ORIGINAL ARTICLE

CONTENTS

Anticancer and apoptotic effect of alogliptin on A549 cancer cell line

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ABSTRACT

Aim: To evaluate the anticancer, apoptotic, and antioxidant effects of Alo on A549 cells, both alone and in combination with CP, and to elucidate the molecular mechanisms underlying the death of cancer cells.

Materials and Methods: The American Type Culture Collection's (ATCC) normal HBL100 cells and human lung A549 cells were used in the investigation. The cells were split into four groups. Following a 72-hour incubation period, ELISA assays were used to quantify the levels of the DPP-4 enzyme, apoptotic regulators (Bax and caspase-3), and oxidative stress marker (malondialdehyde) in lung cancer cell and normal cell lines. One-way ANOVA with significance set at $P < 0.05$ were used in the statistical analysis.

Results: The findings showed that Alo reduced the activity of the DPP-4 enzyme in both cell lines ($P < 0.0001$). Molecular analysis showed a considerable increase in pro-apoptotic markers (BAX, Caspase-3). Higher amounts of malondialdehyde were indicative of increased oxidative stress in both monotherapy and combination. But in HBL 100 cells, Alo decreased BAX, caspase-3, and MDA levels.

ConclusionS: Alo has caused cancer cell death through a variety of mechanisms, such as DPP4 inhibition, apoptotic pathway activation, and oxidative stress enhancement based on DPP-4, BAX, caspase-3, and MDA measurements.

KEY WORDS: A549 cell line, alogliptin, BAX, caspase-3, DPP4 inhibitor, lung cancer, MDA

Wiad Lek. 2026;79(2):346-354. doi: 10.36740/WLek/217851 DOI

INTRODUCTION

One of the most common and deadly types of cancer worldwide, lung cancer is caused by the overgrowth of epithelial cells in the pulmonary system, which impairs respiratory function [1]. It frequently spreads to distant organs such the brain, bones, liver, and adrenal glands as well as regional lymph nodes and pleural tissues due to its aggressive clinical nature [2]. As a result, the prognosis is typically dismal, with median survival spans for localized disease estimated at 13 months and for metastatic cases at roughly five months [3]. More than 2.2 million new cases of lung cancer were diagnosed worldwide in 2020, according to new statistics from the Global Cancer Observatory (GLOBOCAN) [4]. It continues to be the second most deadly cancer in women, behind breast cancer, and the primary cause of cancer-related fatalities in males [5]. Small cell lung cancer (SCLC), which makes up around 15% of cases, and non-small cell lung cancer (NSCLC), which makes up the remaining 85% of cases, are the two main histological subtypes of lung cancer [6]. Surgery, radiation, immunotherapy, and chemotherapy—most notably with cisplatin (CP), an intravenous platinum-based chemotherapeutic agent—are common treatment approaches [7]. Despite Cis's widespread use and clinical efficacy, systemic toxicity, tumor cell resistance, and unfavorable side effects usually restrict its use [8]. Adjunctive medicines that potentially improve efficacy and lower toxicity profiles have drawn attention as a result [9]. In a number of cancer models, the selective dipeptidyl peptidase-4 (DPP4) inhibitor alogliptin (Alo), which is mainly used to treat type 2 diabetes, has demonstrated apoptotic properties [10]. DPP4 inhibitors improve glycemic control and may alter pathways linked to tumor growth by increasing the activity of incretin hormones including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide GIP [11]. In cancer, the DPP4 enzyme CD26 has a context-dependent role: under expression has been connected to tumor suppression in cancers including breast and endometrial cancers, while overexpression has been linked to carcinogenesis in lung, colon, and renal cancers [12]. The significance of this duality lies in the fact that it has the

potential to serve as a therapeutic target in oncology [13]. Comprehensive studies assessing the specific cytotoxic and antioxidant effects of alogliptin against lung cancer cells, namely A549, are scarce, despite earlier research documenting the overall anticancer effects of DPP4 inhibitors [14]. Furthermore, little is known about the precise mechanisms by which alogliptin affects oxidative stress and lung cancer cell survival [15].

AIM

The purpose of this study is to evaluate the apoptotic and anticancer effects of alogliptin both by itself and in conjunction with cisplatin on the A549 lung cancer cell line. The results could help create better treatment plans and shed light on how DPP4 inhibition functions in the treatment of lung cancer.

