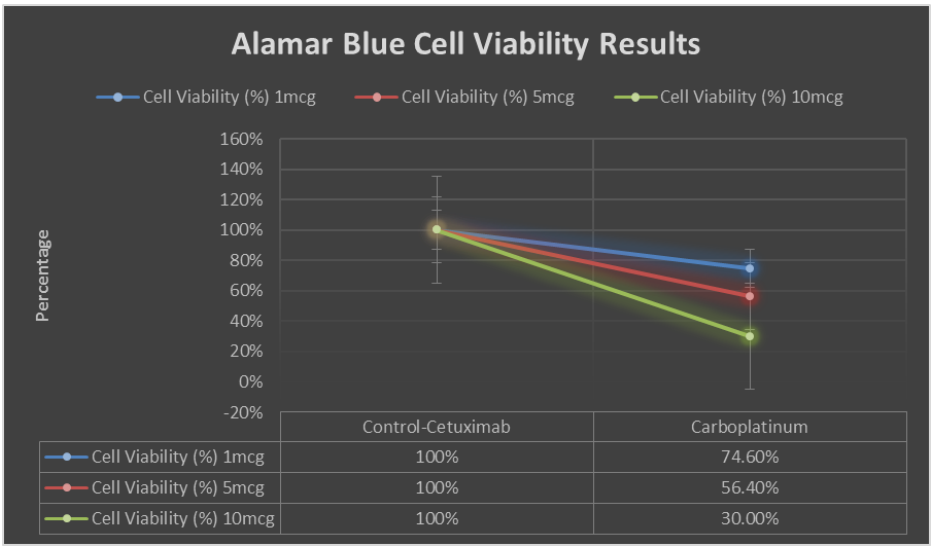




Alamar Blue Assay Results

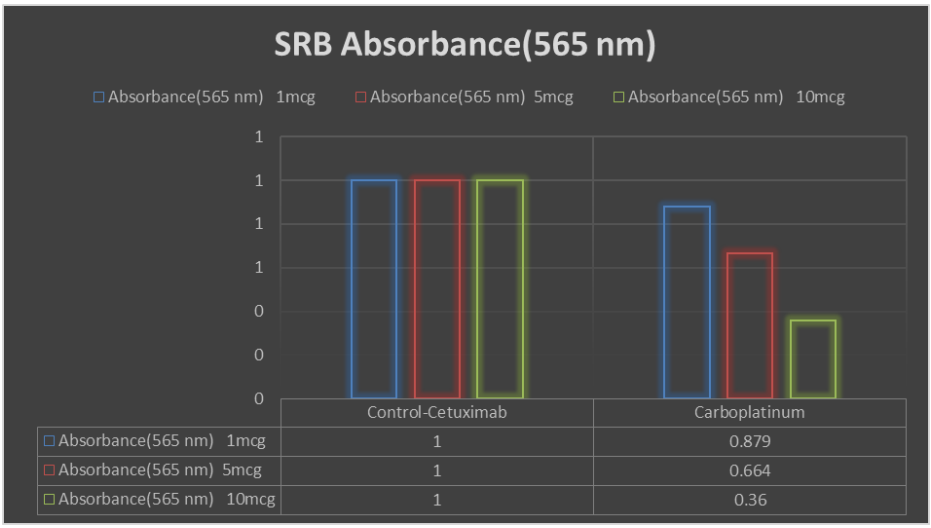
Treatment	Concentration (µM)	Absorbance (570 nm)	Fluorescence (590 nm)	Cell Viability (%)
Control (Cetuximab)	-	1.000	50,000	100%
Carboplatinum	1	0.820	37,310	74.6%
	5	0.620	28,210	56.4%
	10	0.330	15,015	30.0%

