RESEARCH ARTICLE

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Synergistic Cytotoxic Impact of Linagliptin - Ciprofloxacin Combination on Cervical Cancer Cell Line: Insights into Targeting Heat Shock Protein 60

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Abstract

Objective: This study aimed to assess the combined impact of linagliptin and ciprofloxacin on inhibiting cervical cancer cell line proliferation and their ability to target heat shock protein 60. **Methods:** The anticancer properties of the linagliptin-ciprofloxacin combination were assessed employing the HeLa cervical cancer cell line, with incubation periods of 24 and 72 hours. The human fibroblast cell line (HFF) was utilized to evaluate the mixture's safety. The concentrations of linagliptin, ciprofloxacin, and their combination varied between 0.1 and 1000 µg/ml. combination index value was estimated to assess the potential synergistic impact of linagliptin and ciprofloxacin. The study also employs computational molecular docking simulations to evaluate the affinity of linagliptin and ciprofloxacin for binding to heat shock protein 60. **Results:** The study's findings demonstrated that the combination of linagliptin and ciprofloxacin markedly inhibited the proliferation of cervical cancer cells. The inhibitory effect depended on the concentration of the mixture and the incubation duration. It concurrently exhibits a diminished impact on the viability of the HFF cell line. The combination index study indicates that the interaction between linagliptin and ciprofloxacin shows a synergistic effect across all concentrations, particularly after 24 hours of incubation. The computational molecular docking simulation demonstrated that linagliptin and ciprofloxacin can bind with Hsp 60. The docking scores for linagliptin and ciprofloxacin were recorded at -7.6 kcal/mol and -8.1 kcal/mol, respectively. **Conclusion:** Our study findings from the MTT assay, combination index, and computational docking simulations indicate that the combination of linagliptin and ciprofloxacin presents a promising option for treating cervical cancer, considering their defined adverse effects and pharmacokinetic profiles.

Keywords: Linagliptin- ciprofloxacin- cervical cancer- Hela cell line- combination index- heat shock protein 60

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Introduction

Each year, approximately 500,000 women are diagnosed with cervical cancer, resulting in more than 300,000 deaths worldwide. Approximately 90% of cervical cancer cases occur in low- and middle-income nations. Structured screening initiatives have led to an approximate 50% reduction in the incidence and death rates of cervical cancer in high-income countries during the last three decades. The severity of the illness affects treatment options upon diagnosis and the accessibility of local resources. A radical hysterectomy, chemotherapy, or a combination of both may be required [1]. Multiple randomized clinical investigations demonstrated Women with a diagnosis of invasive cervical cancer who are eligible for radiation therapy should opt for concurrent cisplatin-based chemoradiotherapy rather than radiotherapy alone [2-7], A review of 18 research trials across 11 countries demonstrates that combination chemoradiation has a favorable impact on prognosis. The research showed a 12% enhancement in overall survival rates and advancements in managing local and distant disease progression [8-12].

Chemoradiotherapy is considered a primary treatment for cervical cancer; however, the adverse effects linked to chemotherapy highlight the need for safer alternatives. Multiple trials have been undertaken to identify an effective treatment for cervical cancer by repurposing a medication initially designed for another therapeutic use [13-16]. Linagliptin and ciprofloxacin are two drugs that may possess anticancer properties. Several studies have been performed on this topic.

The diabetic drug linagliptin exhibits its ability to inhibit the survival, growth, and movement of

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Glioblastoma multiforme cancer cells. This impact is achieved via a mechanism that regulates the levels of phosphorylated NF-kB, proteins that govern the cell cycle, and proteins involved in cell adhesion [17, 18]. Linagliptin showed the capacity to reduce the viability of osteosarcoma cancer cells by a hypothesized mechanism involving the activation of the apoptotic pathway [19]. A separate study indicated that linagliptin effectively inhibits the proliferation of cervical cancer cells in a time-dependent manner, operating through a mechanism that involves targeting human Hsp 90 [20].

Noteworthy ciprofloxacin at a dose rather than an antibacterial dose shows the capability to lower the duplication of particular cancer cells, such as (colorectal, bladder and prostate) neoplasm, via operating the (pro-apoptotic) mechanism [20-22]. Ciprofloxacin has been studied on various cell lines in vitro, suggesting its potential application for cancer patients through a mechanism involving the induction of apoptosis, the cell cycle's arrest, and the disruption of mitochondrial membrane potential [23] .

