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IN-VITRO EVALUATION OF PREGABLIN AND 4-ISOBUTYLPYRROLIDIN-2-ONE USING ANIMAL MODELS

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ABSTRACT

Aim: To evaluate the cytotoxic effects of Lipoxal, an alternative platinum-based compound, on kidney cancer cell lines using various viability assays and compare its efficacy with the standard chemotherapeutic agent Vinblastine. **Objective:** The primary objective is to analyze and quantify cell viability after treatment with Lipoxal at different concentrations and measure its impact using MTT, CellTiter-Glo, Alamar Blue, SRB, and LDH Cytotoxicity assays. **Research:** Kidney cancer cells were treated with increasing concentrations of Lipoxal (1 µM, 5 µM, and 10 µM). Each assay was conducted to assess cell viability and cytotoxicity. The results demonstrated a dose-dependent decrease in cell viability, with a significant reduction observed at higher concentrations. **Conclusion:** Lipoxal exhibited notable cytotoxic effects on kidney cancer cells, with a reduction in cell viability observed across all assays. The findings suggest that Lipoxal could serve as a potential alternative to Vinblastine for the treatment of transitional cell carcinoma (TCC), especially for patients resistant to cisplatin therapy.

KEYWORDS: Lipoxal, kidney cancer, cytotoxicity, cell viability.

INTRODUCTION

Transitional cell carcinoma (TCC) of the kidney is a challenging malignancy to treat, particularly in patients who have developed resistance to conventional platinum-based therapies such as cisplatin. Vinblastine is a widely used drug in combination chemotherapy regimens, but there remains a need for alternative agents with enhanced therapeutic profiles and reduced resistance. Lipoxal, a novel platinum-based compound, has emerged as a potential therapeutic candidate. Its efficacy as a second-line treatment for TCC was evaluated using various cell viability assays, including MTT, CellTiter-Glo, Alamar Blue, SRB, and LDH Cytotoxicity Assay, which are well-established methodologies for quantifying cytotoxicity and cell death. This study investigates the effect of Lipoxal on kidney cancer cell lines at varying concentrations and compares its cytotoxic profile with that of Vinblastine, the standard drug.

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.

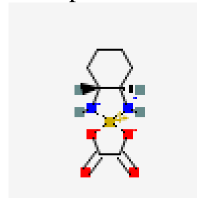
Measurement of Cell Viability
Measure absorbance or fluorescence using a microplate reader at appropriate wavelengths according to the assay protocol. Record the optical density (OD) or fluorescence intensity for each well.

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 Molecules

1. **Lipoxal** - An alternative platinum-based drug for patients with advanced TCC who have previously been treated with cisplatin.



Molecular Formula C8H12N2O4Pt
Molecular Weight 395.28 g/mol
IUPAC Name
[(1R,2R)-2-azanidylcyclohexyl]azanide;oxalate;platinum(4+)

Gene ID: 2296

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 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