MATERIALS AND METHODS

CHEMICALS AND CELL LINE

In order to evaluate the anticancer and apoptotic effects of alogliptin, both alone and in combination with cisplatin, on the A549 lung cancer cell line, this study was conducted. The results could guide the creation of better treatment plans and shed light on the function of DPP4 inhibition in the treatment of lung cancer.

HBL100 CELL LINE

In cancer research studies, the human breast epithelial cell line HBL100 is used as a standard cell control. HBL100 cells, which were initially isolated from healthy breast tissue, offer an essential comparison model for evaluating how well anticancer therapies distinguish between cancerous and healthy cells. For the study, Sigma in the United States of America provided the RIPA lysis buffer, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide dye powder, and dimethyl sulfoxide (DMSO). Fetal bovine serum (10% FBS), phosphate-buffered saline (PBS), and RPMI-1640 medium containing fetal bovine serum were supplied by Gibco (USA). Trypsin-EDTA and trypan blue stain were purchased from Capricorn in the United States of America and Flow Laboratories in the United Kingdom, respectively. Troge, based in Germany, provided the streptomycin and benzylpenicillin antibiotics, respectively. Alogliptin (Alo) and Cisplatin (CP) both target Moi (USA). The amounts of MDA, caspase-3, and BAX in the sample were determined using a human-specific ELISA kit developed by the Bioassay Technology Laboratory in China. An ELISA kit for human caspase-3, BAX, and MDA was acquired from Elabscience under the name "UNITED STATES".

CELL CULTURE

Fetal bovine serum (FBS) was used to neutralize the A549 cells after they had been cultured with trypsin-EDTA for enzymatic detachment and washed with phosphate-buffered saline (PBS). After that, the cells were plated on 96-well culture plates using RPMI-1640 medium that had been treated with 100 µg/mL of streptomycin and 100 units/mL of penicillin until they reached the desired concentration. To achieve about 80% confluence and create a monolayer, cultures were cultured for 24 hours at 37 °C in a humidified environment with 5% CO_2. Trypan blue exclusion was used to calculate the number of viable cells. After the incubation period, the medium was either left untreated for control or replaced with 200 µL of new media containing the test medicines. The four experimental groups consisted of control cells, (cells that had not been treated), cells that had been treated with CP, cells that had been treated with Alo, and cells that had been treated with a combination of CP and Alo. By assessing DPP-4, BAX, caspase-3, and MDA levels after 72 hours, the apoptotic effect, oxidative stress, and DPP-4 expression were evaluated.

STATISTICAL ANALYSIS

We used Microsoft Excel 2019 and GraphPad Prism 10 to analyze all of the experimental data. For statistical comparisons, Tukey's post hoc test was used after a one-way Graph Prism ANOVA. The results were considered to be statistically significant if the p-value was lower than 0.05 percentage points.

RESULTS

Dipeptidyl Peptidase 4 expression in A549 and HBL100 cells

The expression of DPP4 in A549 and HBL100 cells is presented in Table 1

HUMAN DIPEPTIDYL PEPTIDASE 4 DETERMINATION

Upon exposure to the IC50 of CP, the findings of the CP used showed that the concentration of DPP4 did not significantly alter ($P > 0.05$) in the A549 and HBL100 cells when compared to the control group. However, as illustrated in figure (1), after A549 and HBL100 cells were treated with the IC50 of Alo ($P < 0.0001$), the concentration of DPP4 significantly decreased in contrast to the control group.

Table 1: The expression of DPP4 in A549 and HBL100 cells

Cell line	DPP4 level (ng/ml)	Total protein (ng/ml)	DPP4 expression %
A549 control	7.547±0.89	1385059±174348	5.45×10^{-4} **±0.09
HBL100 control	7.792±0.45	1477358±412460	5.27×10^{-4} **±0.10