Recent studies demonstrate that heat shock proteins (HSPs) are frequently overexpressed in various cancer types [24-26] . Recent studies demonstrate that heat shock proteins (HSPs) are crucial in tumor cell proliferation, invasion, differentiation, metastasis, and apoptosis. The main finding is that these proteins resulted in the overexpression of multiple tumors [27]. Androgen receptors play a significant role in the carcinogenesis and progression of prostate cancer. HSP27 influences the androgen receptor's stability, nuclear translocation, and transcriptional activity [28, 29]. Moreover, Hsp27 is crucial for the epithelial-to-mesenchymal transition induced by the epidermal growth factor through regulating the β-catenin/Slug signalling pathway [30]. HSP70 and HSP90 interact with WASF3, a protein associated with the invasion and metastasis of prostate cancer [31].

Hsp 60 plays a crucial role in cancer development. It is vital in transporting and folding mitochondrial proteins and is associated with various cancer types [32]. Numerous studies indicate that HSP60 plays a role in apoptosis by promoting the activation of pro-caspase-3 through various caspases, such as caspase-6. HSP60, located in the cytosol, prevents the translocation of the pro-apoptotic protein Bax into mitochondria, thereby promoting cell survival [33].

The prognostic association of HSP60 with cervical cancer has recently emerged as a crucial area of investigation. The studies conducted assessed the prognostic relevance of HSP60 in cervical cancer through the application of 2-dimensional Electrophoresis (2-DE), semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR), and Western Blot (WB) analyses. The findings indicate that HSP60 is essential in the progression of cervical cancer [34]. Data from patients with advanced prostate cancer revealed a significant correlation between HSP60 expression and tumor progression. The expression of HSP 60 shows a substantial correlation with androgen independence in cases of locally advanced prostate cancer. The extent and range of HSP60 immunoreactivity functioned as indicators for biochemical recurrence in prostate cancer patients. This study indicated that patients exhibiting intense HSP60 staining in biopsy samples had shorter recurrence-free survival than those with weak HSP60 expression. An analysis of patients with prostate cancer demonstrated that HSP60 expression is elevated in prostate cancer tissues relative to normal prostatic tissue [35-38].

Integrating existing medications for non-cancer therapeutic applications offers a promising strategy for developing effective cancer treatments that minimize adverse effects and combat resistance in cancer cells. Numerous studies have explored this subject, with one showing that the pairing of amygdalin and esomeprazole successfully eliminates cervical cancer cells. The success of this combination relied on the medication's concentration and the incubation period [39, 40]. Another study demonstrated that the combination of laetrile and vinblastine significantly reduced the growth of esophageal cancer, exhibiting a synergistic pattern between the components of the mixture [41-43]. At the same time, another study suggested that combining ciprofloxacin and laetrile effectively hinders the growth of esophageal cancer cells [44]. Many studies have investigated this issue; however, they have limitations in illustrating the anticancer properties of the linagliptin–ciprofloxacin combination and their capacity to target Hsp60 in cancer cells. This study investigates the inhibitory effects of the linagliptin–ciprofloxacin combination on the proliferation of cervical cancer cells and evaluates their targeting of Hsp60.

Materials and Methods

Study medications

The Samarra Pharmaceutical Factory provided linagliptin and ciprofloxacin as raw materials. The medications were diluted with RPMI medium to achieve concentrations ranging from 0.1 μg/ml to 1000 μg/ml.

Study cell lines

The HeLa cell line, derived from a malignant cervical carcinoma, and the HFF cell line, originating from human fibroblasts, were initially established at the tissue culture section of ICCMGR. The cells were cultured in 75 cm^2 tissue culture flasks under controlled conditions, maintaining a relative humidity of 37°C and 5% CO2. The cells were incubated in RPMI-1640 medium (Sigma Chemicals, England) containing 10% fetal bovine serum (FBS) and 100 U/mL of penicillin-streptomycin (100 μg/mL streptomycin) [39, 45].

