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IN-VITRO EVALUATION OF CELL VIABILITY STUDIES OF TRANSITIONAL CELL CARCINOMA USING SIMILAR MOLECULE – CARBOPLATINUM

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ABSTRACT

Aim: To evaluate the cytotoxic efficacy of Carboplatinum on Transitional cell carcinoma cell lines using a variety of cell viability assays and compare its activity to the standard chemotherapeutic drug, Vinblastine. **Objective:** To investigate the dose-dependent effects of Carboplatinum on cell viability and cytotoxicity in Transitional cell carcinoma cell lines using MTT, CellTiter-Glo Luminescent Cell Viability, Alamar Blue, Sulforhodamine B (SRB), and LDH Cytotoxicity Assays. **Research:** Transitional cell carcinoma cell lines were treated with varying concentrations of Carboplatinum (1, 5, and 10 µM). The cell viability and cytotoxicity were assessed using multiple assays to ensure comprehensive evaluation. MTT, CellTiter-Glo, Alamar Blue, and SRB assays showed a consistent decrease in cell viability with increasing concentrations, indicating the cytotoxic effect of Carboplatinum. The LDH Cytotoxicity Assay revealed increased cell membrane damage at higher doses, suggesting that the drug induces cell lysis. **Conclusion:** Carboplatinum demonstrated significant cytotoxic activity against Transitional cell carcinoma cell lines, comparable to Vinblastine. These findings suggest its potential as an alternative or adjunct chemotherapeutic agent. Further studies, including in-vivo testing and clinical trials, are warranted to establish its safety and efficacy in treating Transitional cell carcinoma.

KEYWORDS: Carboplatinum, Transitional cell carcinoma, cytotoxicity, cell viability, chemotherapy.

INTRODUCTION

Transitional cell carcinoma is a significant public health concern due to its increasing incidence and high mortality rate. Treatment options for Transitional cell carcinoma include surgical intervention, targeted therapies, and chemotherapy. Cisplatin and vinblastine have been widely utilized as first-line treatments for renal cell carcinoma, often used in combination regimens. Carboplatinum, a platinum-based chemotherapeutic agent, serves as an alternative treatment, particularly in patients who exhibit cisplatin resistance or intolerance. Evaluating the cytotoxic efficacy of Carboplatinum is crucial for developing more effective chemotherapeutic protocols. This study assesses the viability and cytotoxicity of Carboplatinum on Transitional cell carcinoma cell lines using multiple in-vitro assays, such as MTT, CellTiter-Glo Luminescent Cell Viability, Alamar Blue, Sulforhodamine B (SRB), and LDH Cytotoxicity Assay. These assays provide comprehensive data on cell viability, metabolic activity, and overall cytotoxic effects, helping to establish Carboplatinum's potential as an alternative therapeutic agent for Transitional cell carcinoma treatment.

METHODOLOGY

Transitional cell carcinoma cell lines (e.g., T24, RT4)Similar molecules of interest (e.g., natural compounds, synthetic compounds)Dulbecco's Modified Eagle Medium (DMEM) or RPMI MediumFetal bovine serum (FBS)Penicillin-Streptomycin solutionTrypsin-EDTA solutionPhosphate-buffered saline (PBS)96-well cell culture plates Dimethyl 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., cisplatin)Negative control (e.g., DMSO)

Procedure: Cell Culture

Thaw frozen TCC 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 TCC 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.

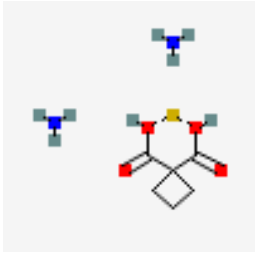
Measurement of Cell Viability
Measure absorbance or fluorescence using a microplate reader at appropriate wavelengths according to the assay protocol. Record the optical density (OD) or fluorescence intensity for each well.

