ORIGINAL ARTICLE

CONTENTS

Exploring the anti-tumor potential of saxagliptin in A549 lung adenocarcinoma cells

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ABSTRACT

Aim: To evaluate the anti-tumor potential of SAXA on A549 cells and assess its combinatory effects with CP on cell viability and apoptosis markers.

Materials and Methods: Four primary groups were utilized from A549 lung cancer cell lines: unprocessed Cells (control), cells subjected to CP treatment, cells subjected to SAXA treatment, and cells treated with CP plus SAXA, thereby obtained a combination of varying concentrations of CP and SAXA. Five used concentrations (62.5, 125, 250, 500 and 1000) µg/mL for SAXA and 0.9, 1.87, 3.75, 7.5, and 15 µg/mL for CP with four duplicates employed for each treated group. Incubated for 72hr., cells gathered, centrifuged, and supernatants were eliminated, while particles were gathered to determine BCL2 and BAX levels using ELISA test kits

Results: SAXA dramatically reduced A549 cell viability in a dose-dependent manner. The combination of SAXA and CP also displayed cytotoxicity; however, no synergistic effect was found above CP alone. Notably, the combined treatment dramatically lowered BCL2 levels ($p < 0.001$), but BAX levels remained stable ($p > 0.05$).

Conclusions: SAXA showed promising anti-cancer action against A549 cells. Although the combination with CP did not boost cytotoxicity, the observed pro-apoptotic reduction in BCL2 implies potential therapeutic efficacy.

KEY WORDS: SAXA, A549 cell-line, Cisplat, MTT assay, Anticancer.

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INTRODUCTION

Cancer is a group of diseases which identified by several defining characteristics, that include persistent cell proliferation, resistance to programmed cell death, promotion of blood vessel development, and invasion of surrounding healthy cells [1]. lung cancer is a type of cancer that begins when aberrant cells proliferate uncontrollably in the lungs [2]. Lung cells normally replicate under controlled condition in order to help in tissue repair and maintain general health. An increase in the frequency of cell division and a lack of control over cellular development, however, may lead to the development of a tumor [3]. Pulmonary carcinoma represents primary reason of Cancer related fatality and incidence globally, with approximately 2 million diagnoses and 1.8 million deaths reported. After prostate and breast cancer, respectively, lung neoplasms are the second most prevalent cancer diagnoses in both men and women [4]. Men are slightly more likely than women to be diagnosed with invasive cancer throughout their lifetime (41.6%) compared to (39.6%). also, there is thought that males are more likely to develop most types of cancer because they are exposed to more carcinogenic environmental and lifestyle variables, such smoking, yet a new study indicates that other unchangeable characteristics also have a significant effect [5]. In accordance with the most recent estimate from the global cancer observatory "GLOBOCAN", there were 2,206,771 recent cases of pulmonary carcinoma detected globally in 2020 [4]. An estimated 33,873 new cases of cancer were reported in Iraq in 2020, with 14,070 cases involving men and 19,803 involving women. 19,786 patients lost their lives by lung cancer [6]. Surgery, radiation, immunotherapy, and chemotherapy, including CP, are among the available therapeutic options [7]. Platinum based chemotherapy (CP) is typically used as the initial treatment for patients with non-small cell lung cancer [8-9]. In biological systems, anticancer medications cause oxidative stress, which results in lipid peroxidation and the production of several electrophilic aldehydes. Since oxidative stress prevents cancer cells from proliferating, its effects can increase the effectiveness of anticancer treatments [10]. CP's use and efficacy are restricted by two intrinsic problems: adverse effects and

drug resistance [11]. CP treatment adverse consequences include vestibulopathy, peripheral neuropathies (which are less common in younger patients), neurotoxicity that manifests as ototoxicity, and severe nephrotoxicity that results in end-stage dialysis [12]. It has been noted that 50% of CP-treated individuals either quickly develop multidrug resistance or develop intrinsic resistance [13]. Dipeptidyl peptidase-4 (DPP-4) inhibitors have recently been shown to have profound anticancer effects on cancer cells. Specifically, the US Federal Drug Agency (FDA) authorized sitagliptin, an anti-diabetic drug, as a DPP-4 inhibitor in 2006 [14]. DPP-4 inhibitors are a group of oral diabetic medications that work by blocking the DPP-4 enzyme. Numerous bioactive peptides, such as Glucagon like Peptide-1" GLP-1" and glucose dependent Insulinotropic Polypeptide "GIP", are disabled by the ubiquitous enzyme DPP-4. In addition to slowing stomach emptying time, inhibiting incorrect post-meal glucagon release, and reducing food intake, GLP-1 primarily works via inducing glucose-dependent insulin release from the pancreatic islets. Consequently, its blockage may have a variety of impacts on glucose regulation [15]. Recent study in 2024 showed that linagliptin (a member of DPP4 inhibitors) has anti-tumor effect on lung cancer by decreasing the cancer cell viability when treated with linagliptin IC50. Additionally, BCL2 level reduced following treatment of A549 cells with IC50 of linagliptin [7]. Other study in 2023 revealed that sitagliptin has an anti-apoptotic effect against Hepatocellular carcinoma based on BCL2 measurement on HepG2 cell line [14].

AIM

The purpose of this article is to evaluate the anti-tumor potential of SAXA on A549 cells and assess its combinatory effects with CP on cell viability and apoptosis markers.