Cytotoxic study

Linagliptin, ciprofloxacin, and a combination were utilized to assess their efficacy in inhibiting cervical cancer cells cultured in a 96-well microtiter plate. The increase in cancer cell proliferation was consistent and gradual throughout the logarithmic growth phase. The toxicity of the evaluated medications was examined over two distinct incubation periods: 24 hours and 72 hours [46].

Each well contains 10,000 cells. Seeding requires a medium containing 10% fetal bovine serum. The plates

underwent 24 hours of incubation at 37°C to facilitate cell attachment. Serial dilutions were performed using a serum-free RPMI medium. Linagliptin, ciprofloxacin, and their combination were diluted in RPMI medium devoid of calf serum. A series of dilutions for each medication was prepared, ranging from 0.1 to 1,000 µg/ml [44, 47].

After 24 hours of cancer cell proliferation, each treatment concentration was assigned to six wells, each receiving 200 µl of RPMI media containing the medication. Control wells received 200 microlitres of maintenance media, with exposure durations varying between 24 and 72 hours. The plates were reinserted into the incubator after being securely affixed with a self-adhesive material. The cells were subsequently treated with MTT dye.

A microtiter plate reader (ELISA reader) was utilized to assess the optical density of each well at a transmission wavelength of 550 nm.

[48, 49] .

A mathematical equation utilized to determine the growth inhibition rate is: [49]

$$Growth\ inhibition\ \% = \frac{optical\ density\ of\ control\ wells - optical\ density\ of\ treated\ wells}{optical\ density\ of\ control\ wells} * 100\%$$

Drug combinations assessment

A study was conducted to investigate the integration of mixture components. The evaluation involved generating concentration-effect curves, which plotted the proportion of cells showing reduced growth against drug concentration after 24 and 72 hours of treatment. The interaction of medications was assessed for synergy, additive effects, and antagonism using Compusyn software (Biosoft, Ferguson, MO, USA), which calculated the combination index and dose reduction index values.

CI values less than 1 indicate synergy, values greater than 1 signify additivity, and values above 1 represent antagonism. The dose reduction index (DRI) quantifies the extent to which the concentration of individual components in a mixture can be lowered while preserving equivalent efficacy compared to each separate medication. A DRI exceeding 1 indicates a favourable concentration reduction, while a DRI below 1 signifies an unfavorable dosage decrease [50, 51].

Molecular docking

Linagliptin and ciprofloxacin's chemical structures were depicted using ChemDraw software (Cambridge Soft, USA) and subsequently refined with the Chem3D version. The molecular structure of Hsp 60 chaperonins (Heat Shock Protein 60) was sourced from the Protein Data Bank (PDB code: 4pj1) .

Protein structures were optimized and modified using AutoDock Tools. The ligands' optimal conformation was determined using AutoDock Tools, which generated a PDBQT file.

After optimization, the structures of the ligands linagliptin and ciprofloxacin, along with the human Hsp 60 chaperone protein, were input into AutoDock-Tools. The docking procedure was subsequently executed utilizing the same program. The docking energy scores and binding interactions were analyzed using PLIP and BIOVIA Discovery Studio [52, 53].

Ethical approval

The scope of this study does not include human subjects.

Statistical Analysis

The MTT test results are the mean ± standard deviation (SD) from six replicates. A one-way analysis of variance (ANOVA) was utilized. The LSD test was employed to analyze the differences among various groups. The study used statistical software version 20, setting a significance threshold at $p < 0.05$ [54].

Results

Cell line cytotoxicity assay

Linagliptin cytotoxicity

The cytotoxic effect of linagliptin on cervical cancer cell proliferation revealed its capacity to inhibit cancer cell growth in a concentration-dependent manner, as evidenced by significant variations across all linagliptin concentrations during each incubation period. The cytotoxicity pattern was contingent upon the incubation duration, evidenced by a notable variation in growth inhibition between the two incubation periods across all concentrations. Furthermore, this effect was elucidated by the reduction in the IC_{50} level during the 72-hour incubation period compared to the 24-hour incubation period: Table 1 and Figure 1.

Table 1. The Impact of Linagliptin on Cervical Cancer Cell Survival atA 24 and 72 hours.