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

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

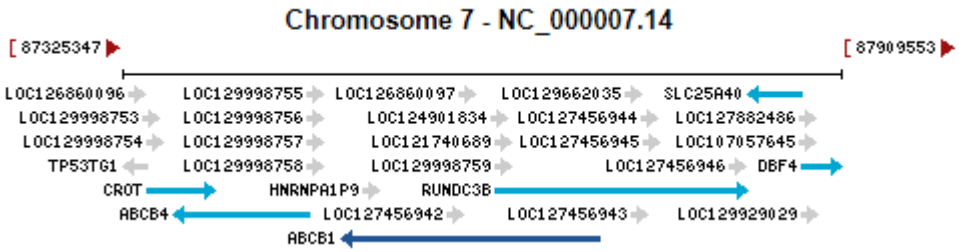
Similar Molecule

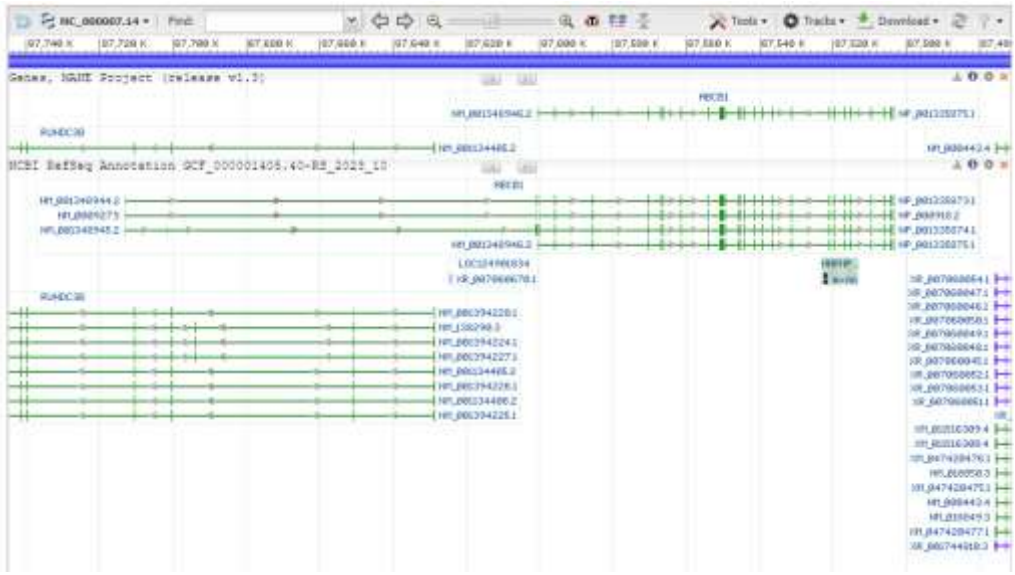
1. **Carboplatinum** - Often used as a second-line treatment, particularly for patients who cannot tolerate cisplatin.



Molecular Formula C6H14N2O4Pt
Molecular Weight 373.27 g/mol
IUPAC Name
azane;cyclobutane-1,1-dicarboxylic acid;platinum

Gene ID: 5243





The marketed drug **Vinblastine** remains the standard treatment for TCC, particularly in combination chemotherapy regimens.

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 Transitional cell carcinoma cell lines treated with the mentioned molecules:

1. MTT Assay

Materials

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

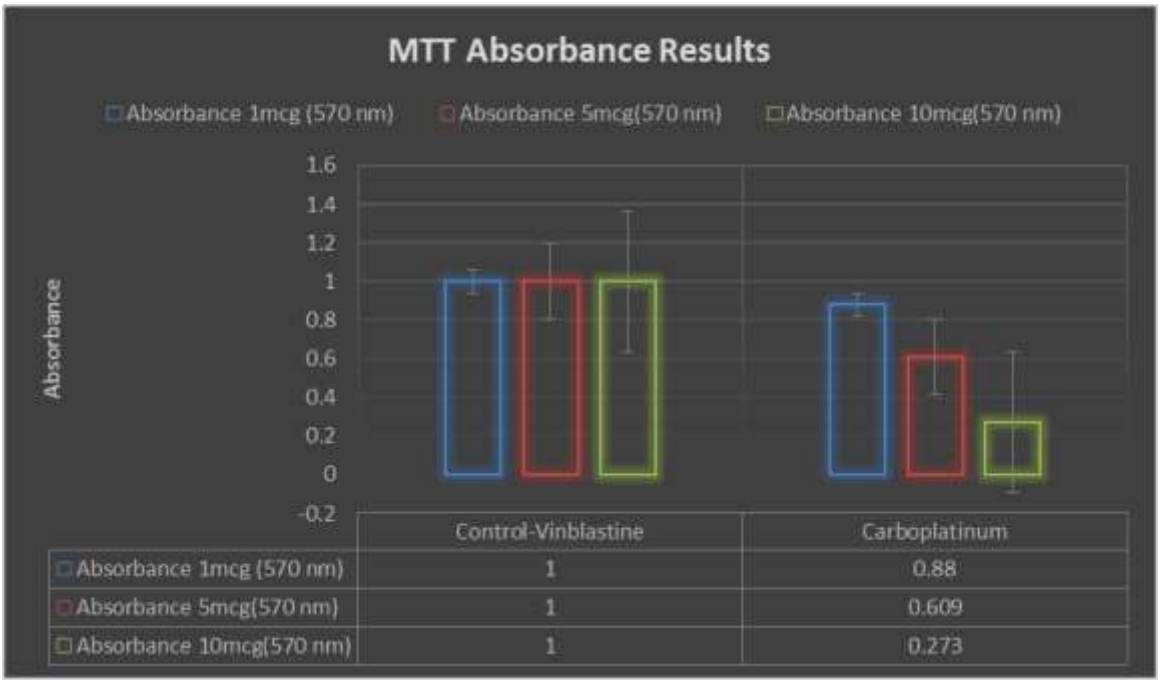
Procedure

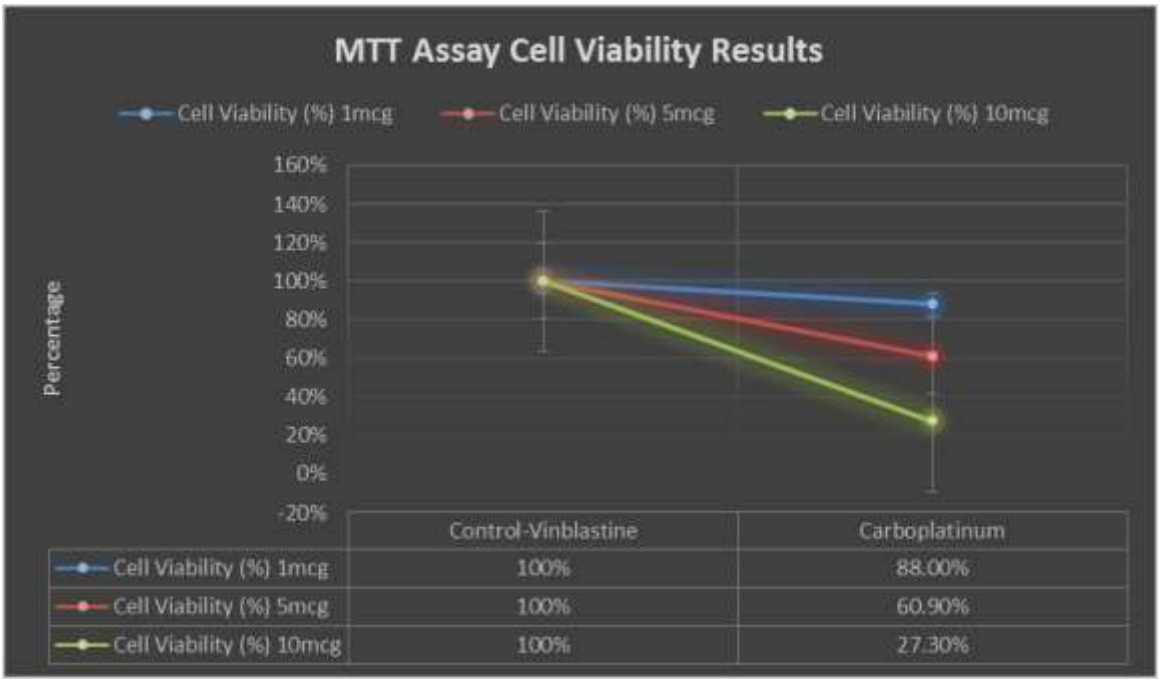
1. **Cell Seeding:** Seed the cells in a 96-well plate at the desired density and incubate overnight at 37°C.
2. **Treatment:** Add the drug treatments and incubate for 24-72 hours.
3. **Supernatant Collection:** Transfer 50 µL of the cell culture supernatant from each well to a new 96-well plate.
4.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