MATERIALS AND METHODS

CHEMICALS AND CELL LINE

The Iraq Biotech Cell Bank Unit in Basrah provided the A549 lung cancer cells, passage number 20. A459 cells were generally obtained in January 1972 from a human alveolar-cell cancer in a 58-year-old white man. They were continuously propagated in vitro for more than three years, producing more than 1,000 cell generations that are frequently used as models for lung cancer [16]. HBL100 cells which is provided from Iraq Biotech Cell Bank Unit in Basrah. The milk of an apparently healthy lady was used to create the epithelial HBL-100 cell line in vitro. It has transformational properties from the start and progresses throughout vitro maintenance until it becomes tumorigenic in nude mice. This immortal cell line is a helpful model for researching the transition of human epithelial cells to cancer [17]. MTT (3-(4,5 Dimethylthiazole-2-yl)-2,5-Diphenyl-2H-tetrazolium Bromide) pigment powder, Dimethyl sulfoxide (DMSO) and RIPA buffer for lysis was ordered from Sigma, USA. Gibco, USA provided the phosphate buffer saline (PBS), 10% fetal bovine serum (FBS), and Roswell Park Memorial Institute-1640 (RPMI-1640). The UK-based Flow Laboratories provided the trypan-blue stain. The suppliers of streptomycin and benzoylpenicillin were Troge, Germany. Capricorn, USA, was the source of trypsin-EDTA. CP was ordered from Pfizer, USA. SAXA had been purchased from Targetmol pharmaceuticals Co, USA. From Bioassay Technology Laboratory of China.

CELL CULTURE AND MTT ASSAY

Trypsin-EDTA as a proteolytic protein, phosphate buffer saline (PBS), and fetal bovine serum (FBS) were used to extract the A549 and HBL100 cell lines. The cells were then incubated in 96 well plate using Roswell Park Memorial Institute-1640 liquid medium containing 100units/mL Penicillin, and 100µg/mL Streptomycin. The sample was incubated for 24 hours at 37 °C, with 5 % Co2, and 95 % humidity, to facilitate formation of the monolayer of cells (80 % growth phase). After that, 200 µL of medium—which contains the test medications and control group—was used to substitute the prior medium [18]. Four main groups were used: cells that were left untreated (control), cells that were treated with CP, cells that were treated with SAXA, and cells that were treated with CP + SAXA, which obtained a combination of CP and SAXA at varying concentrations. Five different concentrations (62.5, 125, 250, 500 and 1000 µg/mL) for SAXA and 0.9, 1.87, 3.75, 7.5, and 15 µg/mL for CP with four duplicates employed for each treated group. The non-specific conversion between formazan and the studied medications was assessed using a blank (which contained only medium). Incubation for 72 hr., then the (4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2Htetrazolium bromide (MTT) test [19] used for determining cell viability, and Non-linear regression utilizing four-parameter logistic Hill equation was utilized to compute dose-effect curves. For every group, the IC50 (concentration required for 50% suppression of cell viability) was determined using GraphPad Prism 10. Subsequent equation was employed to get the percentile of cell viability:

Cell Viability % = (AS-AB)/(AC-AB) × 100 %

Sample absorbance represented by AS, control absorbance represented by AC, and Ab is the absorbing

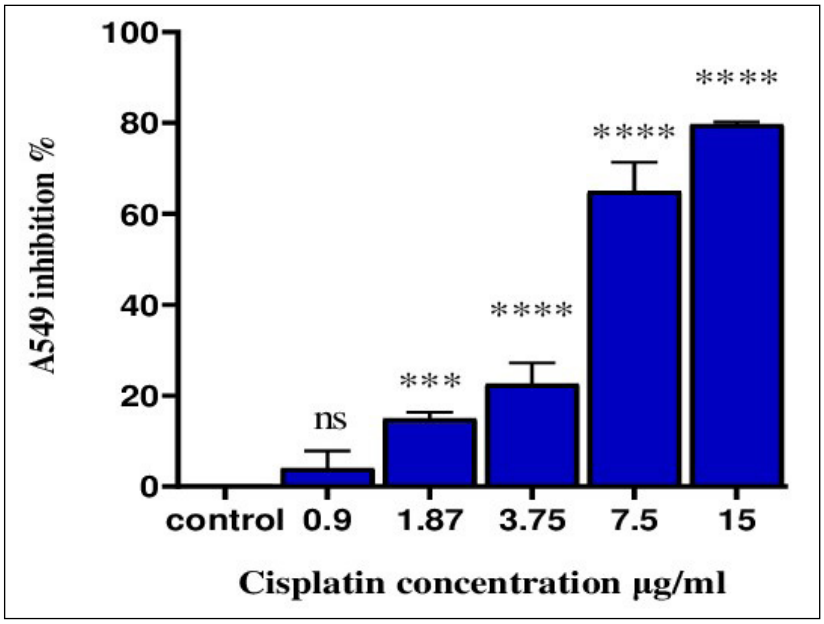

Fig. 1. Cytotoxic effect of different cisplatin concentrations on"A549" cell line. For the examination, (one way) Anova was used. Data are displayed as mean ± SD, ns (P>0.05), ***P<0.001, ****P<0.0001, n=4, 72 hours incubation
Source: Own materials