** (P<0.01) n=4

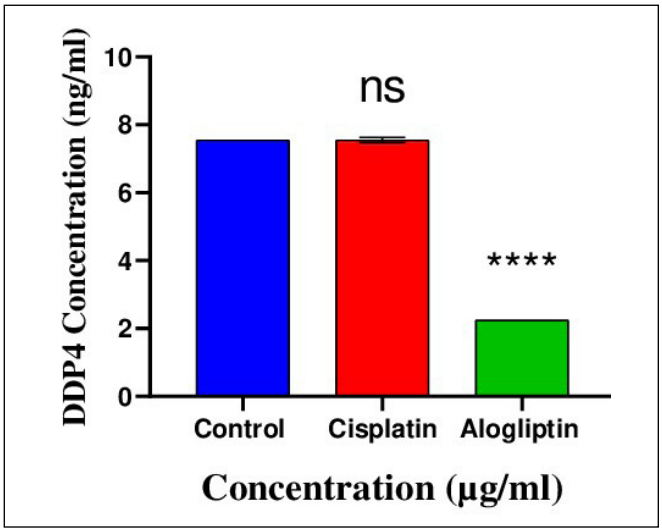

Fig. 1. Cisplatin and alogliptin's effects on the A549 cell line's DPP4 levels
A one-way ANOVA was used in the study, and the results are displayed as mean ± SD with ns = P > 0.05 and **** P < 0.0001 in comparison to the control, figure (2)
Source: Own materials

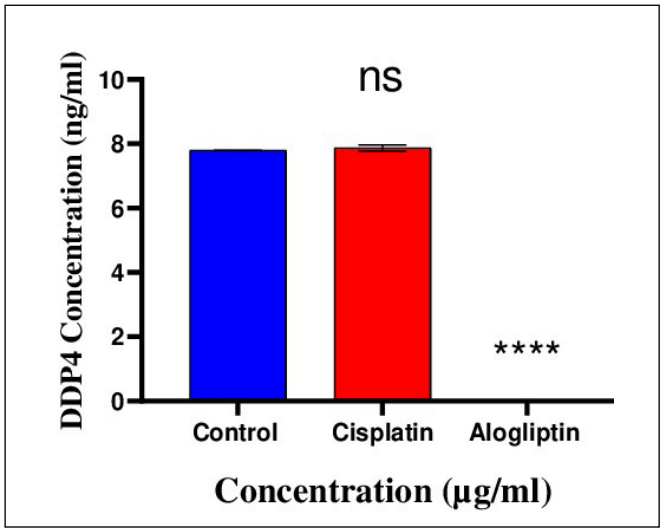

Fig. 2. Cisplatin and alogliptin's effects on the HBL100 cell line's DPP4 values
The analysis was conducted using a one-way ANOVA. In contrast to the control, the data are shown as mean ± SD. ns = P < 0.05, **** P < 0.0001
Source: Own materials

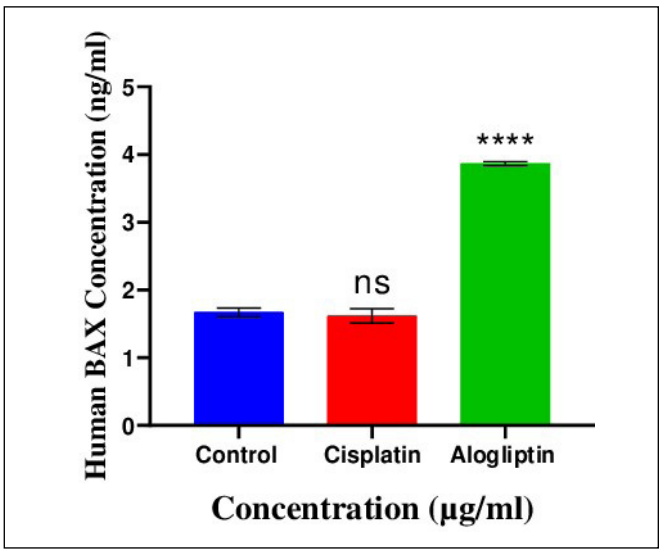

Fig. 3. Changes in human BAX levels in the A549 cell line caused by cisplatin and alogliptin
A one-way ANOVA was used for the analysis. In relation to the control, the results are displayed as mean ± SD. ns indicate P > 0.05, **** P < 0.0001, respectively, figure (4)
Source: Own materials

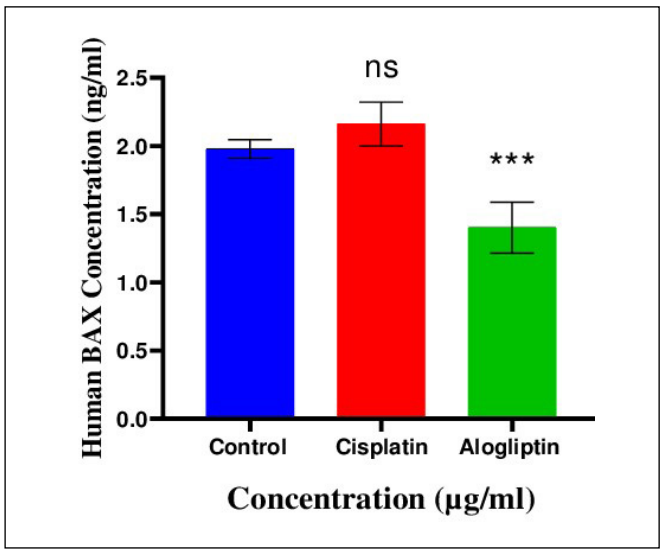

Fig 4. Changes in human BAX levels in the HBL100 cell line caused by cisplatin and alogliptin
The analysis was conducted using a one-way ANOVA. In contrast to the control, the data are shown as mean ± SD. ns = P > 0.05, *** P < 0.001
Source: Own materials

