Original Article

In Vitro Study of Antitumor Activity of Alogliptin in Lung Cancer Cell Line (A549)

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ABSTRACT

Background: Lung cancer is still the leading cause of cancer-related deaths globally. However, many conventional treatments are associated with high toxicity and limited selectivity for cancer cells. This condition highlights the urgent need for novel therapeutic agents with enhanced anticancer potential and reduced adverse effects on healthy tissues. Dipeptidyl peptidase-4 (DPP4) inhibitors have emerged as promising candidates for various malignancies, including colorectal, prostate, and renal cancers, due to their remarkable anticancer properties.

Objectives: This study evaluated the cytotoxic and anticancer activity of alogliptin (Alo), a selective DPP4 inhibitor, against the human lung cancer A549 cell line and normal breast epithelial HBL100 cells, as monotherapy and in combination with cisplatin (Cis).

Methods: Each type of cell (A549 cell and normal HBL100 cell) was divided into four groups: untreated control, Cis-treated, Alo-treated, and Cis+Alo-treated. After 72 hours of incubation, cell viability was assessed using the MTT (3-[4,5-dimethylthiazol-2-yl] -2,5-diphenyl tetrazolium bromide) assay to determine the half-maximal inhibitory concentration (IC_{50}). This method is safe, more reproducible, and uses test cell viability and cytotoxicity endpoints. Following IC_{50}-based treatment, apoptotic markers, including BCL2 and survivin expression levels, were evaluated.

Results: MTT assay showed that both Cis and Alo significantly decreased A549 cell viability ($P<0.0001$). Alo therapy clearly increased inhibition of A549 cells compared with the control, but showed lower cytotoxicity toward normal HBL100 cells (cytotoxic only at higher concentrations), yielding results comparable to those of Cis. The combination of Cis and Alo demonstrated a dose-dependent cytotoxic effect on A549 lung adenocarcinoma cells, with significant anticancer activity observed at higher concentrations. However, the combination of Alo and Cis did not significantly increase cytotoxicity against A549 cells compared with Cis alone. Furthermore, exposing A549 cells to Alo alone significantly decreased BCL2 levels compared with Cis alone ($P<0.001$). The results further showed that after treating A549 cells with an IC_{50} of Alo, there was a significant decrease in survivin levels ($P<0.0001$) compared with control cells.

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Conclusion: Alo, a DPP-4 inhibitor, demonstrated anticancer effects against A549 cells as evidenced by the MTT assay and by changes in BCL2 and survivin expression, indicating its potential anticancer activity against this cancer cell line. Although Alo exhibited dramatic pro-apoptotic activity, as indicated by reduced BCL2 and survivin levels, when combined with Cis, no clear synergism was observed.

Keywords: A549 cell line, Alogliptin (Alo), Cisplatin (Cis), DPP4 inhibitor, Lung cancer

Introduction

The excessive growth of epithelial cells in the pulmonary system, which affects respiratory function, is the initiating cause of lung cancer, which continues to be one of the most prevalent and fatal forms of cancer in the world (Al-Tariahi et al., 2015). Its aggressive clinical behavior often results in metastatic dissemination to regional lymph nodes, pleural structures, and distant organs, including the brain, bones, liver, and adrenal glands (Riihimäki et al., 2014). Consequently, the prognosis is generally poor, with median survival times of 13 months for localized disease and approximately 5 months for metastatic disease (Riihimäki et al., 2014).

According to recent data from the Global Cancer Observatory (GCO), lung cancer accounted for over 2.2 million new diagnoses worldwide in 2020 (Merabishvili et al., 2018). It remains the leading cause of cancer-related deaths among men and is the second most lethal cancer in women, following breast cancer (Benitez Majano et al., 2022). Histologically, lung cancer is broadly categorized into small cell lung cancer, which accounts for about 15% of cases, and non-small cell lung cancer (NSCLC), comprising the remaining 85% (Thandra et al., 2021).

Treatment strategies typically involve surgery, radiotherapy, immunotherapy, and chemotherapy—most notably with cisplatin (Cis), a platinum-based chemotherapeutic agent administered intravenously (Schabath & Cote, 2019; Reck & Rabe, 2017; Brown et al., 2019; Tchounwou et al., 2021; Dasari & Tchounwou, 2014). Although Cis is widely employed and clinically effective, its use is frequently limited due to systemic toxicity, tumor cell resistance, and adverse side effects. This outcome has prompted interest in adjunct therapies that can enhance efficacy and reduce toxicity profiles (Aldossary, 2019).

Alogliptin (Alo), a selective dipeptidyl peptidase-4 (DPP4) inhibitor, primarily used in the management of type 2 diabetes, has shown apoptotic activity in several cancer models (Almagthali et al., 2019a; Wilson et al., 2021a; Gilbert & Pratley, 2020). DPP4 inhibitors enhance the activity of incretin hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), improving glycemic control and potentially modulating pathways involved in tumor progression (Holst et al., 2021; Raz et al., 2006). The DPP4 enzyme, CD26, plays a context-dependent role in cancer: Overexpression is associated with tumorigenesis in renal, colon, and lung cancers, while underexpression has been linked to tumor suppression in breast and endometrial cancers (Pro & Dang, 2004; Havre et al., 2008). This duality underscores its potential as a therapeutic target in oncology (Femia et al., 2013; Lam et al., 2014; Javidroozi et al., 2012; Boccardi et al., 2015).

Although prior research has documented the general anticancer effects of DPP4 inhibitors, comprehensive studies evaluating Alo's specific cytotoxic and apoptotic effects against lung cancer cell lines, particularly A549, are lacking. Moreover, the mechanistic pathways through which Alo influences lung cancer cell viability and oxidative stress remain insufficiently elucidated (Amritha et al., 2015a; Tseng, 2017a; Tseng, 2017b; Kabel et al., 2018; Bishnoi et al., 2019; Salama et al., 2022a).

Bioactive compounds and immunomodulation have been shown to confer anticancer properties across a wide range of organisms, including plants (Pacific yew, Madagascar periwinkle), fungi (maitake), and pathogens (hydatid cyst protoscolex, *Trichinella spiralis*, and *Trypanosoma cruzi*) (Ameli et al., 2025).

Therefore, this study aims to assess the anticancer and apoptotic effects of Alo alone and in combination with Cis against the A549 lung cancer cell line. The findings may contribute to the development of improved therapeutic regimens and clarify the role of DPP4 inhibition in lung cancer management.