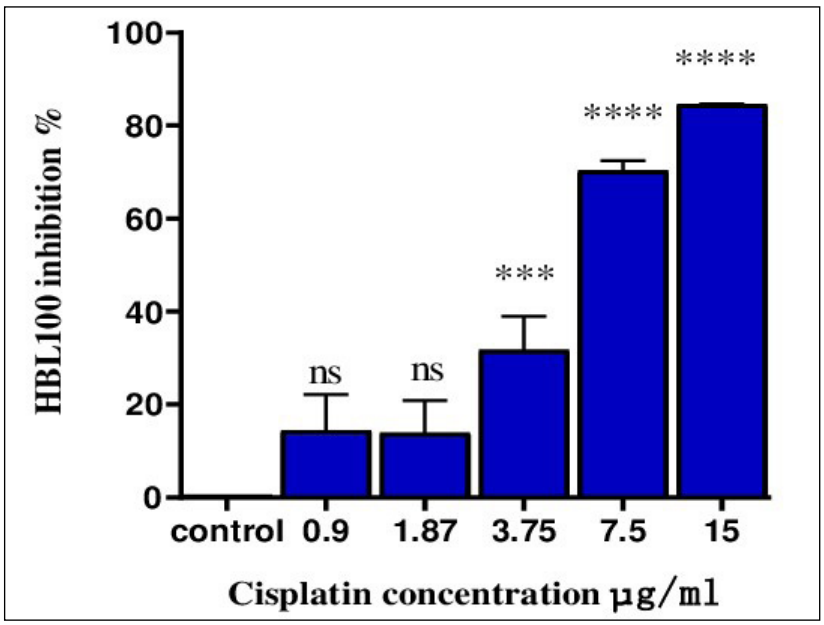

Fig. 2. Cytotoxic effect of different cisplatin concentrations against HBL100 cell line. For the examination, (one way) Anova was used. Data displayed as mean ± SD, ns =P>0.05, ***P<0.001, ****P<0.0001, n=4, 72 hours incubation
Source: Own materials

capacity of the blank. Four copies of each determination were made. The following formula was used to estimate the inhibition rate, or the percentage of cytotoxicity [20].

Inhibition%=100-Viability x 100%

EVALUATION OF BCL2 CONCENTRATION

A549 and HBL100 cells were cultivated in four flasks and subjected to the IC50 of Cp, SAXA, and IC50 of CP plus SAXA for 36 hours. Following treatment, the supernatant was extracted from the cells after they were harvested and centrifuged. After extracting the proteins from the cell pellets using a lysis buffer, they were inserted into an Eppendorf sterile tube (1.5 mL) and kept at –20 °C until they could be examined with a Bcl2 ELISA test kit. ELISA kit for human Bcl2 ordered from SUNLOG Biotech CO "Hang Zhou, China" was utilized for examination. The testing procedure was conducted in accordance with the SUNLOG Biotech CO protocol. The absorbance value for each well was measured with a microplate reader calculated to 450 nm.

EVALUATION OF BAX CONCENTRATION

The extracted protein that mentioned in the previous paragraph was examined with a BAX ELISA assay kit, which is obtained from SUNLOG Biotech CO (Hang Zhou, China). The SUNLOG Biotech CO methodology was followed for conducting the test. To determine each well's absorbance value, a microplate reader set to 450 nm was used.

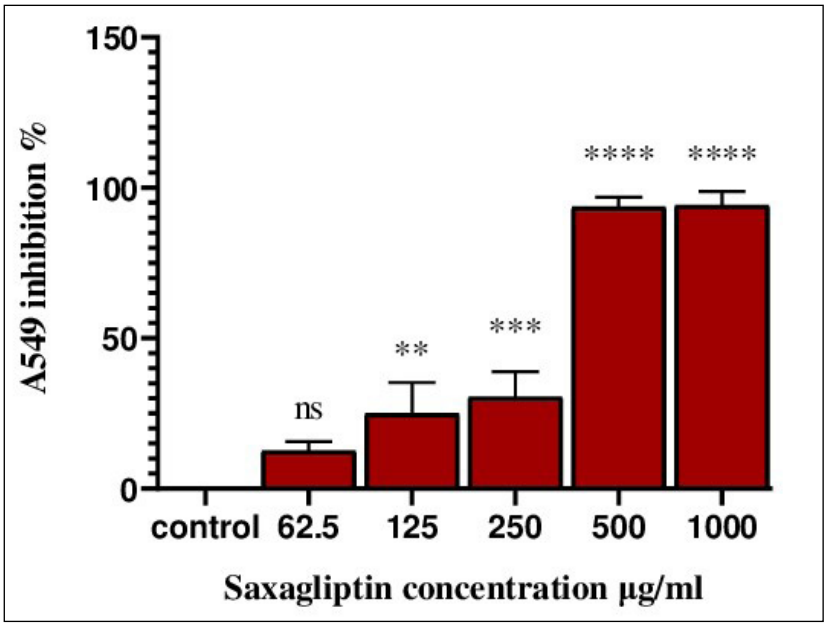

Fig. 3. Cytotoxic effect of different concentrations of saxagliptin against "A549" cell line . For the examination, (one way) Anova was used. Data displayed as mean ± SD, ns=P 0.05, **p<0.01, ***P<0.001, ****p<0.0001, n=4, 72 hours incubation
Source: Own materials