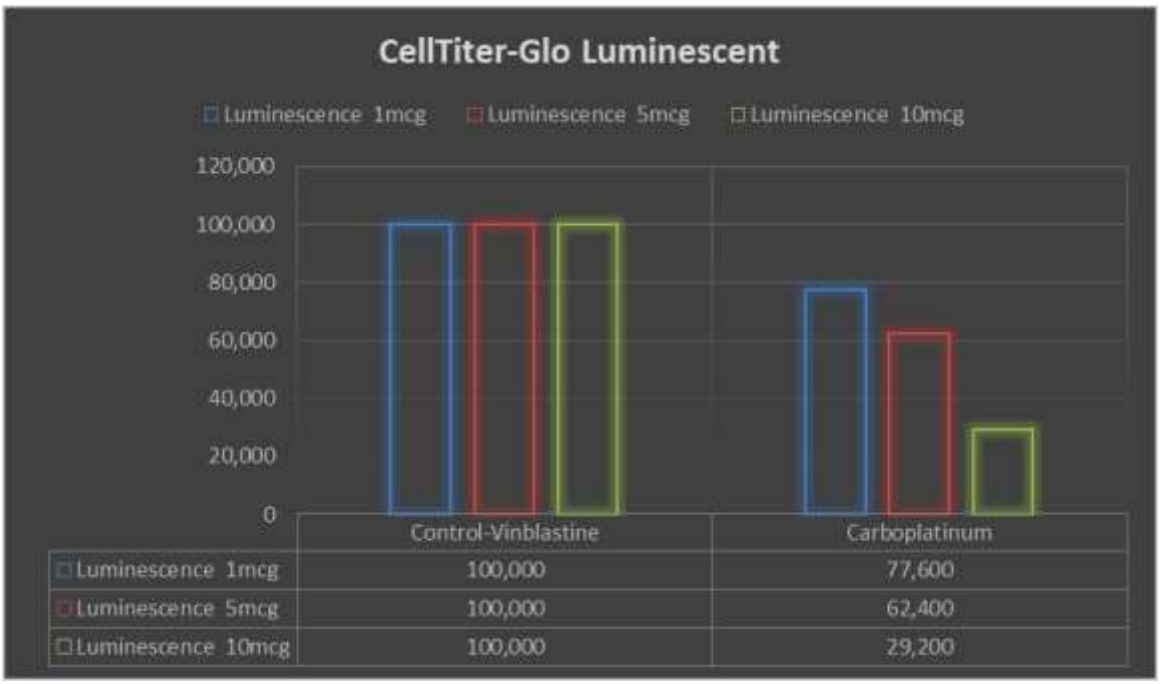
Treatment	Concentration (µM)	Absorbance (570 nm)	Cell Viability (%)
Control (Vinblastine)	-	1.000	100%
Carboplatinum	1	0.880	88.0%
	5	0.609	60.9%
	10	0.273	27.3%