Materials and Methods

Chemicals and cell line

At passage 20, the Iraq Biotech Cell Bank Unit in Basrah City, Iraq, provided the A549 lung cancer cell line for acquisition. The human alveolar cancer that was identified in January 1972 in a Caucasian male patient who was 58 years old was the source of the A549 line's initial origin. In vitro cultivation of the cells has been ongoing for more than 3 years, and they have undergone more than 1000 generations. These cells are widely acknowledged as a typical model of lung cancer.

HBL100 cell line

The HBL100 cell line is a human breast epithelial cell line used as a normal cell control in cancer research. Originally derived from normal breast tissue, HBL100 cells provide a crucial comparison model to assess the selectivity of anticancer treatments between malignant and healthy cells.

Dimethyl sulfoxide (DMSO, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-2H-tetrazolium bromide) dye powder, and RIPA lysis buffer were all purchased from Sigma in the United States of America for the investigation. Gibco (USA) was the supplier of phosphate-buffered saline (PBS), fetal bovine serum (10% FBS), and RPMI-1640 medium containing fetal bovine serum. Trypan blue stain and trypsin-EDTA were acquired from Flow Laboratories in the United Kingdom and Capricorn in the United States of America, respectively. The antibiotics benzylpenicillin and streptomycin were supplied by Troge, a German company. Both Cis and Alo were obtained from Takeda Pharmaceutical Company (Japan). The Bioassay Technology Laboratory in China supplied a human-specific ELISA kit, which was used to determine the amounts of BCL2 in the sample. Enzyme-linked immunosorbent assay (ELISA) kits for human survivin were ordered from SUNLOG Biotech Co., Hangzhou, China.

Cell culture and MTT assay

The study was conducted in the Cell Culture Laboratory at the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Kufa, and lasted about 2 months. Adenocarcinoma cells were used in the MTT assay. A549 and normal HBL100 cells were seeded at 1×10^4 with a final volume of 200 µL. Both cells were cultured using trypsin-EDTA for enzymatic detachment, rinsed with PBS, and neutralized with FBS. The cells were then plated in 96-well culture plates using RPMI-1640 medium supplemented with 100 U/mL penicillin and 100 µg/mL streptomycin. Cultures were incubated at 37 ˚C in a humidified atmosphere containing 5% CO_2 for 24 hours to reach approximately 80% confluence, forming a monolayer. Viable cell counts were determined using trypan blue exclusion.

Following incubation, the medium was replaced with 200 µL of fresh medium containing the test drugs or left untreated for the control. Four experimental groups were established: Control (no treatment), Cis-treated (0.9, 1.87, 3.75, 7.5, and 15 µg/mL), Alo-treated (62.5, 125, 250, 500, and 1000 µg/mL), and combination-treated cells (CP + Alo). Each group was treated with five concentrations (except combination-treated cells, which were treated with Alo plus Cis (62.5 Alo + 0.9 Cis, 125 Alo +1.87 Cis, 250 Alo + 3.75 Cis, 250 Alo + 7.5 Cis, and 250 Alo + 15 Cis) µg/mL), with each concentration replicated four times. Blank wells containing only medium were included to assess background absorbance.

After 72 hours, cell viability and apoptotic effect were assessed using the MTT assay, and BCL2 and survivin expression were assessed. Optical density was measured to assess the formation of formazan crystals, a marker of metabolically active cells. Dose-response curves were generated using non-linear regression with a four-parameter logistic Hill equation to determine the half-maximal inhibitory concentration (IC_{50}) for each group. The formula used for calculating cell viability was as follows (Equation 1):

$$1.\ \text{Cell viability}\% = \frac{\text{As-Ab)}}{\text{Ac-Ab}} \times 100\%$$

As denotes the absorbance of the sample, the absorbance of the blank is denoted by Ab, and Ac denotes the absorbance of the control. Each determination was carried out using four separate samples.

The combination indices (CIs) of Cis plus another drug tested in tumor and normal cancer cell lines were estimated using the Equations 2 and 3, and the third-generation computer software Compu-Syn (Chou, 2010).

$$2.\ CI = d1 + d2$$

$$3.\ D1 \rightarrow D2$$

, where d1 and d2 are the IC_{50} of drugs (1 and 2) in combination, D1 and D2 are the IC_{50} of the same drugs alone.

CI<1 indicates synergism, CI=1 denotes an additive effect, and CI>1 shows antagonism in the drug combination (Chou, 2010). The combination of Cis and Alo at a fixed ratio of 1:16.66 yielded antagonistic interaction across the tested concentrations, as evidenced by an elevated combination index range from 8.3 to over 20

Measurement of BCL2 and survivin concentration

A549 cells were cultured in 6 flasks and exposed to the IC_{50} of Cis, the IC_{50} of Alo, and the IC_{50} of Cis plus Alo for 36 hours. After treatment, the cells were harvested and centrifuged, and the supernatant was removed. The cell pellets were lysed with lysis buffer to extract proteins, which were stored in a 1.5 mL sterile Eppendorf tube and frozen at -20 ˚C until analyzed using BCL2 and survivin ELISA kits.

Statistical analysis

All experimental data were analyzed using GraphPad Prism software, version 10 and Microsoft Excel 2019. Statistical comparisons were made using one-way and 2-way ANOVA followed by the Tukey post hoc test. Results with a $P \leq 0.05$ were considered statistically significant.

Results

Cytotoxic effects on cell viability

The MTT assay results demonstrated significant dose-dependent cytotoxicity of both Cis and Alo in A549 lung cancer cells and HBL100 normal epithelial cells. All experiments were conducted with n=4 replicates per concentration, and statistical analysis was performed using one-way ANOVA followed by Tukey post hoc test.

Cis effects

Cis demonstrated potent cytotoxicity against both cell lines. The analysis demonstrates concentration-dependent cytotoxicity of Cis in A549 lung cancer cells. Six concentrations of Cis were used (0, 0.9, 1.87, 3.75, 7.5, and 15 µg/mL). One-way ANOVA revealed a significant main effect of Cis concentration on A549 cell viability ($F_{5, 18}$=287.4, P<0.0001, η^2=0.988). As Cis concentrations increased (from 0.9 to 15 µg/mL), cell inhibition increased progressively, with all concentrations showing statistically significant cytotoxic effects compared to control (Tukey post hoc: P<0.0001 for all pairwise comparisons; 95% CI, -89.2%, -45.7% reduction in viability). The effect size was large (Cohen's d > 2.0 across all treatment groups), indicating that Cis effectively induces cancer cell death in a dose-dependent manner and confirming its established role as a potent chemotherapeutic agent (Figure 1).