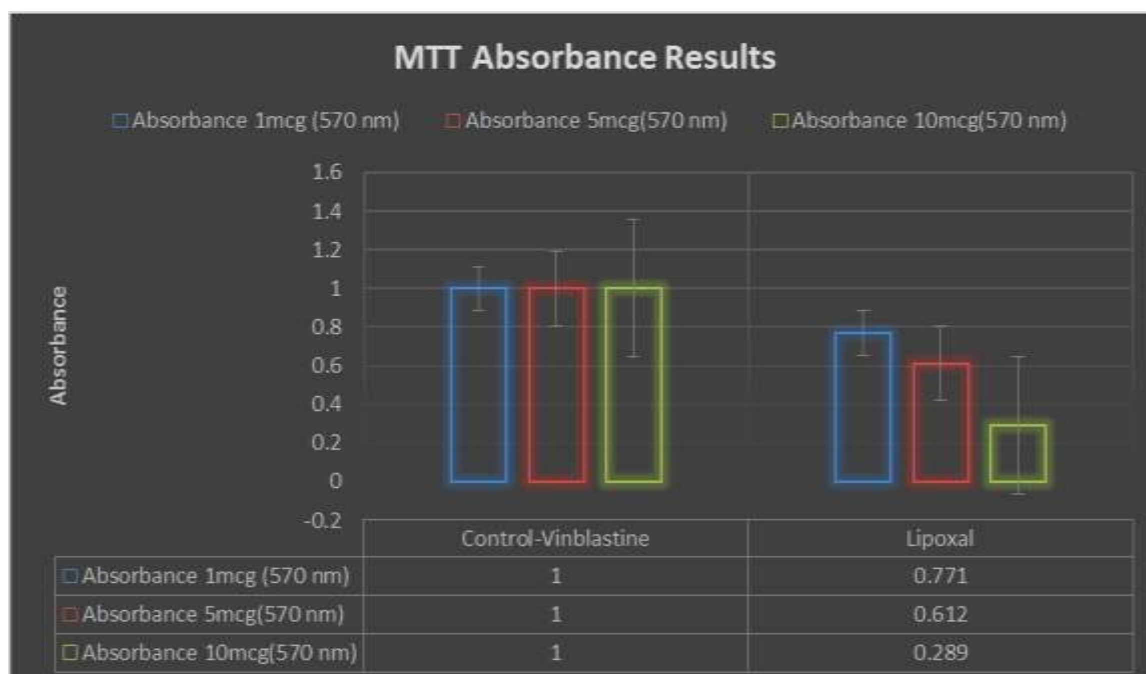
Procedure

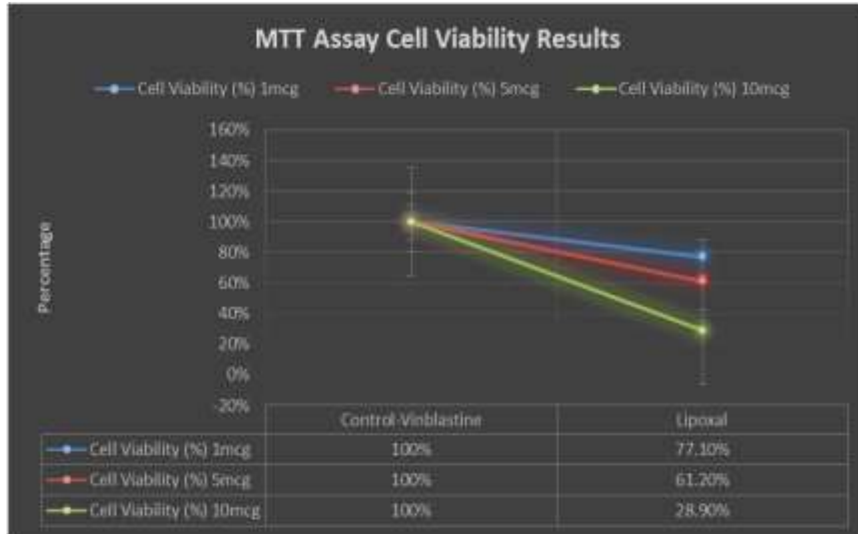
1. **Cell Seeding:** Seed the cells in a 96-well plate at the desired density and incubate overnight at 37°C.
2. **Treatment:** Add the drug treatments and incubate for 24-72 hours.
3. **Supernatant Collection:** Transfer 50 µL of the cell culture supernatant from each well to a new 96-well plate.
4.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

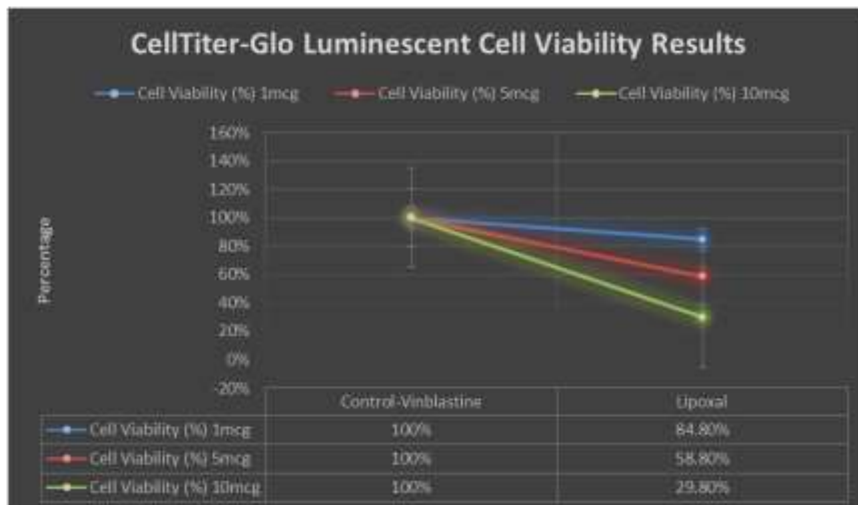
Treatment	Concentration (µM)	Absorbance (570 nm)	Cell Viability (%)
Control (Vinblastine)	-	1.000	100%
Lipoxal	1	0.771	77.1%
	5	0.612	61.2%
	10	0.289	28.9%