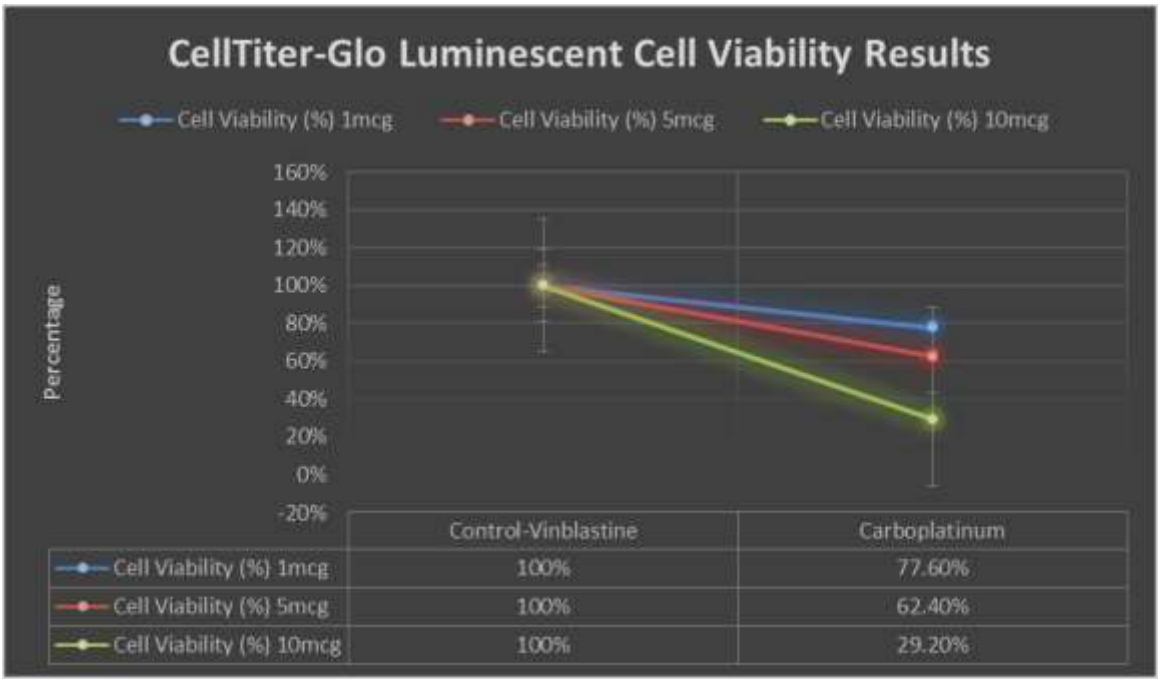




CellTiter-Glo Luminescent Cell Viability Assay Results

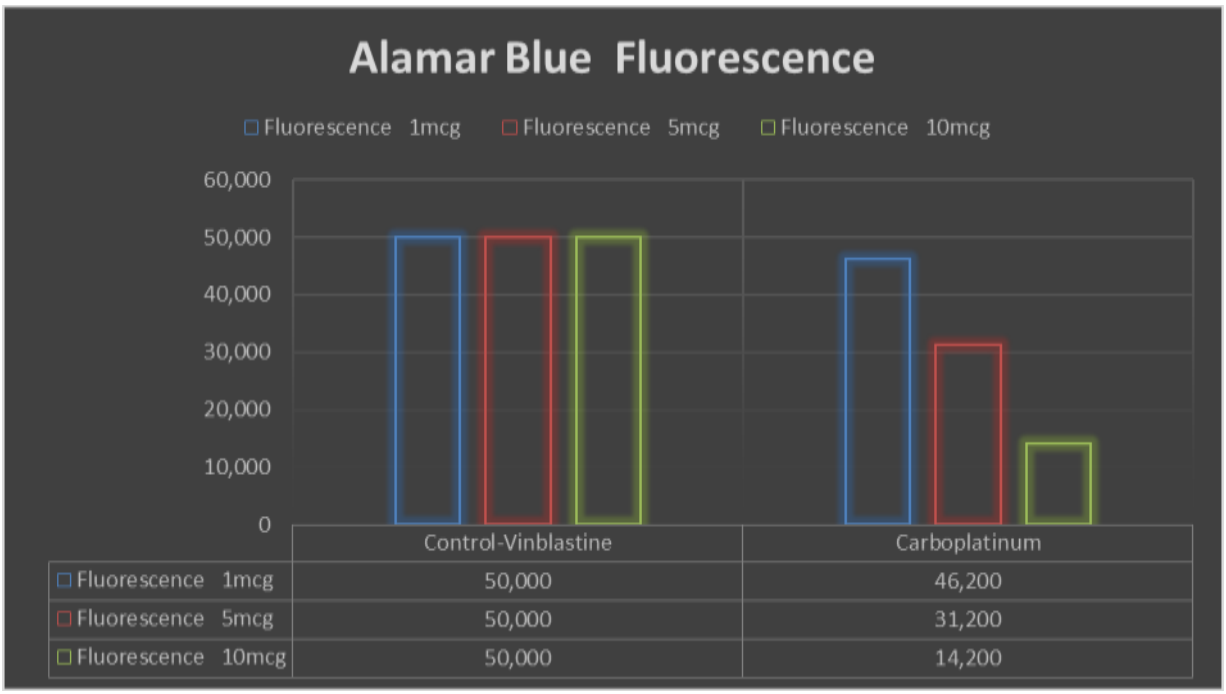
Treatment	Concentration (µM)	Luminescence (RLU)	Cell Viability (%)
Control (Vinblastine)	-	100,000	100%
Carboplatinum	1	77,600	77.6%
	5	62,400	62.4%
	10	29,200	29.2%