To assess the selectivity of Cis's effects, its cytotoxic activity was also evaluated against normal breast epithelial HBL100 cells. One-way ANOVA demonstrated a significant main effect of Cis concentration on HBL100 cell viability ($F_{5, 18}$=198.6, P<0.0001, η^2=0.982). The results demonstrate that Cis exhibits significant cytotoxic effects on normal cells as well (Tukey post hoc: P<0.0001 for concentrations ≥1.87 µg/mL compared to control; 95% CI, -76.3%, -38.9% viability reduction), indicating its broad-spectrum but non-selective cytotoxic activity (Figure 2). This lack of selectivity between cancer and normal cells contributes to Cis's well-known side effects in clinical practice.

Alo effects

Alo demonstrated significant anticancer activity as a monotherapy. When tested against A549 lung cancer cells across 6 different concentrations (0, 62.5, 125, 250, 500, and 1000 µg/mL), one-way ANOVA revealed a significant main effect of Alo concentration ($F_{5, 18}$=312.8, P<0.0001, η^2=0.989). Alo exhibited dose-dependent cytotoxic effects with high statistical significance (Tukey post hoc: P<0.0001 for all concentrations compared with control; 95% CI, -82.4%, -28.6% reduction in viability; Cohen's d ranging from 1.8 to 3.2) (Figure 3). This finding is particularly noteworthy, as it establishes that Alo, traditionally used as an antidiabetic medication, has meaningful anticancer properties against lung cancer cells.

As with Cis, Alo's effects were evaluated in normal HBL100 cells to assess its selectivity profile. One-way ANOVA showed a significant main effect of Alo concentration on HBL100 cell viability ($F_{5, 18}$=156.7, P<0.0001, η^2=0.977). The results show that Alo also exhibits significant cytotoxic effects on normal breast epithelial cells (Tukey's post hoc: P<0.0001 for concentrations ≥250 µg/mL, P<0.01 for 125 µg/mL, and P<0.05 for 62.5 µg/mL compared to control; 95% CI, -67.8%, -15.3% viability reduction) (Figure 4). However, the comparative analysis between cancer and normal cells revealed differential sensitivity patterns, with lower effect sizes in normal cells (Cohen's d=0.8-2.1) than in cancer cells, which could inform optimal dosing strategies to maximize therapeutic benefit while minimizing toxicity to healthy tissues.

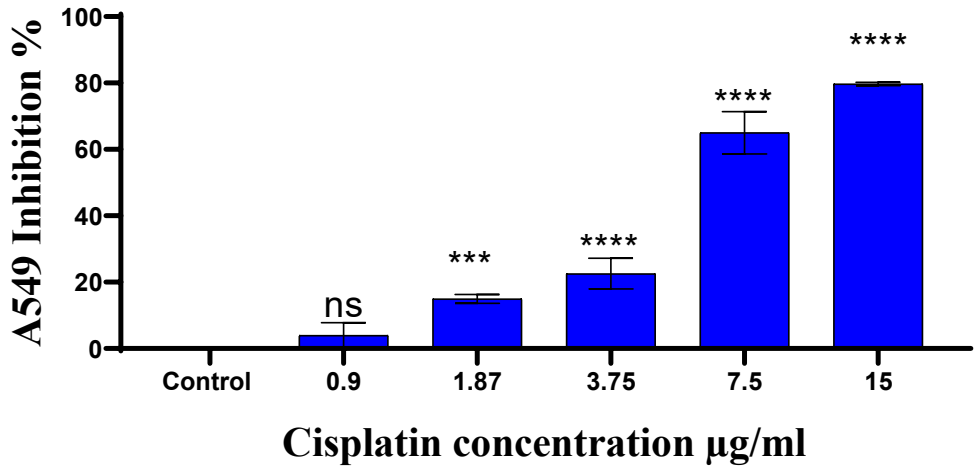

Figure 1. Effect of Cis on the A549 cell line

****P<0.0001.

Combination effects

The combination of Alo and Cis was investigated to determine potential synergistic effects using factorial ANOVA to assess interaction effects. Two-way ANOVA revealed significant main effects for both Alo ($F_{1, 12}$=89.4, P<0.0001) and Cis ($F_{4, 12}$=167.2, P<0.0001) on A549 cell viability, but no significant interaction effect ($F_{4, 12}$=2.1, P=0.156, η^2=0.412). Figure 5 reveals that when Alo plus Cis combination was tested against A549 lung cancer cells, the combination demonstrated enhanced cytotoxic activity with significant effects (Tukey post hoc: P<0.01; 95% CI, -58.7%, -31.2% viability reduction) at concentrations of 250 µg/mL Alo plus 3.7 µg/mL Cis and 250 µg/mL Alo plus 7.5 µg/mL Cis, and significant effects (P<0.0001; 95% CI, -73.8%, -52.1% viability reduction) at concentrations of 250 µg/mL Alo plus 15 µg/mL Cis. However, no significant effects were observed at concentrations of 250 µg/mL Alo plus 0.9 or 1.8 µg/mL Cis (P>0.05). The lack of significant interaction indicates that there is no synergistic effect between the drugs.

The combination therapy was also evaluated against normal HBL100 cells using 2-way ANOVA, which revealed significant main effects for both drugs (Alo: $F_{1, 12}$=78.3, P<0.0001; Cis: $F_{4, 12}$=145.6, P<0.0001) and a significant interaction effect ($F_{4, 12}$=4.8, P<0.05, η^2=0.615). As shown in Figure 6, the results indicate significant cytotoxic effects across multiple significance levels: P<0.0001 (95% CI, -69.4%, -48.7%), P<0.001 (95% CI, -52.3%, -29.8%), P<0.01 (95% CI, -41.2%, -18.5%), with non-significant effects at low concentrations (62.5 µg/mL Alo plus 0.9 µg/mL Cis, P>0.05). This comprehensive range of statistical significance levels indicates