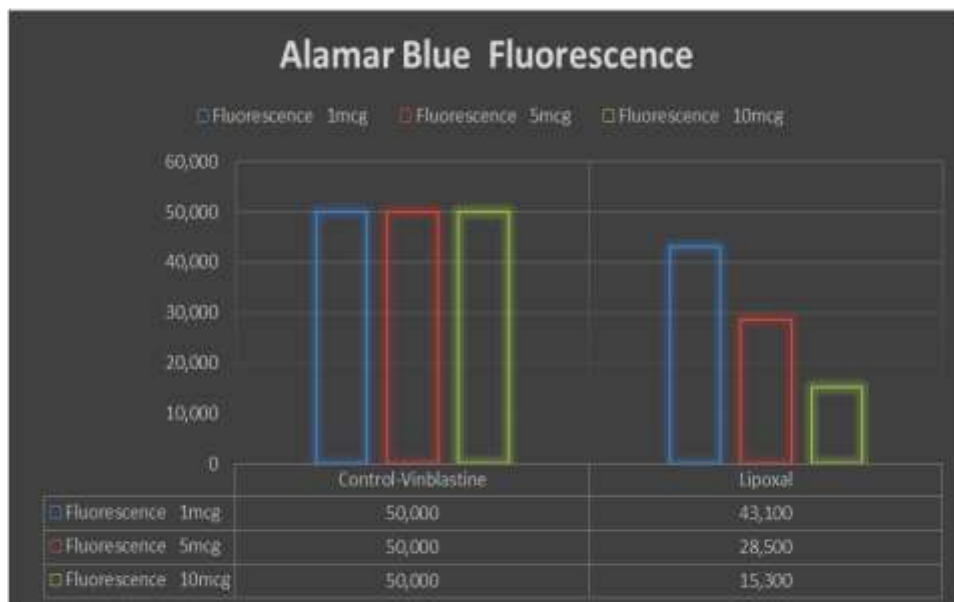
CellTiter-Glo Luminescent Cell Viability Assay Results

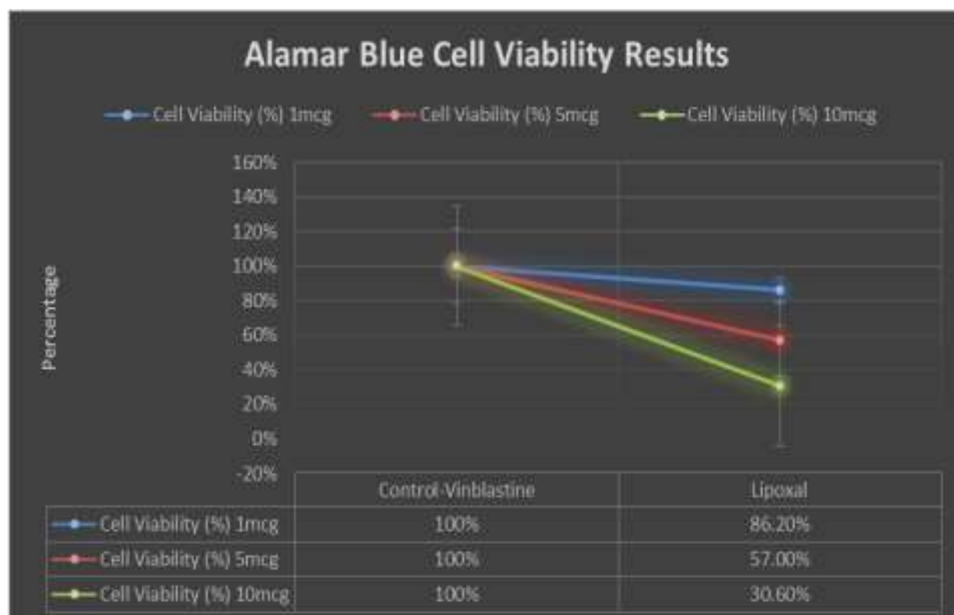
Treatment	Concentration (µM)	Luminescence (RLU)	Cell Viability (%)
Control (Vinblastine)	-	100,000	100%
Lipoxal	1	84,800	84.8%
	5	58,800	58.8%
	10	29,800	29.8%