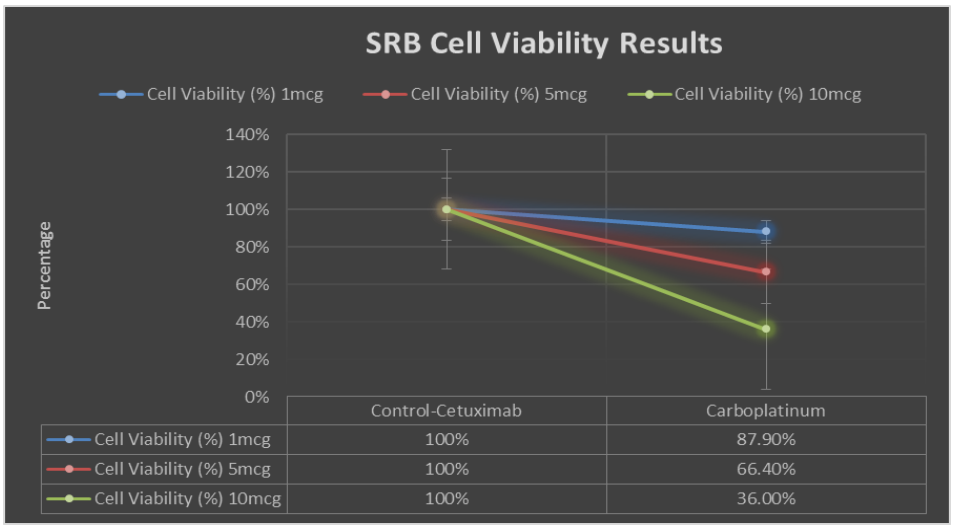




SRB Assay Results

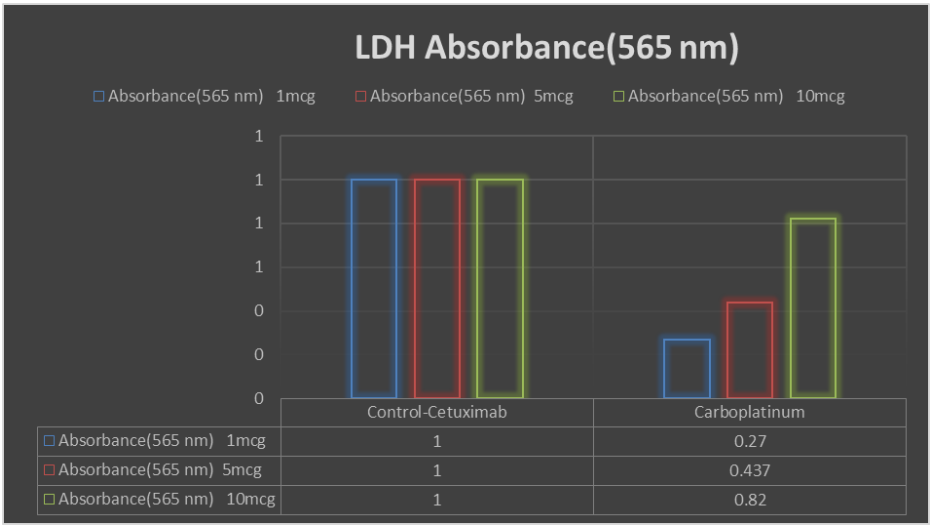
Treatment	Concentration (µM)	Absorbance(565 nm)	Cell Viability (%)
Control (Cetuximab)	-	1.000	100%
Carboplatinum	1	0.879	87.9%
	5	0.664	66.4%
	10	0.360	36.0%

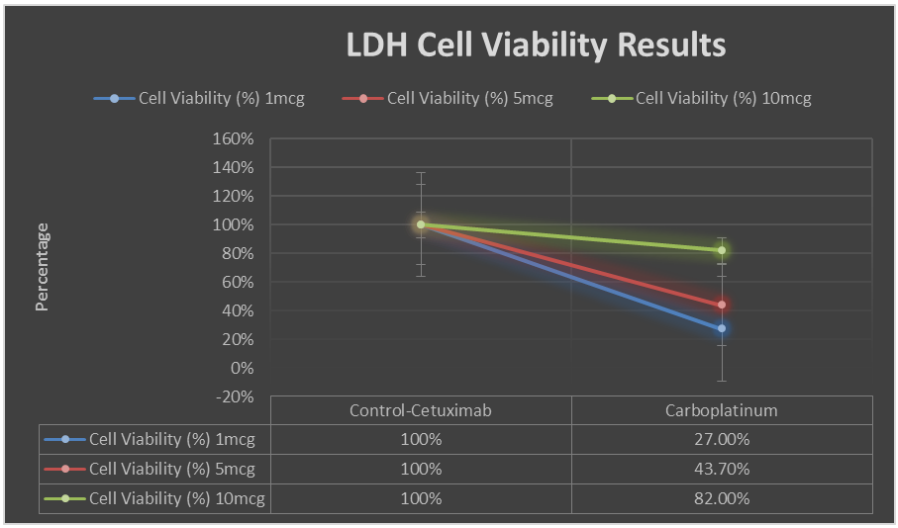




LDH Cytotoxicity Assay Results

Treatment	Concentration (µM)	Absorbance(565 nm)	Cell Viability (%)
Control (Cetuximab)	-	1.000	100%
Carboplatinum	1	0.270	27.0%
	5	0.437	43.7%
	10	0.820	82.0%





	Control-Cetuximab	Carboplatinum
Cell Viability (%) 1mcg	100%	27.00%
Cell Viability (%) 5mcg	100%	43.70%
Cell Viability (%) 10mcg	100%	82.00%

DISCUSSION

The results from this study reveal a dose-dependent decrease in cell viability across all assays used, confirming Carboplatinum's cytotoxic effects on kidney cancer cells. At a concentration of 10 µM, all assays showed significant reduction in cell viability, indicating effective inhibition of cell proliferation and induction of cytotoxicity. The SRB assay, which measures total protein content, showed a more pronounced reduction in cell viability, suggesting that Carboplatinum significantly disrupts cellular protein synthesis pathways. The LDH assay, used to measure cytotoxicity based on cell membrane integrity, showed the highest cytotoxicity at 10 µM concentration, indicating that Carboplatinum may cause severe membrane damage in kidney cancer cells. These findings suggest that Carboplatinum's anti-cancer effects are mediated through multiple pathways, including DNA damage, protein synthesis inhibition, and membrane disruption.

CONCLUSION

Carboplatinum demonstrated significant cytotoxic effects on kidney cancer cell lines, as evidenced by reduced cell viability in a dose-dependent manner. The comprehensive use of various assays highlighted its potential as a promising anti-cancer agent. The SRB and LDH assays were particularly effective in detecting its cytotoxic effects, making them valuable tools for further pharmacological research. Future studies may focus on evaluating Carboplatinum's efficacy in combination with other therapeutic agents to enhance its anti-cancer properties while minimizing potential side effects.

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