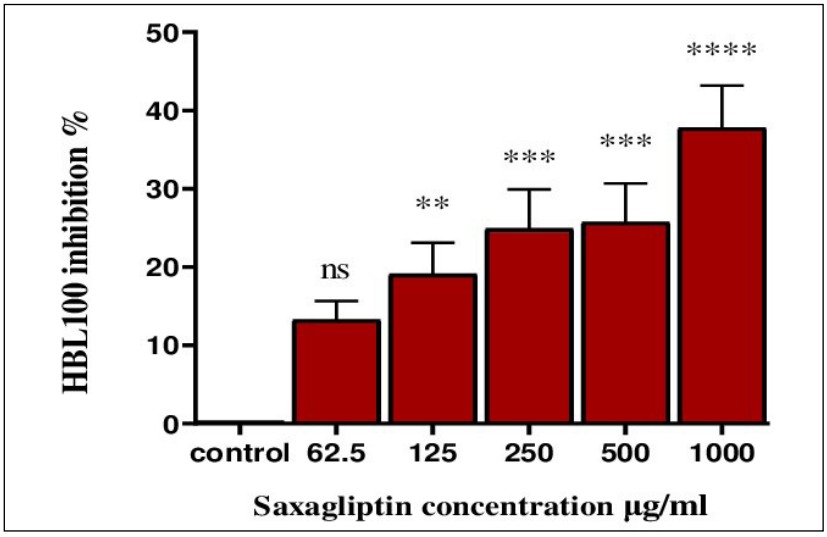

Fig. 4. Cytotoxic effect of different concentrations of saxagliptin against HBL100 cell line. For the examination, (one way) Anova was used. Data displayed as mean ± SD, ns=P>0.05, **P<0.01, ***p 0.001, ****P<0.0001, n= 4, 72 hours incubation
Source: Own materials

STATISTICAL ANALYSIS

The results were gathered and analyzed utilizing GraphPad Prism Edition10 and Microsoft Office Excel2019. Significant variations between the data means were evaluated using a post hoc (Tukey) analysis and one-way ANOVA test. A p-value of 0.05 or lower signifies a statistically significant variance.

RESULTS

CISPLATIN CYTOTOXICITY AGAINST A549 AND HBL100 CELL LINE

Cisplatin showed no significant cytotoxicity in concentration 0.9 µg/mL for both A549 and HBL100 cell lines. At 1.87 µg/mL, CP revealed significant cytotoxic activity in A549 cell line (p= 0.0003) while in HBL100 there is no significant cytotoxicity. Other concentrations (3.75, 7.5, 15 µg/mL) displayed a significant inhibition in A549 and HBL100 cell lines viability (P < 0.0001) in comparison to control group, Figures (1-2).

SAXAGLIPTIN CYTOTOXIC EFFECT AGAINST A549 AND HBL100 CELL LINES

SAXA demonstrated dose-dependent cytotoxicity against A549 lung cancer cells and HBL100 normal cell lines. SAXA demonstrated no notable cytotoxicity at 62.5 µg/mL in both A549 (lung cancer) and HBL100 (non-tumorigenic breast) cell lines; nevertheless, significant decrease of cell viability commenced at 125 µg/mL (p<0.01). SAXA had notable cytotoxic effects on A549 and HBL100 cell lines at a dose of 250 µg/mL (P>0.001). In addition, SAXA at a dose of 500 µg/mL significantly inhibited cell viability in the A549 and HBL100 cell lines, with p-values of < 0.0001 and <0.001,

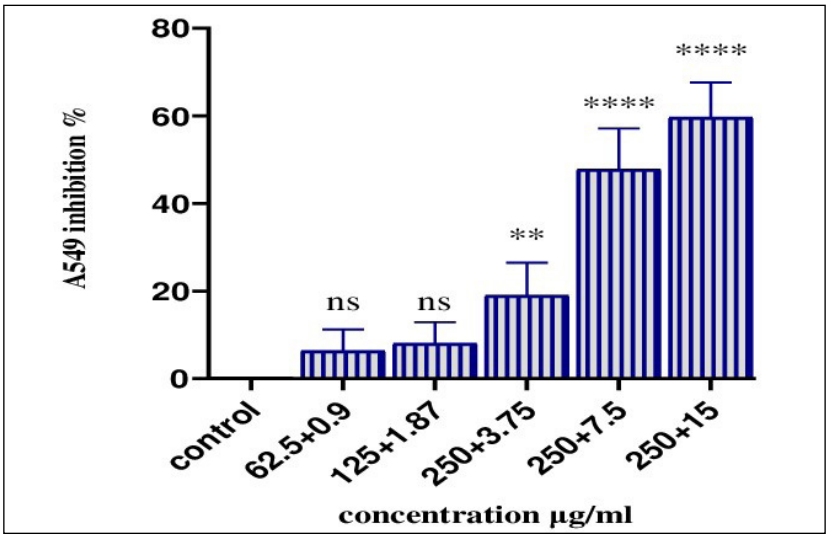

Fig. 5. Cytotoxicity Of Different Concentrations Of saxagliptin plus cisplatin against "A549" Cell Line. For the examination, (one-way) Anova was used. Data displayed as mean ± SD, ns=P>0.05, **p<0.01, *** P<0.0001, n= 4, 72-hours incubation
Source: Own materials