DETERMINATION OF HUMAN APOPTOSIS REGULATOR (BAX)

When compared to the control group, the CP did not significantly increase BAX levels after giving the IC50 to A549 and HBL100 cells (P > 0.05). However, as Figure 3 illustrates, BAX levels dramatically rose (P < 0.0001) in comparison to the control group following treatment of A549 cells with Alo's IC50. On the other hand, Figure 4 illustrates that Alo

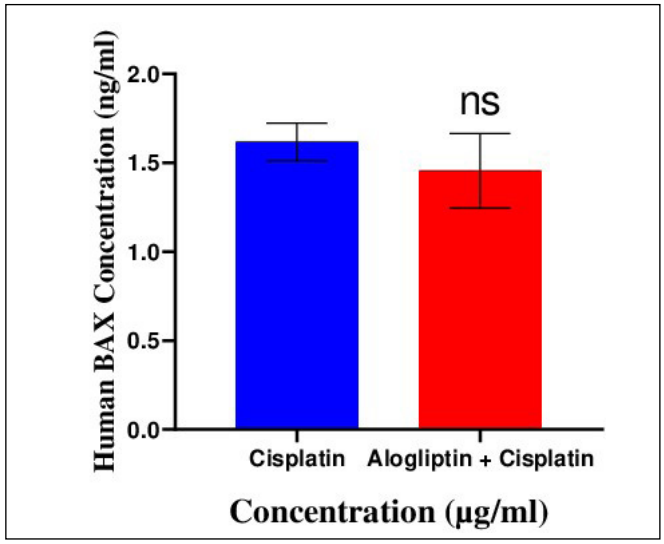

Fig. 5. In the A549 cell line, the effects of cisplatin alone and cisplatin + alogliptin combos on the human BAX level are compared
An ANOVA in one direction was used in the analysis. Mean ± SD is used to illustrate the results, *P is less than 0.05
Source: Own materials

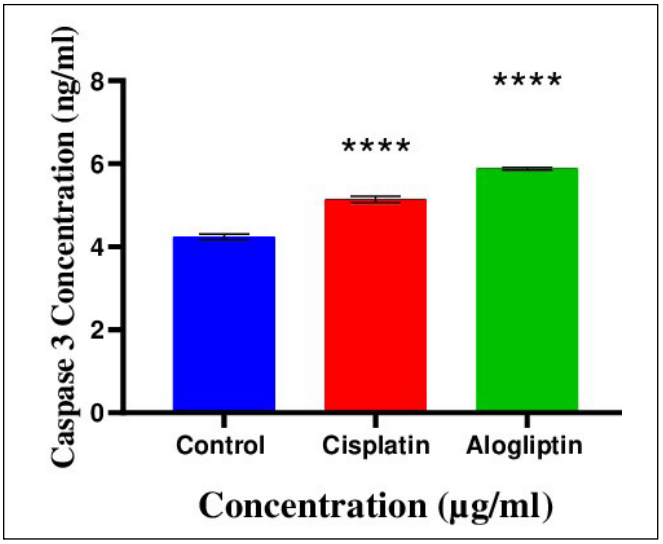

Fig. 6. Caspases 3 levels in the A549 cell line are affected by cisplatin and alogliptin
An ANOVA in one direction was used for the analysis. The mean ± SD is used to display the data. **** P < 0.0001 in comparison to the control
Source: Own materials