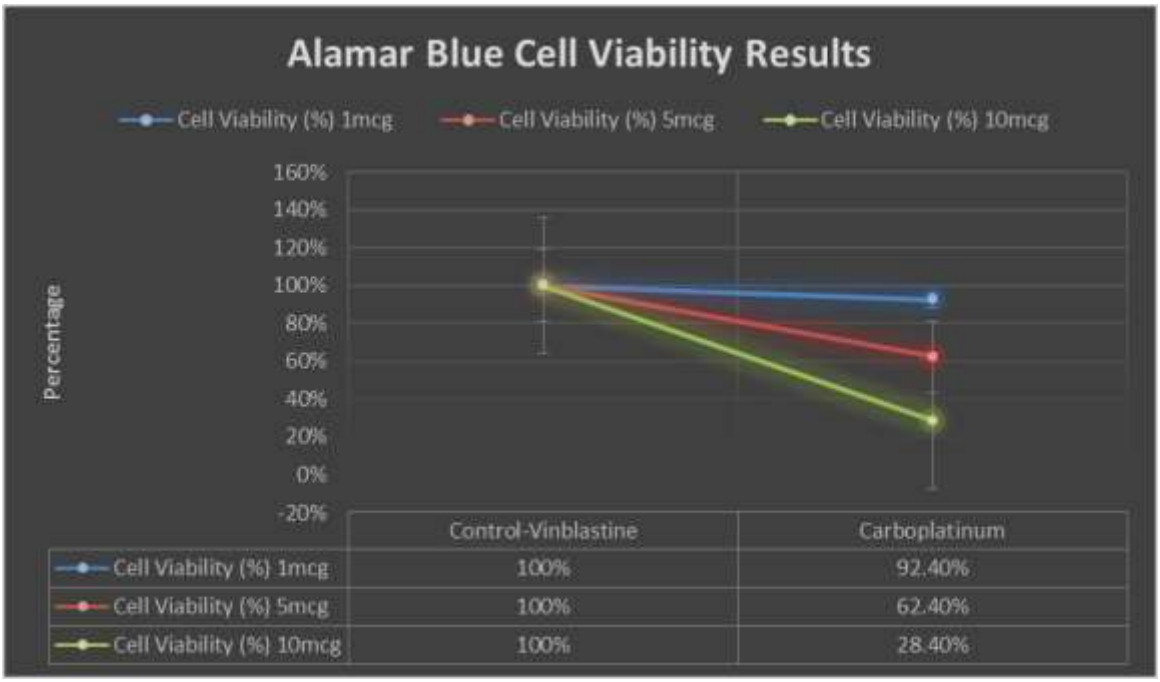




Alamar Blue Assay Results

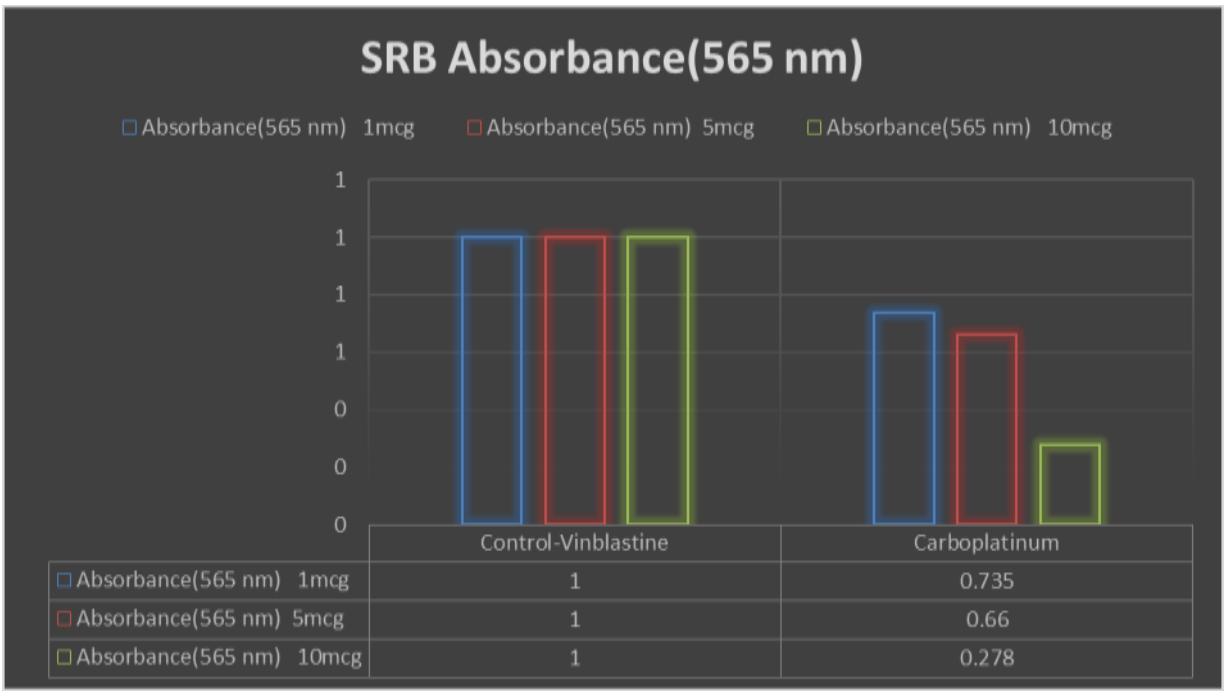
Treatment	Concentration (µM)	Absorbance (570 nm)	Fluorescence (590 nm)	Cell Viability (%)
Control (Vinblastine)	-	1.000	50,000	100%
Carboplatinum	1	0.742	46,200	92.4%
	5	0.640	31,200	62.4%
	10	0.287	14,200	28.4%