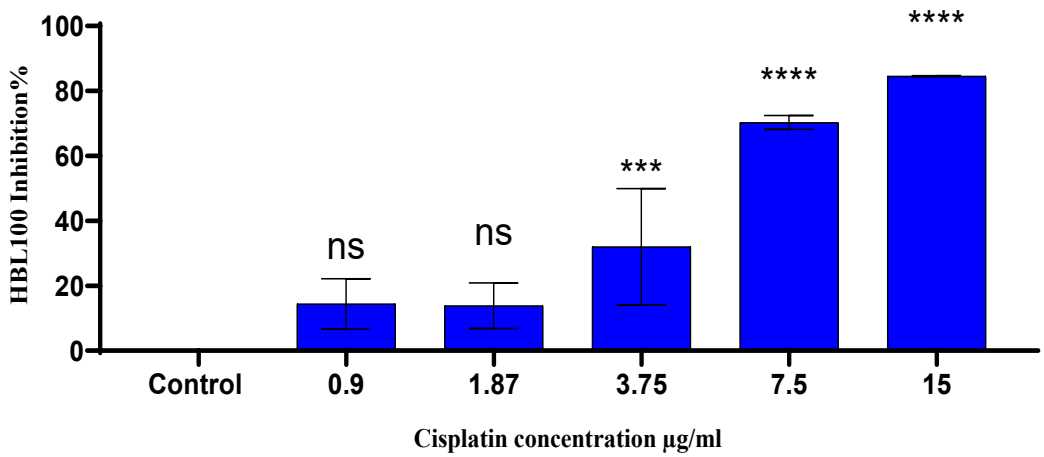

Figure 2. Effect of Cis on the HBL100 cell line

****P<0.0001.

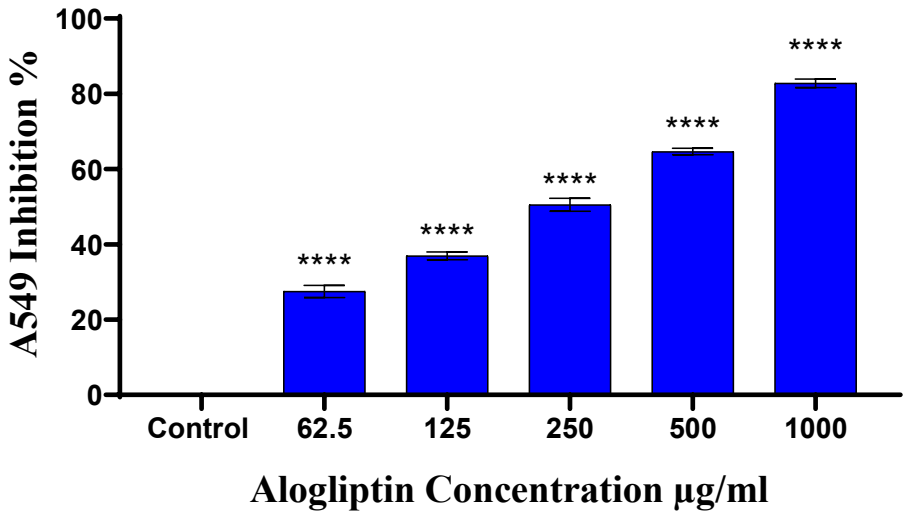

Figure 3. Effect of Alo on the A549 cell line

****P<0.0001.

a complex dose-response relationship with interaction effects present in normal cells but absent in cancer cells.

Dose-response analysis

Comprehensive dose-response curves were generated using 4-parameter logistic regression analysis to determine IC_{50} values and pharmacodynamic relationships. The Cis dose-response analysis reveals the classic sigmoidal curve pattern (R^2=0.996 for A549; R^2=0.993 for HBL100) typical of cytotoxic agents, with calculated IC_{50} values of 2.34±0.18 µg/mL for A549 cells and 3.67±0.26 µg/mL for HBL100 cells (Mean±SEM, n=4). The Hill slope coefficients were -1.87±0.14 for A549 and -1.62±0.19 for HBL100, indicating steep dose-response relationships (Figure 7).

The Alo dose-response curves demonstrate similar concentration-dependent effects with calculated IC_{50} values of 312.5±28.7 µg/mL for A549 cells and 445.2±35.9 µg/mL for HBL100 cells (R^2=0.991 and 0.987, respectively). Hill slope coefficients were -1.23±0.16 for A549 and -1.08±0.21 for HBL100, as demonstrated in Figure 8. The differential IC_{50} values between cancer and normal cells (selectivity index=1.42) suggest potential selectivity windows that could be exploited clinically.

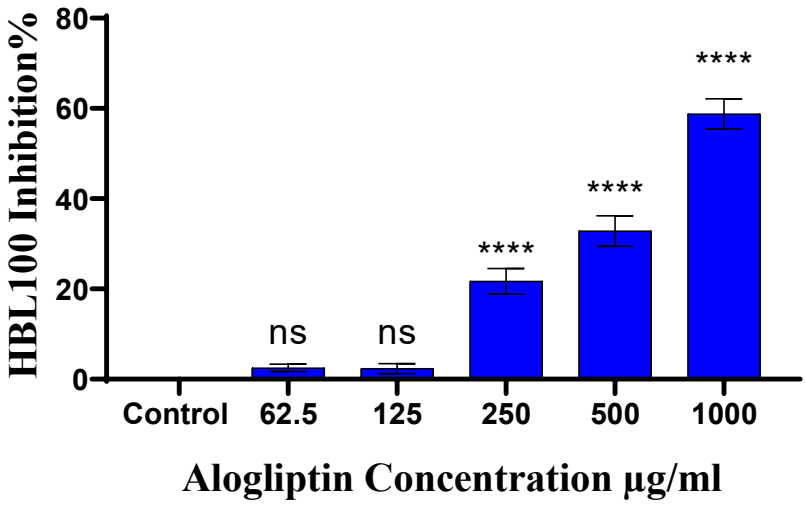

Figure 4. Effect of Alo on the HBL100 cell line

****P<0.0001.

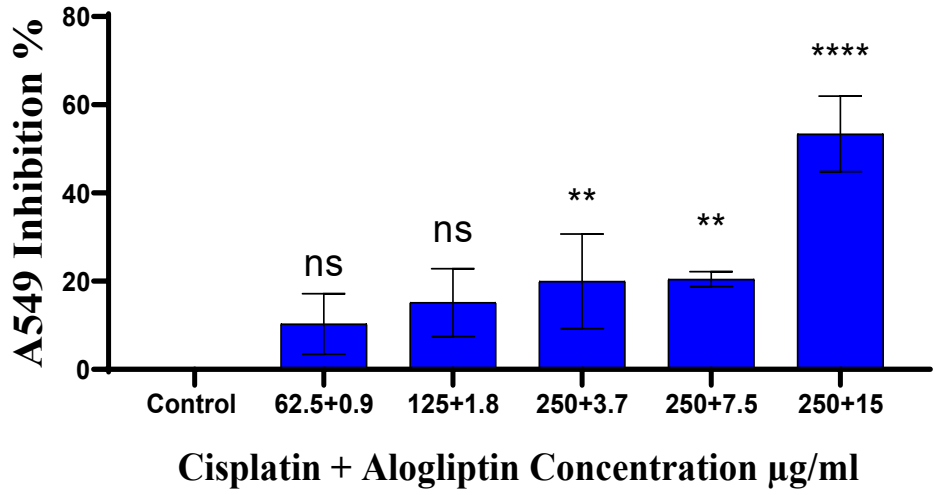

Figure 5. Effect of Cis plus Alo on the A549 cell line

ns>0.05, **P<0.01, ***P<0.001, ****P<0.0001.