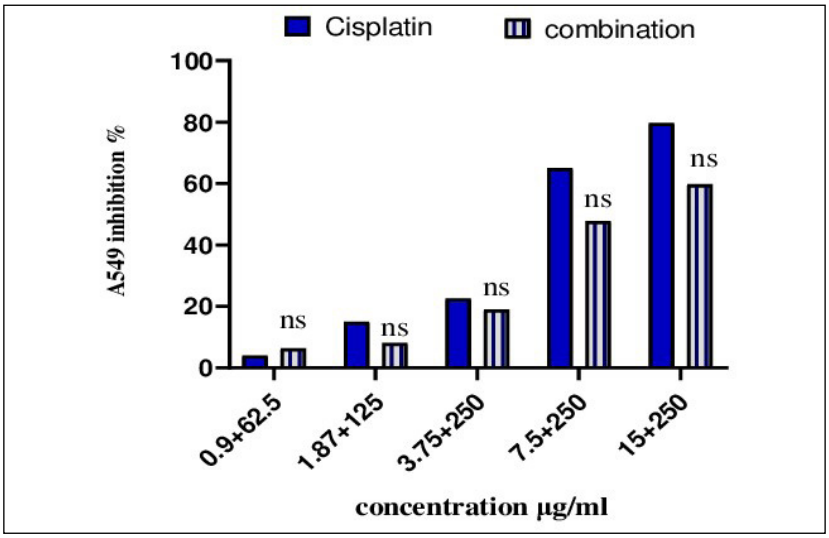

Fig. 6. Comparison between cytotoxicity of cisplatin alone against cisplatin plus saxagliptin Combination on "A549" Cell line. For the examination, (one way) Anova was used. Data displayed as mean ± SD, ns=P>0.05, n=4, 72 hours incubation
Source: Own materials

respectively. Ultimately, both cell lines exhibited comparable responses at the maximum dose (1000 µg/mL, p<0.0001), Figures (3-4).

CYTOTOXIC ACTIVITY OF A COMBINATION OF CISPLATIN PLUS SAXAGLIPTIN ON A549 CELL LINE

The combination of CP and SAXA demonstrated a dose-proportional cytotoxic effect on A549 lung adenocarcinoma cells, with significant anti-cancer activity observed at higher concentrations. At the lowest tested doses (0.9 µg/mL CP + 62.5 µg/mL SAXA and 1.87 µg/mL CP + 125 µg/mL SAXA), no significant cytotoxicity was detected. However, at 3.75 µg/mL CP + 250 µg/mL SAXA, a statistically significant cytotoxic effect was observed (P < 0.01). Most notably, at the highest tested concentrations (7.5 µg/mL CP + 250 µg/mL SAXA and 15 µg/mL CP + 250 µg/mL SAXA), the combination induced highly significant cytotoxicity (P < 0.0001) compared to the control group, Figure (5).

COMPARISON BETWEEN THE CYTOTOXICITY OF CISPLATIN ALONE AGAINST CISPLATIN PLUS SITAGLIPTIN COMBINATION ON THE A549 CELL LINE

Figure (6) demonstrates a comparison between the cytotoxicity of CP alone versus SAXA plus CP combination against A549 cells at various concentrations. Combination between SAXA and CP did not significantly increase the cytotoxic effect against A549 (P>0.05), in comparison with CP alone.

MEASUREMENT OF HUMAN BCL2 LEVELS

CISPLATIN AND SAXAGLIPTIN EFFECT ON BCL2 LEVEL

The results of the investigation showed that after treating A549 cells with IC50 of CP and SAXA, the level of BCL2 decreased with P value (<0.001) for CP and (<0.01) for SAXA compared with control group, Figure (7).

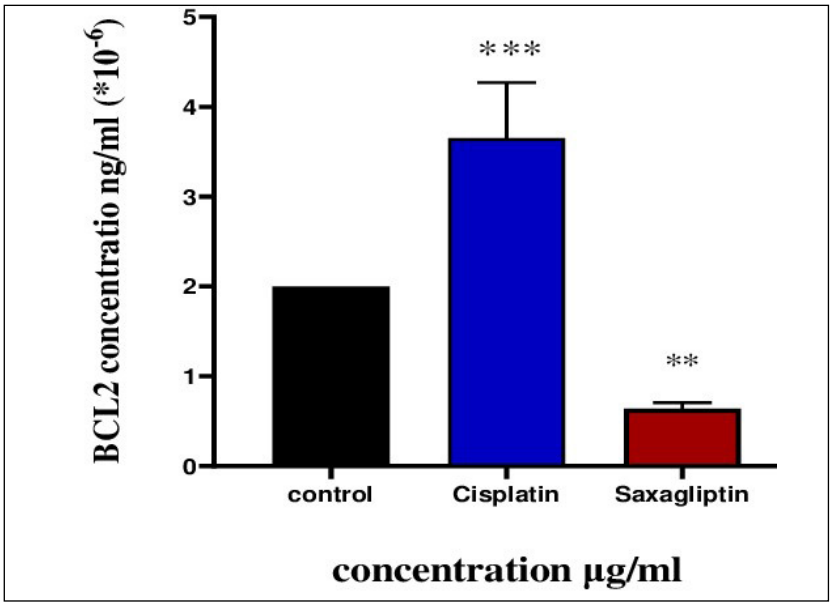

Fig. 7. Cisplatin and saxagliptin activity on BCL2 concentration in "A549" cell line for the examination, one way Anova was used. Data displayed as mean ± SD, **p<0.01, ***P<0.001 Compared with Control
Source: Own materials