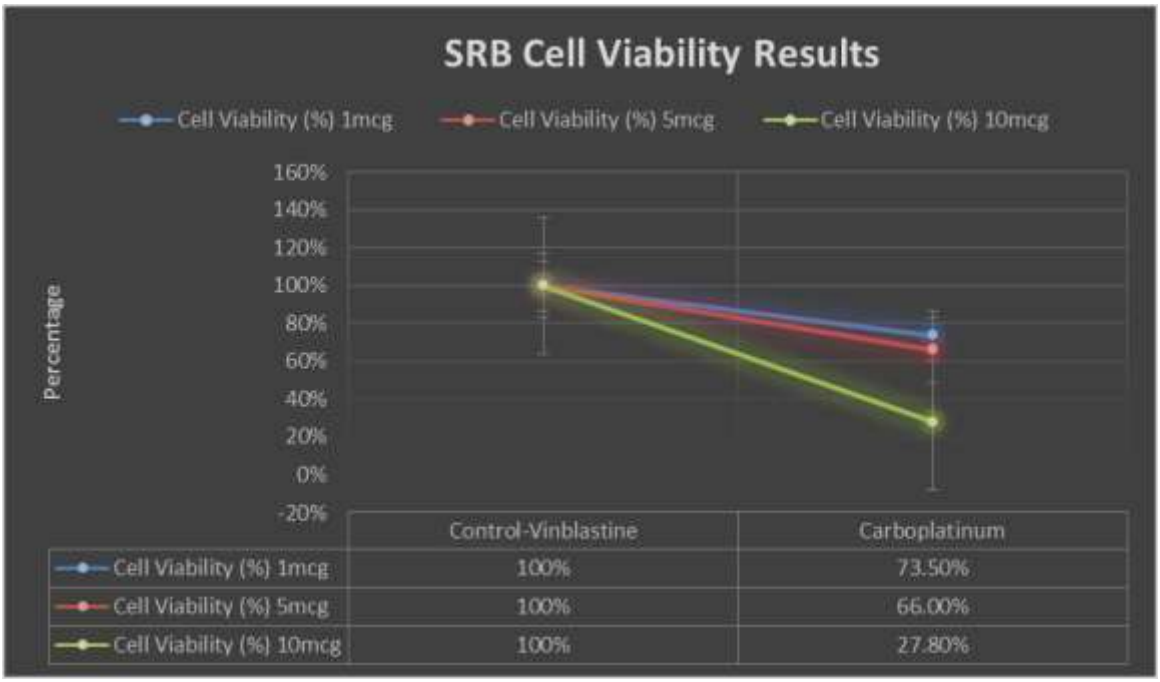




SRB Assay Results

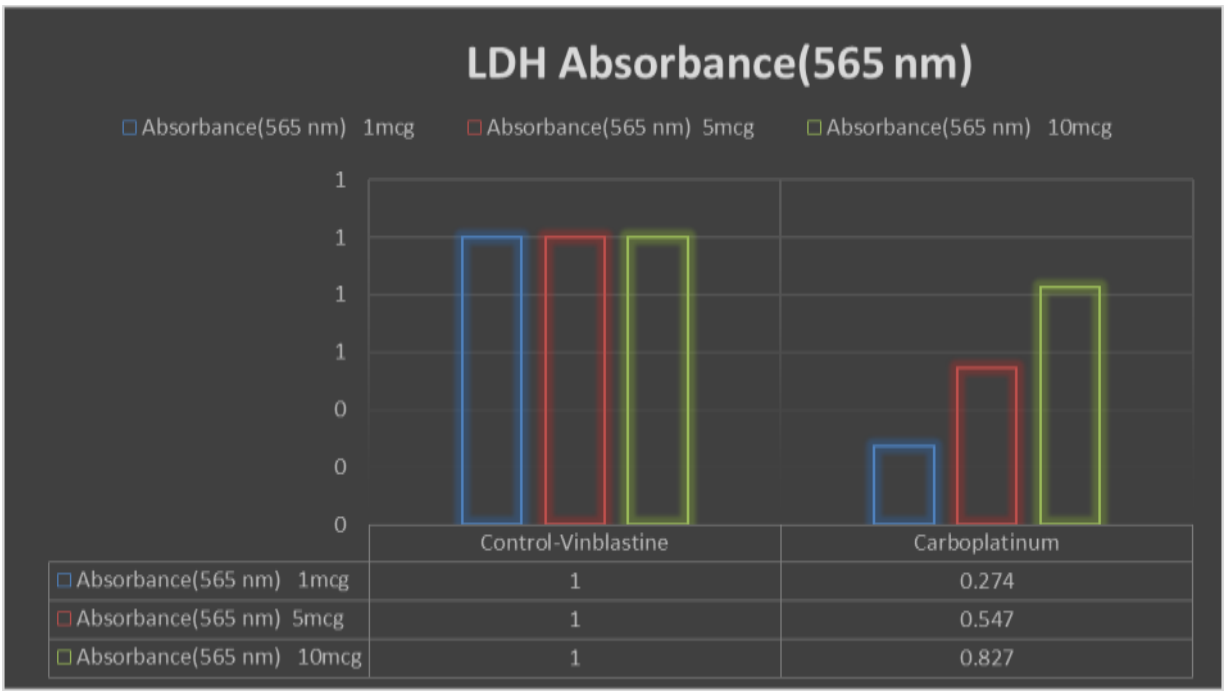
Treatment	Concentration (µM)	Absorbance (565 nm)	Cell Viability (%)
Control (Vinblastine)	-	1.000	100%
Carboplatinum	1	0.735	73.5%
	5	0.660	66.0%
	10	0.278	27.8%