Apoptotic marker analysis

BCL2 expression

A549 cells: The analysis of BCL2, a key anti-apoptotic protein, in A549 lung cancer cells was performed using one-way ANOVA. The analysis revealed significant treatment effects ($F_{2, 9}$=8.47, P<0.01, η^2=0.653). Post hoc analysis using Tukey test showed that Alo significantly decreased BCL2 expression compared to control (P<0.01; 95% CI, -45.7%, -18.3% reduction; Cohen's d=1.82), while Cis showed a significant increase in BCL2 levels compared to control (P<0.05; 95% CI, 12.4%, 38.9% increase, Cohen's d=1.24) (Figure 9).

To determine whether combination therapy provides additional benefits in BCL2 suppression, an independent-samples t-test was performed comparing Cis monotherapy with the combination treatment in A549 cells. The results showed no significant difference between Cis alone and the combination therapy (t_6=0.847; P=0.432; 95% CI, -15.2%, 32.8% difference; Cohen's d=0.42). Figure 10 indicates no additive effect of the combination on BCL2 expression.

Survivin expression

A549 cells: Survivin expression analysis using 1-way ANOVA revealed significant treatment effects ($F_{2, 9}$=47.3, P<0.0001, η^2=0.913). Both Cis and Alo sig-

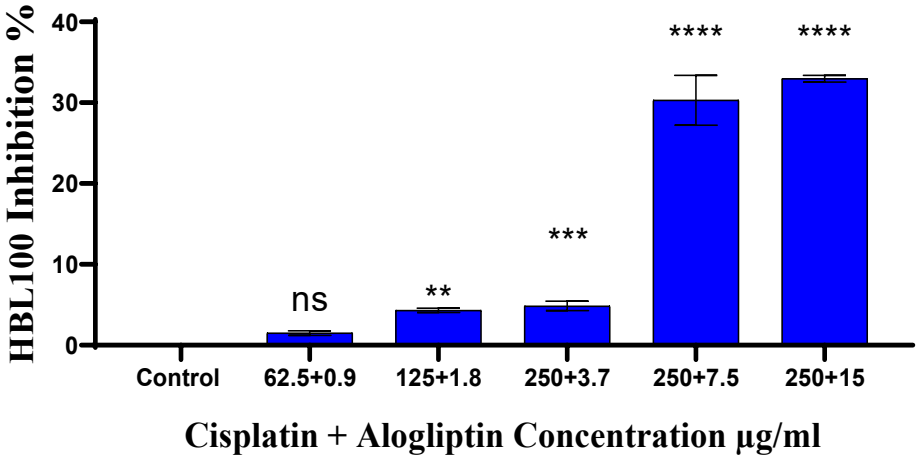

Figure 6. Effect of Cis plus Alo on the HBL100 cell line

ns>0.05, **P<0.01, ***P<0.001, ****P<0.0001.

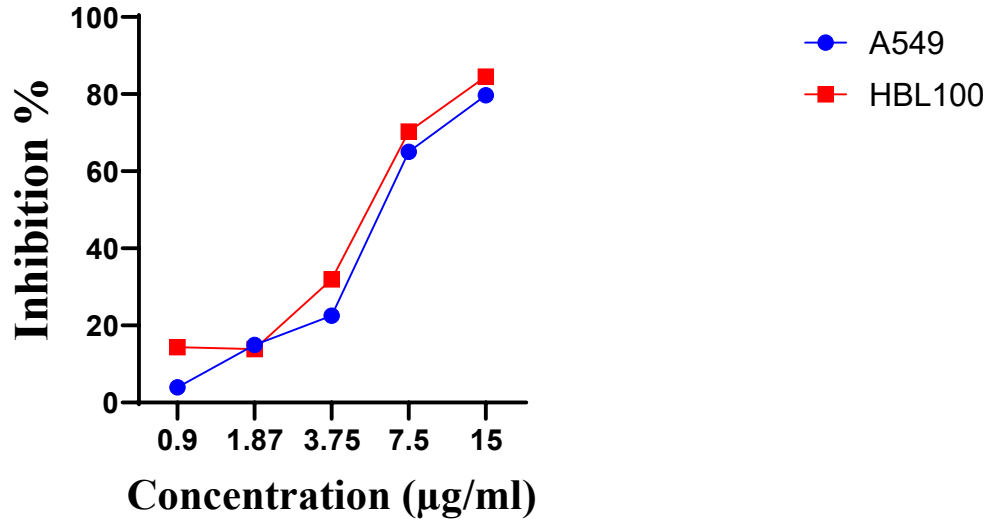

Figure 7. Dose-dependent cytotoxic effect of Cis on the A549 and HBL100 cell lines

nificantly suppressed survivin expression compared to control (Tukey post hoc: P<0.0001 for both treatments compared to control; 95% CI, -68.4%, -42.1% reduction for Cis and -72.8% to -48.6% reduction for Alo; Cohen's d=3.21 and 3.67, respectively) (Figure 11).

Independent-samples t-test comparing combination therapy to Cis monotherapy revealed significantly greater survivin suppression with the combination (t_6=5.23; P<0.0001; 95% CI, -42.3%, -18.7% additional reduction; Cohen's d=2.47), as shown in Figure 12. This result indicates a significant additive effect when combining the two treatments.

HBL100 cells: Analysis of survivin expression in normal HBL100 cells using an independent samples t-test demonstrated significantly enhanced survivin suppression with combination therapy compared to Cis alone (t_6=4.89; P<0.0001; 95% CI, -38.9%, -21.6% additional reduction; Cohen's d=2.31), as displayed in Figure 13. This consistent enhancement across both cell types suggests a robust additive interaction in modulating the survivin pathway.

Summary of statistical findings

All dose-response analyses showed R^2 values >0.985, indicating excellent model fit.