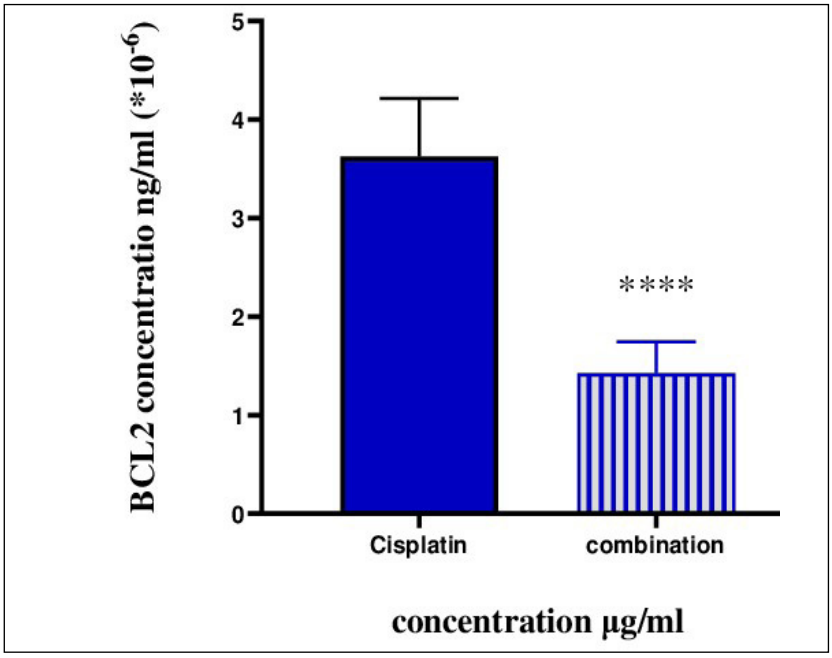

Fig. 8. Comparison between the activity of cisplatin alone and cisplatin plus saxagliptin on BCL2 concentration in "A549" Cell Line. For the examination, (one way) Anova was used. Data displayed as mean ± SD, ****P<0.0001 Compared to Cisplatin
Source: Own materials

COMPARISON BETWEEN THE EFFECTS OF CISPLATIN ALONE AGAINST CISPLATIN PLUS SAXAGLIPTIN COMBINATIONS ON THE BCL2 LEVEL

The results of the study showed that after treating A549 cells with IC50 of the combination CP plus SAXA, the level of BCL2 decreased p<0.0001 in comparison to the CP alone, Figure (8).

CISPLATIN AND SAXAGLIPTIN EFFECT ON BCL2 LEVEL

The results of the investigation showed that after treating A549 cells with IC50 of CP, there is no significant increase in BAX level (P > 0.05) in comparison to the control group. At the same time, the findings showed there is a Significant increase In BAX level P<0.0001 after exposing A 549 to the Ic50 Of SAXA, Compared with Control group, Figure (9).

COMPARISON BETWEEN THE EFFECTS OF CISPLATIN ALONE AGAINST CISPLATIN PLUS SAXAGLIPTIN COMBINATIONS ON THE BAX LEVEL

The results of the study revealed that after treating A549 cells with IC50 of the combination CP plus SAXA, there is no significant increase in BAX level P>0.05 in comparison to the CP alone, Figure (10).

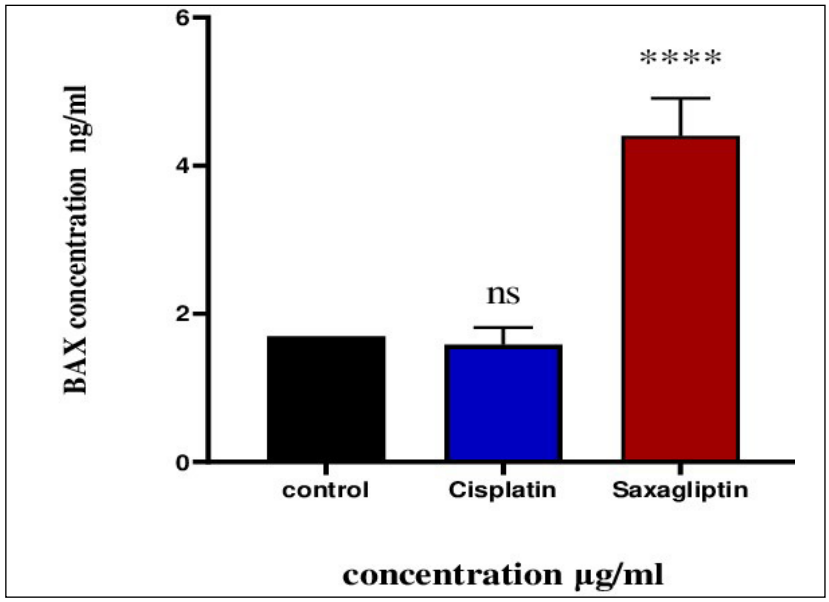

Fig. 9. Cisplatin and saxagliptin "activity" On BAX concentration in "A549" Cell Line. For the examination, (one way) Anova was used. Data displayed as mean ± SD, ****P<0.0001, ns (P>0.05) Compared with control
Source: Own materials

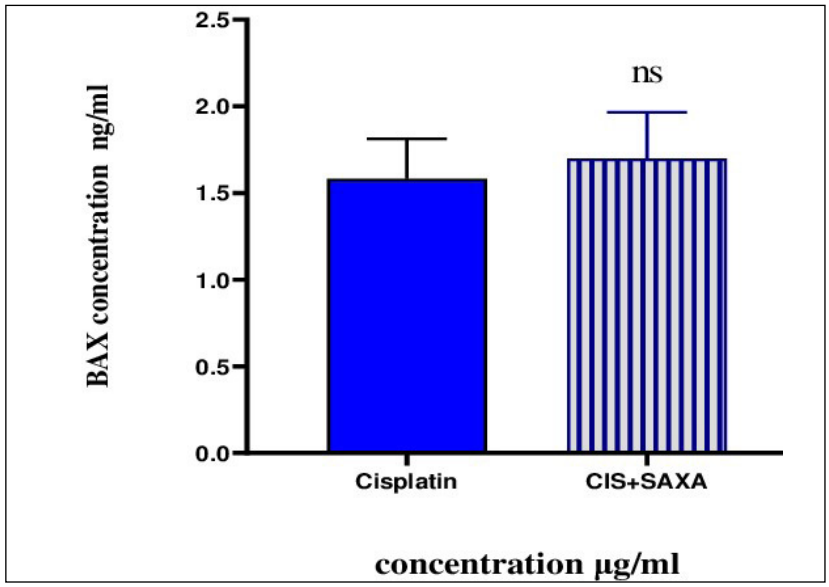

Fig. 10. Comparison between the activity of cisplatin alone and cisplatin plus saxagliptin on BAX Concentration In "A549" Cell Line for the examination, (one way). Anova was used. Data Displayed as mean ± SD, ns (P>0.05) Compared with Cisplatin
Source: Own materials