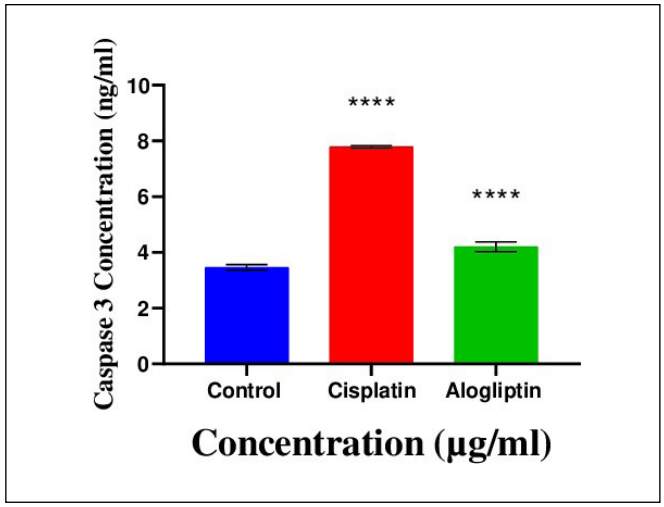

Fig. 7. Alogliptin and cisplatin's effects on the HBL100 cell line's caspase 3 levels
An ANOVA in one direction was used for the analysis. It displays the data as mean ± SD. **** P < 0.0001, in comparison to the control group
Source: Own materials

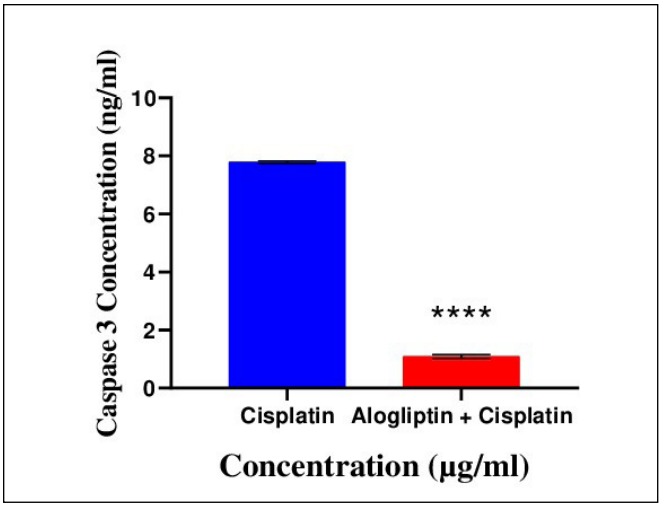

Fig. 8. The impact of cisplatin alone and cisplatin + alogliptin combos on the HBL100 cell line's Caspase 3 level is compared
In the analysis, a one-way analysis of variance was used. Presented in the form of mean ± standard deviation, the data are utilized. **** The value of P is lower than 0.0001
Source: Own materials

significantly reduced the concentration of BAX (P > 0.001) after administering the IC50 of Alo to HBL100 cells in comparison to the control group, figure (3).

COMPARISON BETWEEN THE ACTIVITY OF ALOGLIPTIN PLUS CISPLATIN VERSUS CISPLATIN ALONE ON BAX CONCENTRATION

As illustrated in figures (5-6), the Alo plus CP combination did not significantly raise the BAX concentration in A549 cells (P > 0.05), but the HBL100 cells' BAX level significantly decreased (P < 0.01) when exposed to the combination's IC50 as opposed to cells exposed to CP alone.

HUMAN CASPASE-3 DETERMINATION

Following treatment with the IC50 of CP, the quantity of Caspase-3 increased significantly (P < 0.0001) in A549 and HBL100 cells compared to the control group. Comparing A549 and HBL100 cells to the control group, the amount of Caspase-3 rose dramatically (P < 0.0001) when the IC50 of CP was administered. In contrast to

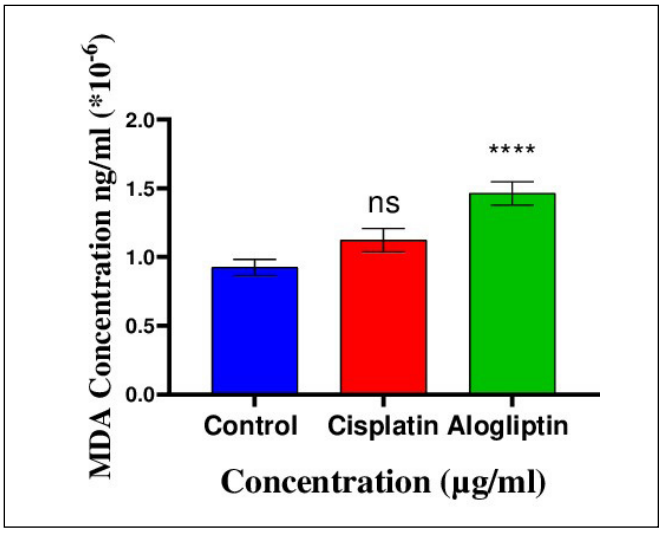

Fig. 9. MDA levels in the A549 cell line are affected by cisplatin and alogliptin To conduct the analysis, a one-way analysis of variance was utilized. The data is presented in the form of mean ± standard deviation. The significance level (ns) is set at P > 0.05, and the significance level (****) is set at P < 0.0001 in comparison to the control
Source: Own materials