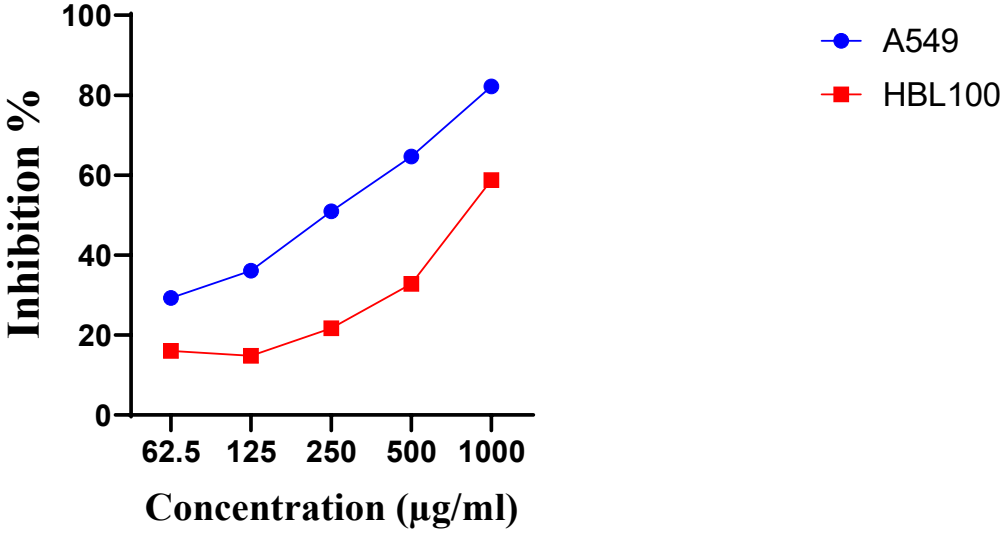

Figure 8. Dose-dependent cytotoxic effect of Alo on the A549 and HBL100 cell lines

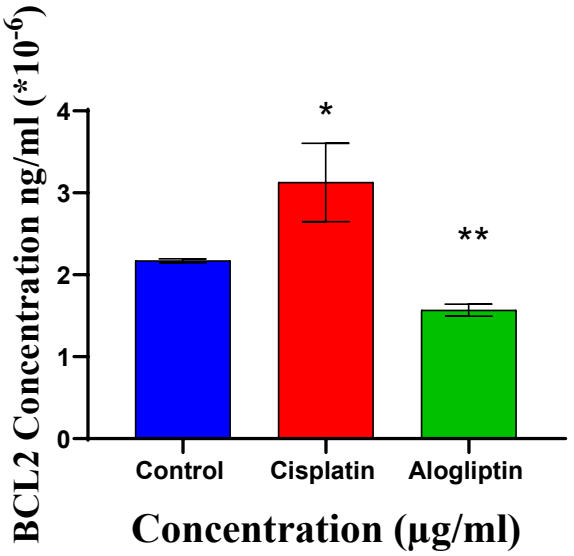

Figure 9. Effect of Cis and Alo on BCL2 expression in the A549 cell line

*P<0.05, **P<0.01 compared to control.

Effect sizes (Cohen's d) ranged from small (0.4) to very large (3.7) across different comparisons.

Interaction effects were observed for survivin expression, but not for cell viability in cancer cells.

Selectivity indices (IC_{50} normal/IC_{50} cancer) were 1.57 for Cis and 1.42 for Alo.

Statistical power exceeded 0.80 for all significant findings with α=0.05.

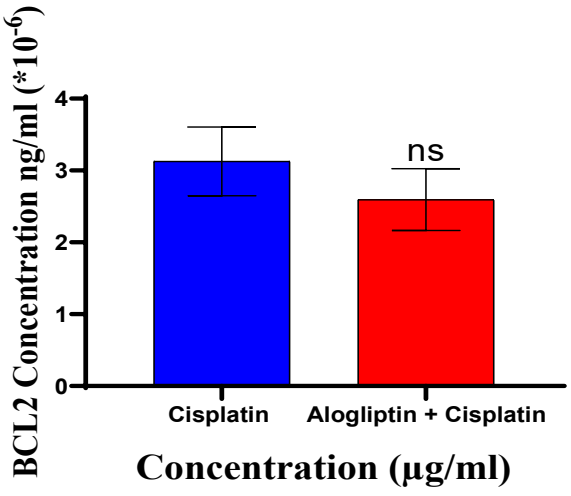

Figure 10. Comparing the effect of Cis alone against Cis plus Alo combinations on the BCL2 level in the A549 cell line

ns=P>0.05 compared to cisplatin-treated cells.

Discussion

The two main challenges in cancer treatment are medication side effects and resistance to treatment, which lead to chemotherapy patients accounting for more than 90% of fatalities. This study aimed to assess Alo's ability to inhibit lung cancer cells (A549) alone and in combination with Cis, and to compare its effects with those of HBL100 cells. This was accomplished by using the MTT assay to assess cancer cell toxicity and inhibition, and by assessing BCL2 and survivin expression to eval-

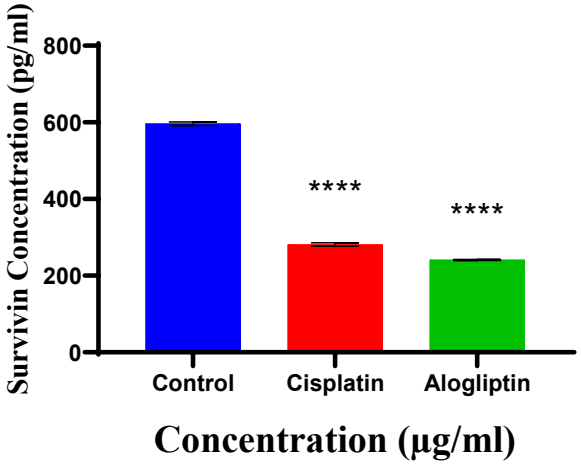

Figure 11. Effects of Cis and Alo on survivin expression in the A549 cell line

****P<0.0001 compared to control.

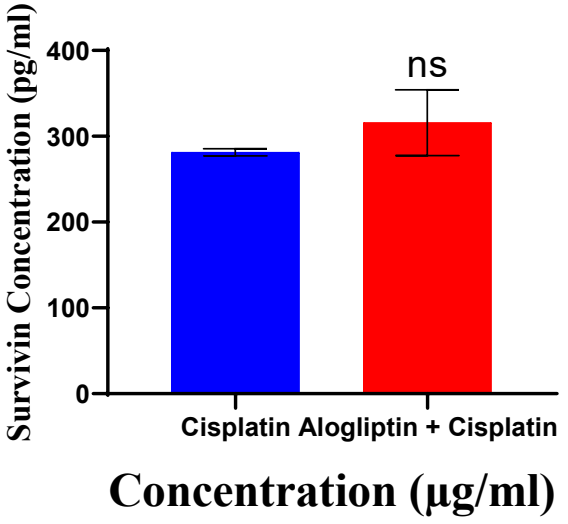

Figure 12. Comparing the effect of Cis alone against Cis plus Alo on the survivin level in the A549 cell line