DISCUSSION

The primary obstacles in the treatment of cancer are drug side effects and treatment resistance, which cause Over 90% of deaths occur in patients receiving chemotherapy [21]. The current research sought to evaluate the cancer suppression activity of SAXA on Lung Cancer cells (A549), alone and combined with CP and comparison with HBL100 cells. This was achieved by employing the MTT assay to evaluate the cancer cells' toxicity and vitality. The anticancer properties of CP have already received approval, and this Chemotherapy Is commonly utilized in the treatment of pulmonary carcinoma. CP's main biological target is DNA [22]. As part of its cytotoxic effect on tumor cells, CP disrupts DNA synthesis and repair processes in cancer cells. It creates 1–2 and 1–3 intra-strand adducts and inter-strand cross-links between purine bases by forming covalent bonds at the N7 position of adenine and guanine. DNA adduct halts the cell cycle at the G2 phase and trigger apoptosis by inhibiting DNA replication [23]. Our study found that SAXA, a drug typically used to treat diabetes, shows promising anti-cancer effects against A549 lung cancer cells in the lab. This discovery adds to a growing body of research suggesting that DPP-4 inhibitors—commonly prescribed for type 2 diabetes - might also play a role in fighting cancer. A prior study by [24] indicates that the Expression of DPP 4 enzyme was significantly elevated in pulmonary carcinoma when compared with normal lung tissue. Therefore, it seems That DPP 4 Inhibitors may be able to impede the growth of lung cancer by blocking the related DPP4 enzyme. The current study findings indicate that SAXA exhibit significant

anti-cancer activity against A549 cells in comparison to the control group, as assessed by MTT assay. These findings agree with that stated by [25], the MTT assay was utilized to assess the anti-neoplastic properties Of DPP 4 Inhibitors SAXA and sitagliptin (SITA) on human ovary (A2780), human breast (MCF-7), and human prostate (PC-3 and LNCaP) cancer cell lines. The results indicated that both drugs exhibited significant beneficial effects on cancer in comparison with Control, which functioned as a Cytotoxic chemical to cancerous cells. Furthermore, retrospective studies indicated that sitagliptin may decrease the risk of breast cancer in type 2 diabetic patients after one year of use, and it may also reduce the risk of prostate and oral cancer. The effects of sitagliptin may be influenced by the dosage and duration of your treatment [26]. Additionally, a study published in 2024 showed that DPP-4 suppression has the potential to augment anti-cancer immune responses through the enhance functioning of cDC1s (type 1 conventional dendritic cells). For instance, sitagliptin improved cDC1 antigen presentation, which Aided T cell activation and the consequent suppression of tumors [27]. Given the safety profile of DPP-4 inhibitors in diabetic patients, their repurposing for cancer therapy could offer a low-toxicity adjunct treatment option.

EFFECT OF COMBINATION THERAPY ON MTT ASSAY

Using combination of CP plus SAXA demonstrated a concentration-dependent cytotoxic effect on A549 lung cancer cell line, with significant anti-cancer activity seen at higher concentrations. At the lowest tested concentrations (0.9 µg/mL CP + 62.5 µg/mL SAXA and 1.87 µg/mL CP + 125 µg/mL SAXA), no significant cytotoxicity was observed, suggesting that a threshold concentration must be reached for the drugs to exert a measurable anti-cancer effect. However, at 3.75 µg/mL CP+ 250 µg/mL SAXA, a statistically significant cytotoxic effect was observed ($P<0.05$). Most notably, at the highest tested concentrations (7.5 µg/mL CP + 250 µg/mL SAXA and 15 µg/mL CP + 250 µg/mL SAXA), the combination induced highly significant cytotoxicity ($P < 0.0001$) compared to the control group.

EFFECT OF COMBINATION THERAPY VERSUS CISPLATIN ALONE ON MTT ASSAY

The observed lack of a significant increase in the cytotoxic effect of CP when combined with SAXA against A549 cells suggests a potential no synergism between the two drugs. This finding aligns with prior research indicating that dipeptidyl peptidase-4 (DPP-4) inhibitors, such as sitagliptin, may interfere with the anticancer activity of chemotherapeutic agents [28] demonstrated that sitagliptin, when used alone, modulated proteins associated with metastasis and apoptosis in SKOV-3 ovarian cancer cells. However, in combination with paclitaxel, sitagliptin reduced the cytotoxic efficacy of the chemotherapy drug at certain concentrations. No synergism could be attributed to the complex biological roles of DPP-4, which is involved not only in glucose metabolism but also in immune regulation, cell adhesion, and apoptosis [29-30]. DPP-4 inhibitors may interfere with these pathways, potentially counteracting the pro-apoptotic or cytotoxic effects of chemotherapy agents like CP or paclitaxel. One possible explanation for the no synergism effect is that DPP-4 inhibition may alter intracellular signaling pathways that are critical for CP-induced apoptosis. For instance, DPP-4 has been implicated in the regulation of NF-κB, PI3K/Akt, and MAPK pathways, which are also targeted by CP [31], If SAXA modulates these pathways in a way that promotes cell survival or reduces drug uptake, it could diminish CP's efficacy. Additionally, SAXA might influence the tumor microenvironment by affecting immune cell activity or cytokine production, indirectly reducing CP's cytotoxic impact. Further mechanistic studies are needed to elucidate the exact molecular interactions between SAXA and CP. Evaluating changes in key apoptotic markers (e.g., caspase activation, Bcl-2 family proteins) or drug transport mechanisms (e.g., copper transporter CTR1, which is involved in CP uptake) could provide insights into the observed no synergism. Additionally, exploring different dosing regimens or sequences of administration might help determine whether the interaction is schedule dependent. Based on that, the combination of SAXA and CP does not enhance cytotoxicity in A549 cells and may exhibit antagonism, consistent with previous findings on DPP-4 inhibitors and chemotherapeutic agents. These results highlight the need for careful evaluation when combining antidiabetic drugs like SAXA with anticancer therapies, as their interaction may inadvertently reduce treatment efficacy. Future studies should investigate whether this effect is cell line-specific or a broader phenomenon affecting other cancer types.