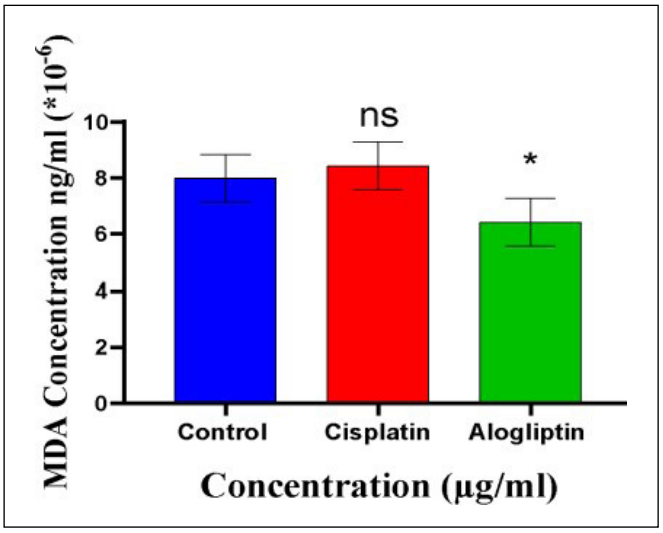

Fig. 10. Alogliptin and cisplatin's effects on the HBL100 cell line's MDA levels An ANOVA in one direction was used for the analysis. It displays the data as mean ± SD. * P <0.05, in comparison to the control group
Source: Own materials

the control group, figures (6-8) show that the quantity of caspase-3 in HBL100 cells treated with the IC50 of Alo dropped dramatically, P<0.0001.

DETERMINATION OF HUMAN MALONDIALDEHYDE LEVEL

After exposing the A549 and HBL100 cells to CP's IC50 in contrast to the control group, CP did not significantly raise the MDA concentration (P > 0.05), as Figures 9 and 10 demonstrate. After treating A549 cells with Alo's IC50, Alo demonstrated a significant rise in MDA concentration (P < 0.0001) compared to the control group, as shown in figure (9). After treating HBL100 cells with the IC50 of Alo, MDA levels significantly decreased (P < 0.05) compared to the control group, figure (10).

COMPARISON BETWEEN ALOGLIPTIN AND CISPLATIN VERSUS CISPLATIN ALONE ON MDA CONCENTRATION

In contrast to cells exposed to the IC50 of CP alone, figure (11) demonstrated that the Alo plus CP combination significantly increases MDA concentration after treating the A549 and HBL100 cells with the combination's IC50 (P < 0.0001 and P < 0.001, respectively).

DISCUSSION

One of the primary causes of cancer-related death globally is still lung cancer, and the effectiveness of current treatments is sometimes hampered by serious side effects and medication resistance [16]. Thus, novel therapy strategies are desperately needed [17]. Given their known safety profiles and well-characterized pharmacokinetics, repurposing currently approved FDA medications, such as DPP-4 inhibitors, may be a viable approach [18].

DPP-4 ENZYME INHIBITION AND MOLECULAR TARGETS

DPP-4 EXPRESSION AND INHIBITION

The findings validate Alo's main mode of action by showing that it effectively reduced DPP-4 enzyme activity in both cell lines [19]. DPP-4 levels were not significantly impacted by CP. This outcome is consistent with our understanding of Alo, a potent and precise DPP-4 inhibitor. We found that A549 lung carcinoma had an overexpression of the DPP-4 enzyme. This suggests that DPP-4 is essential for lung cancer cell survival and proliferation, as evidenced by the substantial decrease in DPP-4 activity caused by Alo. According to literature, DPP-4's expression varies according to the type of cancer and is implicated in immunological control, glucose metabolism, signaling, and apoptosis [20]. The significant decrease in DPP-4 activity after Alo therapy raises the possibility that this enzyme could be used as a therapeutic target and biomarker in the treatment of lung cancer. Furthermore, studies identified DPP-4 as a unique possible diagnostic marker in gastric cancer, and showed that DPP-4/CD26 expression patterns can function as prognostic markers in a variety of hemato-