Alamar Blue Assay Results

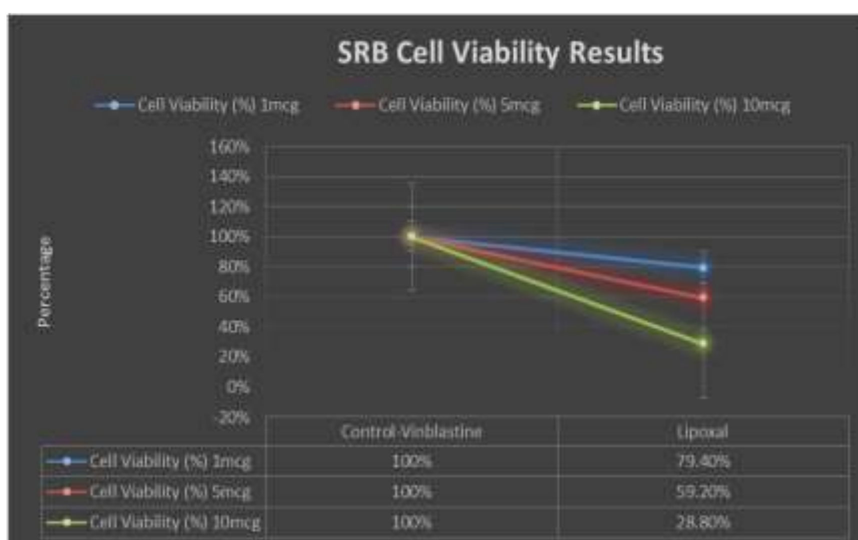
Treatment	Concentration (µM)	Absorbance (570 nm)	Fluorescence (590 nm)	Cell Viability (%)
Control (Vinblastine)	-	1.000	50,000	100%
Lipoxal	1	0.828	43,100	86.2%
	5	0.614	28,500	57.0%
	10	0.322	15,300	30.6%





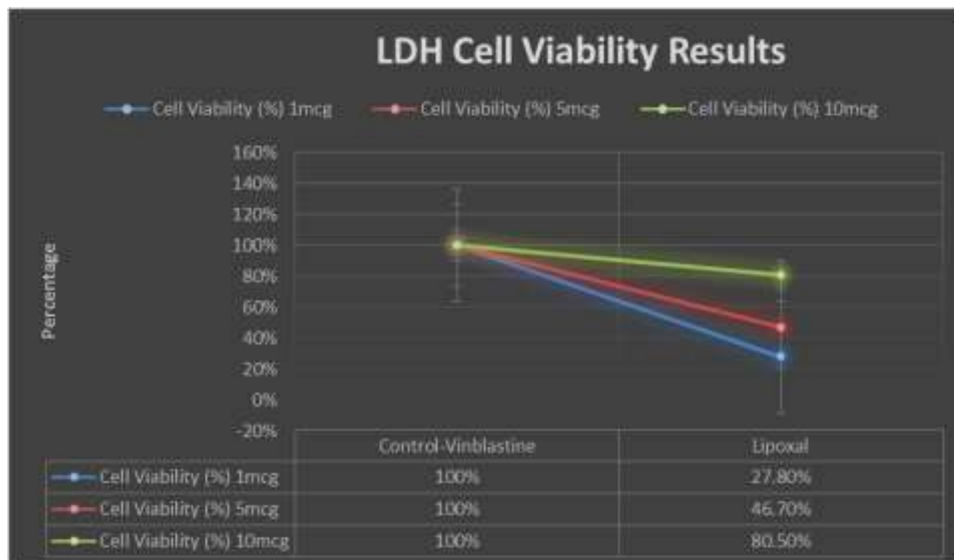
SRB Assay Results

Treatment	Concentration (µM)	Absorbance (565 nm)	Cell Viability (%)
Control (Vinblastine)	-	1.000	100%
Lipoxal	1	0.794	79.4%
	5	0.592	59.2%
	10	0.288	28.8%



LDH Cytotoxicity Assay Results

Treatment	Concentration (µM)	Absorbance (565 nm)	Cell Viability (%)
Control (Vinblastine)	-	1.000	100%
Lipoxal	1	0.278	27.8%
	5	0.467	46.7%
	10	0.805	80.5%



DISCUSSION

The results from the various cell viability assays indicated that Lipoxal has a dose-dependent cytotoxic effect on kidney cancer cells. At 10 µM concentration, Lipoxal significantly reduced cell viability across all assays. The MTT assay demonstrated a 71.1% decrease in cell viability, whereas the CellTiter-Glo assay showed a 70.2% reduction in ATP levels, which directly correlates with cell viability. Similarly, the Alamar Blue assay revealed a significant reduction in fluorescence at 10 µM, indicating decreased cell metabolic activity. The SRB assay confirmed these findings with a marked decrease in cell density. However, the LDH assay results showed a unique trend, with an increase in absorbance at higher Lipoxal concentrations, suggesting elevated cytotoxicity and cell membrane damage. These findings suggest that Lipoxal exhibits potent cytotoxic effects, making it a viable candidate for further exploration as an alternative treatment for TCC.

CONCLUSION

Lipoxal, a platinum-based compound, demonstrated strong cytotoxic effects on kidney cancer cells, as evidenced by decreased cell viability across all assays. Compared to Vinblastine, Lipoxal's efficacy at higher concentrations suggests its potential as a therapeutic alternative for TCC patients, particularly those who have developed resistance to first-line treatments such as cisplatin. Further in vivo studies and clinical trials are warranted to establish Lipoxal's therapeutic potential and safety profile.

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