EFFECT ON BCL2

This study revealed that A549 cells were somewhat resistant to CP treatment. This was explained by the fact that A459 cells treated with CP had higher levels of BCL2 than the control group. These results are similar to study that displayed the development of CP resistance in a variety of malignancies was facilitated by Bcl-2. Such as in non-small cell lung cancer (NSCLC), THE

cytoplasmic repressor/activator protein-1-mediated CP resistance was linked to an increase in Bcl-2 level [32]. These results are supported by a study displayed CP resistance by A549 cells which are outlined by the increasing BCL2 level in comparison to control group in A549 cells exposed to CP's IC50 [7]. Concerning the effect of SAXA on BCL2 level, our findings indicate that SAXA exerts varying effects on BCL2 expression based upon the cell type. SAXA treatment in A549 cells results in a notable reduction in the anti-apoptotic protein BCL2. The reduction in BCL2 level indicates the pro apoptotic effect of SAXA that had selectivity toward cancer cells. In parallel with previous findings, LINA inhibits colorectal cancer cell growth by facilitating cell apoptosis via cell cycle arrest and the inhibition of BCL-2 expression [33]. However, when A549 cells were treated with CP plus SAXA combination, the BCL2 level decreased dramatically compared with those treated with CP's IC50 alone. This result potentiating CP cytotoxicity on cancer cells and exhibited synergistic effect on A549 cells. These results are supported by a study that demonstrated reduced BCL2 level in HepG2 liver cancer cell line when exposed to sitagliptin plus CP in comparison to control and CP alone [14].

EFFECT ON BAX

This study showed that A549 cells were somewhat resistant to CP treatment. This was explained by the fact that A459 cells treated with CP had no change than the control group. This aligns with previous research by [34], who observed that CP alone did not induce a noticeable increase in BAX (a pro-apoptotic protein) levels, suggesting a mechanism behind CP resistance in tumor cells. Resistance to CP is a well-documented challenge in cancer therapy, often attributed to defective apoptotic signaling, enhanced DNA repair mechanisms, or increased drug efflux [35]. Concerning the impact of SAXA on A549 cells, our results show that SAXA has a pro-apoptotic effect on A549 cells, as demonstrated by the substantial rise in BAX levels after treatment with its IC50. This indicates that SAXA may facilitate programmed cell death in this lung adenocarcinoma cell line. Remarkably, other cancer types have also been shown to have the pro-apoptotic effect of DPP4 inhibition, which is SAXA's target. [36] a study on Papillary thyroid carcinoma (PTC) cells, revealing that DPP4 silencing resulted in decreased levels of the anti-apoptotic marker BCl-2 and diminished phosphorylation of ERK1/2, JNK1, and P38 MAPK pathways. Concurrently, there was an increase in the expression of E-cadherin and the pro-apoptotic protein BAX. Additionally, our study showed that there was no discernible change in BAX levels as compared to the control when A549 cell line were treated with IC50 of the combination of SAXA and CP. This observation is consistent with the results of the MTT assay, which demonstrated that the two medications did not exhibit any synergistic cytotoxic effects. Notably, no recent studies addressing this specific interaction on BAX levels in this cell type were found, making this study an original contribution to our understanding of the combined impact of SAXA and CP on BAX expression in A549 cell lines. To conclude, SAXA, a DPP-4 inhibitor, demonstrated anti-cancer effects against A549 cells in the MTT assay, indicating its potential as a therapeutic agent. However, when combined with CP, no synergism effect was observed, reducing the expected cytotoxic impact. This suggests that SAXA may interfere with CP's mechanism of action, possibly by modulating survival pathways or altering drug uptake. Despite the no synergism, SAXA has a pro-apoptotic effect on A549 cells, as demonstrated by the substantial rise in BAX levels and reduction in BCL2 level, SAXA plus CP combination exhibited pro-apoptotic activity, as indicated by reduced BCL2 levels (an anti-apoptotic protein).

FUTURE DIRECTIONS

Further research is needed to:

- Elucidate the exact molecular mechanisms (e.g., immune modulation, apoptosis induction, or metabolic effects).
- Validate these findings in vivo using animal models.
- Assess the efficacy of SAXA and other DPP-4 inhibitors in lung cancer patients, particularly in combination with standard therapies.
- Assess the alternative combinations (If sequential, rather than concurrent, administration improve efficacy?

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CONFLICT OF INTEREST

The Authors declares no conflict of interest

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