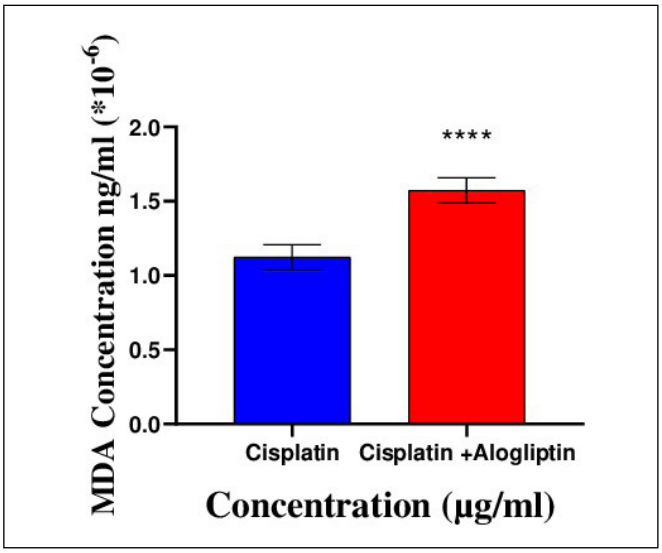

Fig. 11. In the A549 cell line, the effects of cisplatin alone and cisplatin plus alogliptin combos on the MDA level are compared
In the analysis, a one-way analysis of variance was used. Presented in the form of mean ± standard deviation, the data are utilized. **** The value of P is lower than 0.0001
Source: Own materials

logical malignancies [21]. As demonstrated by researchers, who demonstrated that long-term DPP-4 inhibitor treatment decreased colon carcinogenesis in animal models, the therapeutic implications of DPP-4 inhibition go beyond glucose homeostasis [22]. Hypoxia-inducible factor-1a (HIF-1a) VEGFA production was increased by overexpression of DPP-4, according to another endometrial cancer investigation [23]. Both in vitro and in vivo, DPP-4 overexpression caused a change in the morphology of the cell as well as an acceleration of invasion, proliferation, and carcinogenesis. These effects were mitigated by DPP-4 knockdown or pharmacological suppression with sitagliptin [24]. The possibility of dipeptidyl peptidase-4 (DPP-4) inhibitors as anticancer drugs has been investigated in recent research. DPP-4 inhibitors, which were first developed to treat type 2 diabetes, have shown promising results in improving progression-free survival in advanced colorectal and airway malignancies and lowering the growth of cancer cells [25]. In another study of urothelial carcinoma (UC), DPP-4 overexpression and destructive tumor features were seen. Overexpression of DPP-4 stimulates cell invasion, migration, proliferation, and growth [26]. Reduced UC aggressiveness and increased apoptosis were linked to DPP-4 inhibition. Based on particular biomarkers such as DPP-4, a new study implies that targeting senescent cells in cancer for the purpose of addressing tumor dormancy, recurrences, and resistance to conventional chemotherapy and radiation therapies offers a novel therapeutic approach [27]. Senescent cells multiply as people get older, which can result in inflammation and tissue dysfunction. Additionally, senescent cells can cause cell cycle arrest in response to stress, which can make DNA more vulnerable to damage [28]. STX-1 is a first-in-class antibody-drug combination (ADC) that can be used to treat a number of cancer indications, according to the outcomes of the inquiry.

APOPTOTIC PATHWAY MODULATION

PRO-APOPTOTIC BAX EXPRESSION

The study illustrates how Alo has varying impacts on the production of BAX, a crucial pro-apoptotic protein in cancerous cells [29]. Apoptotic pathways were selectively activated in malignant cells while normal HBL100 cells were protected by Alo treatment, which markedly elevated BAX expression in A549 cancer cells while lowering it in mammary epithelial cells (HBL100). This differential effect supports the idea of taking advantage of cancer cells' weaknesses while preserving healthy tissues, which is essential for therapeutic selectivity [30]. The molecular mechanism put out by researchers, in which pro-apoptotic members of the BCL-2 family promote mitochondrial outer membrane permeabilization, resulting in cytochrome c release and BAX activation, is supported by the observed increase in BAX expression in cancer cells after Alo therapy. This effect's selectivity supports Alo's promise as a tailored treatment with better safety records than traditional chemotherapeutics [31]. Studies who detailed the intrinsic apoptotic route involving BAX-mediated mitochondrial malfunction, provides more support for this mechanism. Furthermore, research highlighted the significance of BAX as a therapeutic target by underscoring its crucial role in developmental and therapeutic apoptosis [32]. The idea of cancer-selective apoptosis induction is consistent with the distinct impacts seen in cancer and healthy cells. These results are consistent with another study that demonstrated that the percentage of thyroid cancer cells that undergo apoptosis rises as the ratio of BAX to BCl-2 increases due to an increase in BAX protein levels while Bcl2 levels stay unchanged following gemigliptin treatment of the cells. By increasing cell survival and modifying the BAX/BCl-2 ratio, Huang et al.'s parallel studies found that pretreatment of SH-SY5Y neuroblastoma cells with the DPP4 inhibitor teneligliptin reduces MPP+-induced cytotoxicity [33].