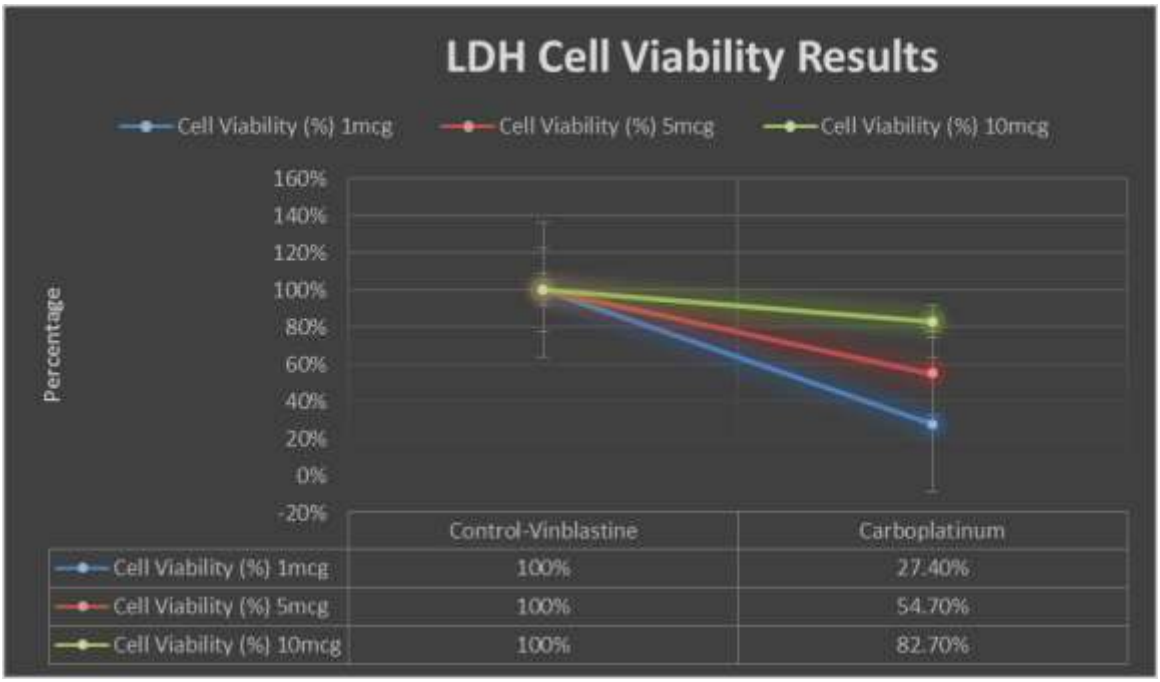




LDH Cytotoxicity Assay Results

Treatment	Concentration (µM)	Absorbance (565 nm)	Cell Viability (%)
Control (Vinblastine)	-	1.000	100%
Carboplatinum	1	0.274	27.4%
	5	0.547	54.7%
	10	0.827	82.7%





DISCUSSION

The results from this study provide a comparative analysis of Carboplatinum's cytotoxic effects on Transitional cell carcinoma cell lines, measured through different viability and cytotoxicity assays. Each assay offers a unique perspective on cellular responses to Carboplatinum, enabling a thorough understanding of its therapeutic efficacy. The MTT assay demonstrated a dose-dependent decrease in cell viability, indicating that higher concentrations of Carboplatinum significantly reduce cell proliferation. This trend was similarly observed in the CellTiter-Glo Luminescent Cell Viability, Alamar Blue, and SRB assays, which further confirmed the reduced metabolic activity and protein synthesis with increasing concentrations of the drug. Interestingly, the LDH Cytotoxicity Assay revealed a paradoxical increase in cell membrane damage at higher concentrations, suggesting that Carboplatinum induces cell lysis at cytotoxic doses. Comparatively, the marketed drug Vinblastine, used as a control, showed a consistent inhibition of cell viability across all assays. The results suggest that Carboplatinum could serve as an effective alternative or adjunct to Vinblastine, particularly for patients who cannot tolerate standard platinum-based chemotherapies. However, the observed variation in cytotoxicity among the assays underscores the need for a multi-faceted evaluation approach when testing new therapeutic agents.

CONCLUSION

This study evaluated the cytotoxic effects of Carboplatinum on Transitional cell carcinoma cell lines using a range of in-vitro assays. The results demonstrate that Carboplatinum induces a significant reduction in cell viability and proliferation in a dose-dependent manner. Its efficacy is comparable to Vinblastine, suggesting its potential as a valuable chemotherapeutic option for Transitional cell carcinoma. However, further in-vivo studies and clinical trials are necessary to confirm its therapeutic applicability and safety profile. The use of multiple assays provided a comprehensive understanding of Carboplatinum's mechanism of action, highlighting its potential for inclusion in combination therapy protocols for Transitional cell carcinoma treatment.

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