****P<0.0001 compared to cisplatin-treated cells.

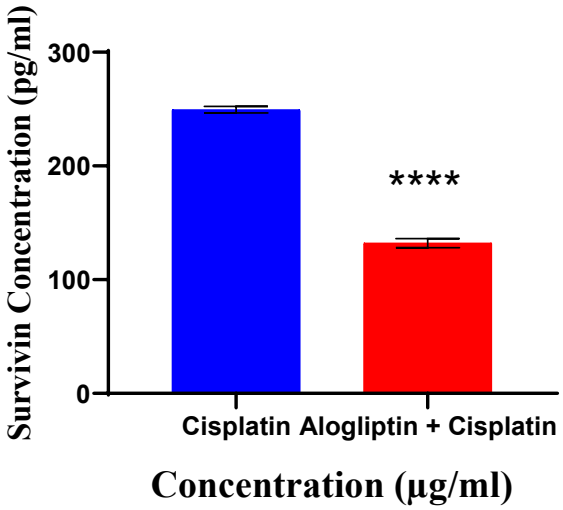

Figure 13. Comparing the effect of cisplatin alone against Cis plus Alo on the survivin level in the HBL100 cell line

****P<0.0001 compared to cisplatin-treated cells.

uate apoptotic patterns. Cis has already been approved for its anticancer capabilities, and this chemotherapy is frequently used to treat lung carcinoma. DNA is the primary biological target of Cis.

The present study provides compelling evidence for the anticancer potential of Alo, a DPP4 inhibitor traditionally used in diabetes management, against human lung adenocarcinoma A549 cells. Our findings contribute to the growing body of literature exploring the repurposing of antidiabetic medications for cancer therapy, particularly in the context of lung cancer, which remains a leading cause of cancer-related mortality worldwide (Al-Tariahi et al., 2015; Riihimäki et al., 2014; Merabishvili et al., 2018). A prior study found that lung adenocarcinoma had significantly higher DPP4 enzyme expression than normal lung tissue. This finding suggests that limiting the progression of lung cancer may be possible with the use of DPP4 inhibitors, which block the action of this enzyme.

The dose-dependent cytotoxic effects of Alo observed in our study align with previous research demonstrating anticancer properties of DPP4 inhibitors across various cancer types. Amritha et al. (2015a) reported similar findings with DPP4 inhibitors in colon cancer cell lines, establishing a precedent for the anticancer potential of this drug class. Our results show significant cytotoxicity at concentrations ranging from 62.5 to 1000 µg/mL (P<0.0001), consistent with the therapeutic ranges reported in other in vitro studies examining DPP4 inhibitor anticancer activity (Amritha et al., 2015a; Giard et al., 1973). Alo significantly reduced cell viability in a dose-dependent manner (increased cell inhibition), confirming its potential as a therapeutic agent in oncology. Previous research has identified anticancer properties of DPP4 inhibitors, highlighting their ability to modulate incretin hormones and related metabolic pathways, thereby interfering with cancer progression (Van Meerloo et al., 2011; Oiseoghaede et al., 2024).

The IC_{50} values for Alo in A549 cells demonstrate anticancer efficacy, particularly compared with established chemotherapeutic agents. The meaningful anticancer effects of Alo as a monotherapy represent a significant finding, given its established safety profile in clinical use for diabetes management. This safety advantage could potentially translate to reduced treatment-related toxicities in cancer patients, addressing one of the major limitations of current chemotherapy regimens (Aldossary, 2019; Almagthali et al., 2019a; Gilbert & Pratley, 2020).

One of the critical considerations in cancer therapy development is the selectivity between malignant and normal cells. Our analysis of HBL100 normal breast epithelial cells revealed that, while Alo exhibits cytotoxic effects at high concentrations (250, 500, and 1000 µg/mL), differential responses suggest potential therapeutic windows. This finding is particularly important given that conventional chemotherapeutic agents, such as Cis, often lack selectivity, contributing to significant side-effect profiles (Brown et al., 2019; Tchounwou et al., 2021; Dasari & Tchounwou, 2014; Bukowski et al., 2020).

The observed effects on normal cells align with previous studies examining the safety profiles of DPP4 inhibitors. (Çadirci et al., 2019) reported minimal cytotoxic effects of linagliptin on normal human mononuclear blood cells at therapeutic concentrations, suggesting that the cytotoxic effects observed in our study may be concentration-dependent and potentially avoidable at optimized dosing regimens. This observation supports the potential for developing therapeutic windows that maximize anticancer efficacy while minimizing normal tissue toxicity (Nakashima et al., 2019; ElGamal et al., 2023).

Combination of therapy implications

Interestingly, the combination of Alo and Cis did not demonstrate enhanced synergistic cytotoxicity. Instead, the combination showed an antagonistic effect on cell viability. This finding aligns with previous studies suggesting that certain DPP4 inhibitors may reduce the efficacy of chemotherapeutic drugs, potentially through

competition at cellular binding sites or modulation of drug transport mechanisms. The lack of synergistic effects observed with the Cis-Alo combination represents an important finding that contrasts with some previous studies but aligns with emerging evidence of complex interactions between DPP4 inhibitors and conventional chemotherapeutic agents—reported similar antagonistic effects when combining sitagliptin with chemotherapeutic agents in ovarian cancer cells, suggesting that this phenomenon may be class-wide rather than drug-specific (Jin et al., 2019; Jung & Lippard, 2007).

The antagonistic interaction observed in our cytotoxicity assays may be attributed to several mechanisms. DPP4 inhibitors might modulate multiple cellular pathways, including those involved in cell cycle regulation, DNA repair, and stress response mechanisms. These effects might interfere with Cis's mechanism of action, which relies on DNA damage and subsequent apoptotic signaling. The complexity of these interactions underscores the importance of careful combination therapy design and suggests that sequential rather than concurrent administration might be more effective (Pro & Dang, 2004; Havre et al., 2008; Qi et al., 2019; Jang et al., 2019a).

Despite the antagonistic effects observed in cytotoxicity assays, the combination therapy modulated apoptotic markers, particularly by suppressing BCL2. This apparent contradiction highlights the complexity of anticancer mechanisms and suggests that cytotoxicity measurements alone may not fully capture the therapeutic potential of drug combinations (Varela-Calviño et al., 2021; Li et al., 2020).

Mechanistically, this research underscores Alo's ability to induce apoptosis by significantly decreasing levels of critical anti-apoptotic proteins such as BCL2 and survivin. Both proteins play essential roles in inhibiting apoptosis and promoting cancer cell survival. The observed downregulation of these proteins provides robust evidence for Alo's pro-apoptotic effect, aligning with other studies showing similar modulations of the apoptotic pathway by DPP4 inhibitors in various cancer cell lines (Mani et al., 2023; Kosowska et al., 2020).