CASPASE-3 ACTIVATION

The study illustrated the impact on caspase-3, the primary apoptotic effector caspase [34]. Alo administration

caused down-regulation effects on normal cells and markedly increased caspase-3 levels in A549 carcinoma cells, suggesting that the apoptotic machinery was selectively activated in malignant lung tissues. The discovery that DPP4 inhibitors may enhance Caspase-3 activity in hepatocellular carcinoma cells lends credence to this conclusion [35]. Alo's anticancer action is largely due to the specific activation of Caspase-3 in cancer cells. DNA fragmentation and cellular shrinkage are two hallmarks of programmed cell death that result from the activation of Caspase-3, the primary protein regulating apoptosis. The results also concur with observations made by researchers, who explained that executioner caspases, such as caspase-3, are crucial targets for cancer treatment since they constitute the last common pathway in apoptotic cell death [36]. But when A549 and HBL100 cells were treated with CP, their caspase-3 levels significantly increased in comparison to the control group. In one study, Niazmand investigated the effects of sitagliptin, either alone or in conjunction with paclitaxel, on the growth and metastasis of EOCs, or epithelial ovarian cancer cells.

MALONDIALDEHYDE (MDA) LEVELS

The MDA test was used in this study to assess the levels of oxidative stress that the monotherapy drugs and their combinations induced in tumor and normal cells [37]. Alo raised MDA levels in A549 cancer cells while lowering them in normal HBL100 cells, according to the evaluation of oxidative stress by MDA assay. Another way that Alo affects cancer cells specifically is through its differential action on oxidative stress [38]. According to research, increased oxidative stress in cancer cells might overwhelm their antioxidant defenses and cause apoptosis. As evidenced by the significantly lower levels of MDA in the group that was treated with alogliptin in comparison to the group that was not treated with the drug and the significantly higher levels of GSH in the group that was treated with the drug, alo had a nephroprotective effect in diabetic rats and may be considered a promising treatment for diabetic kidney disease. Alo may use the cancer cells' existing high levels of oxidative stress to cause selective cytotoxicity, as evidenced by the enhanced MDA levels after therapy [39]. This process is corroborated by researchers, who found that it is possible to control oxidative stress in the tumor microenvironment to encourage the death of cancer cells while preserving healthy tissues. Measuring oxidative stress biomarkers like MDA also provide important information about cellular damage and the effectiveness of treatments. According to a different study, pretreating human neuroblastoma cell line SH-SY5Y cells with the DPP4 inhibitor teneligliptin improved cell survival and decreased MPP+-induced cellular damage. Teneligliptin also prevented reactive oxygen species (ROS) from forming, restored glutathione (GSH), decreased malondialdehyde (MDA) levels, and prevented the neuroblastoma cells' mitochondrial membrane potential from degrading.

CONCLUSIONS

As a monotherapy, Alo exhibits a potent anticancer impact on A549 lung adenocarcinoma cells and selectively selects malignant cells over healthy HBL100 cells, according to the findings of this in vitro investigation. Numerous mechanisms, such as DPP4 inhibition, apoptotic pathway modulation, and increased oxidative stress levels, contribute to Alo's anticancer action. Alo demonstrated preferential toxicity against cancer by significantly increasing the levels of oxidative stress in the A549 cells both when used alone and in conjunction with CP. Alo showed antioxidant activity by dramatically lowering oxidative stress levels in HBL100 normal cells. Furthermore, apoptotic modulators (caspase-3 and BAX) were significantly reduced in Alo.

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Ethical considerations

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.

ACKNOWLEDGMENTS

The authors appreciate and thank everyone who helped with laboratory and theoretical devices.

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. Each author approved the final version of the manuscript for submission.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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RECEIVED: 14.07.2025
ACCEPTED: 29.01.2026