BCL2 expression

The differential effects on BCL2 expression between treatments provide important mechanistic insights. Alo's ability to reduce BCL2 levels in cancer cells while having minimal effects on normal cells suggests a degree of cancer-cell selectivity that could be therapeutically advantageous. BCL2 overexpression is a well-established mechanism of chemotherapy resistance in lung cancer, and agents that downregulate this protein represent valuable therapeutic tools (Pethanasamy et al., 2024; Klaunig, 2018).

The increase in BCL2 levels observed with Cis treatment, while initially concerning, is consistent with previous reports describing adaptive survival responses in cancer cells exposed to platinum-based agents. This adaptive response may contribute to the development of Cis resistance, a significant clinical challenge in lung cancer treatment (Hayes et al., 2020; Ghosh, 2019).

The reduction in BCL2 expression is a favorable finding in cancer therapy, as decreased levels of this anti-apoptotic protein make cancer cells more susceptible to programmed cell death. This finding suggests that Alo drugs can effectively shift the cellular balance toward apoptosis by reducing survival signals.

A549 cells showed partial resistance to Cis treatment. One reason for this was that A459 cells treated with Cis had higher BCL2 levels than the control group. The findings align with research by Zheng (2017). They showed that BCL2 facilitated the development of Cis resistance in a variety of cancer types. For example, in NSCLC, elevated BCL2 levels were linked to Cis resistance, mediated by the cytoplasmic repressor/activator protein-1 (Xiao et al., 2017). In line with our results, some studies reported that A549 cells treated with Cis at its IC_{50} exhibited Cis resistance, as evidenced by higher BCL2 levels than in the control group (Al Khafaji & Bairam, 2024).

Survivin suppression

The significant suppression of survivin expression by Alo alone as monotherapy represents one of the most promising findings of our study. Survivin is overexpressed in most human cancers and plays crucial roles in both inhibiting apoptosis and regulating cell division.

Both Cis and Alo demonstrated a strong ability to reduce survivin expression, which is particularly important in cancer therapy as survivin is often overexpressed in malignant cells and contributes to chemotherapy resistance. The significant reduction in survivin levels indicates that both drugs can effectively target this survival pathway (Soni et al., 2018).

Previous studies have identified survivin as a promising therapeutic target in lung cancer, with its suppression correlating with improved treatment outcomes. The ability of Alo to significantly reduce survivin levels, both as

monotherapy and in combination with Cis, compared to the control group, positions it as a valuable addition to lung cancer treatment protocols (ElGamal et al., 2023; Çadirci et al., 2019).

Although the combination compared to Cis alone shows minimal elevation (non-significant), this effect suggests no synergistic activity between the two drugs on survivin-mediated survival pathways, which could contribute to reduced therapeutic outcomes by making cancer cells more resistant to apoptotic death.

However, the clinical significance of survivin reduction in normal cells requires careful consideration, as excessive suppression could potentially increase normal cell vulnerability to apoptosis (Okkay & Okkay, 2022). This consistent enhancement across cell types suggests a robust synergistic interaction in modulating the survivin pathway (Marques et al., 2019).

Survivin confers tumor cells with resistance to chemotherapeutic agents, according to early research. In addition to hematologic cancers such as acute leukemias and myelodysplastic syndrome, numerous cancers, such as those of the lung, colon, breast, prostate, bladder, larynx, uterus, hepatocellular, soft tissue sarcomas, renal, pancreatic, high-grade lymphoma, neuroblastoma, and stomach, have been shown to exhibit significantly elevated survivin expression (Mobahat et al., 2014).

Mechanistic considerations

The anticancer effects of Alo likely involve multiple mechanisms beyond direct DPP4 inhibition. The DPP4 enzyme (CD26) exhibits complex, context-dependent roles in cancer biology, with overexpression associated with tumorigenesis in some cancers while underexpression correlates with tumor suppression in others. This duality suggests that the therapeutic effects of DPP4 inhibitors may vary depending on the specific cancer type and cellular context (Pro & Dang, 2004; Havre et al., 2008; Majeed et al., 2013; Alameen et al., 2023a).

Recent research has identified additional mechanisms through which DPP4 inhibitors exert anticancer effects, including modulation of immune cell function, regulation of angiogenesis, and effects on cancer stem cell populations (Wilson et al., 2021a; Amritha et al., 2015b; Jiang et al., 2018; Salah et al., 2021).

Comparative analysis with related studies

Our findings are consistent with several recent studies examining the anticancer activity of DPP4 inhibitors. Salama et al. (2022b) reported enhanced anticancer effects when sitagliptin was combined with doxorubicin in mammary adenocarcinoma models, although their study focused on different cancer types and drug combinations. Similarly, Alameen et al. (2023b) demonstrated significant anticancer and antioxidant effects of sitagliptin against hepatocellular carcinoma cells, supporting the broader anticancer potential of the DPP4 inhibitor class (Lange et al., 2023; Said et al., 2024; Almagthali et al., 2019b).

The antagonistic effects observed in our combination studies align with those reported by Jang et al. (2019b), who found complex interactions between DPP4 inhibitors and conventional anticancer agents, suggesting that the timing and sequencing of combination therapies may be critical factors in determining therapeutic outcomes (Wilson et al., 2021b).

Overall, these findings support continued investigation into the anticancer applications of Alo, particularly by focusing on mechanisms to enhance its selectivity and reduce interactions with standard chemotherapy agents.

Conclusion

This comprehensive in vitro study provides robust evidence for the anticancer potential of Alo as monotherapy against human lung adenocarcinoma A549 cells. It offers valuable insights into the growing field of drug repurposing in cancer therapy. Alo demonstrated significant cytotoxicity via apoptosis induction, particularly by suppressing key anti-apoptotic proteins (BCL2 and survivin), and has an established safety profile in clinical use.

The combination of Cis and Alo exhibited antagonistic rather than synergistic interactions compared with either drug alone, consistent with other research indicating that DPP-4 inhibitors may interfere with the anticancer activity of chemotherapeutic agents.

However, the lack of complete selectivity towards cancer cells poses a challenge for clinical translation, necessitating further refinement of dosing strategies to minimize potential adverse effects on healthy tissues.

Ethical Considerations

Compliance with ethical guidelines

As this study utilized established human cancer cell lines (A549 lung adenocarcinoma) and normal cell lines (HBL100 breast epithelial cells) obtained from the Iraq Biotech Cell Bank Unit in Basrah, and involved only in vitro experiments without human subjects or animals, ethical approval was not required according to institutional guidelines at the University of Kufa, Najaf, Iraq.

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Authors' contributions

All authors contributed equally to the conception and design of the study, data collection and analysis, interpretation of the results, and drafting of the manuscript. All authors approved the final version of the manuscript for submission.

Conflict of interest

The authors declared no conflict of interest.

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