Concentration (µg/ml)	Inhibition of cellular proliferation (mean ± SE [a])		P- value
	24 hr.	72 hr.	
0.1	D 4.00±3.000	C 16.00±4.000	0.014*
1	CD 6.00±1.000	B 27.00±3.000	0.0001*
10	C 14.00±2.000	B 31.00±1.000	0.0001*
100	B 24.00±4.000	AB 36.00±2.000	0.010*
1000	A 32.00±2.000	A 43.00±3.000	0.006*
[b] LSD value	9.48	10.16	
IC_{50}	1767.4 µg/ml	1362.7 µg/ml	

[a], standard error; [b], least significant difference; statistically significant differences are shown by variations in capital letters within the same column; *, significant at (P<0.05)

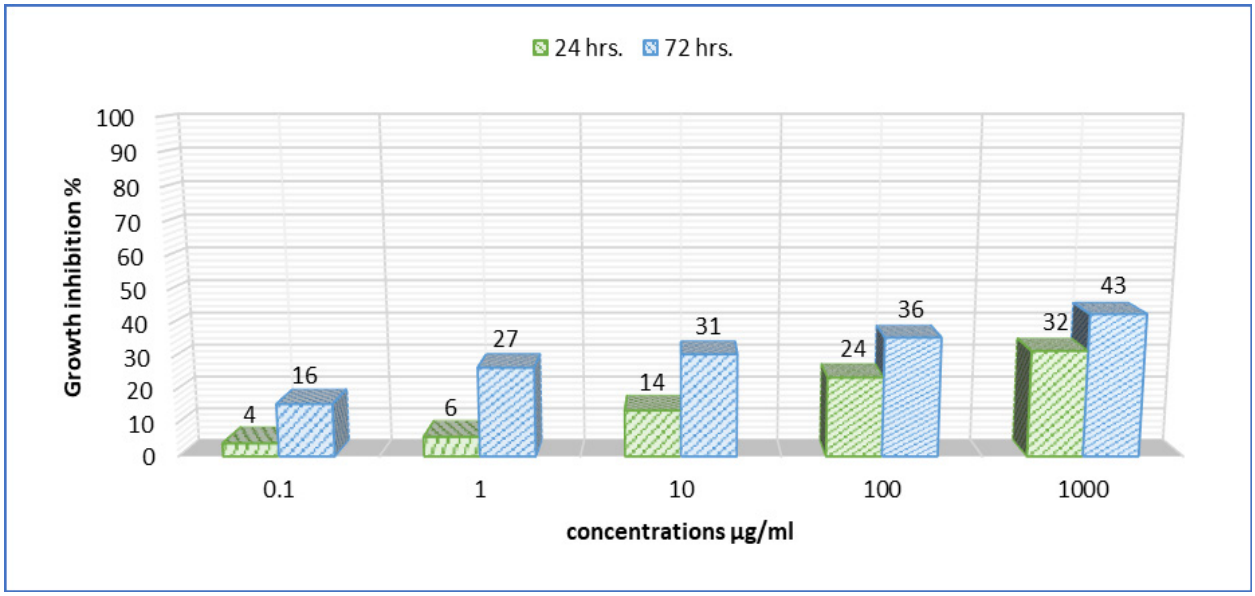

Figure 1. The Impact of Linagliptin on Cervical Cancer Viability at 24 and 72 hours

Ciprofloxacin cytotoxicity

The cytotoxic effect of ciprofloxacin on cervical cancer proliferation demonstrates its ability to inhibit cancer growth in a concentration-dependent manner, as indicated by significant variations across all ciprofloxacin concentrations during each incubation period. The cytotoxicity pattern depended on the incubation duration, as demonstrated by substantial differences in growth inhibition between the two incubation periods at all concentrations. This effect was shown by the decrease in the IC_{50} level during the 72-hour incubation period relative to the 24-hour incubation period: Table 2, Figure 2.

(Linagliptin -ciprofloxacin) combination cytotoxicity

The cytotoxic effect of the linagliptin-ciprofloxacin combination on cervical cancer proliferation illustrates its capacity to inhibit cancer growth in a concentration-dependent manner, evidenced by significant variations across all mixture concentrations during each incubation period. The incubation duration influenced the cytotoxicity pattern, evidenced by substantial differences in growth inhibition across most mixture concentrations, particularly at 10.100 and 1000 µg/ml between the two incubation periods. This effect was demonstrated by the decrease in the IC_{50} level during the 72-hour incubation period relative to the 24-hour incubation period. (Table 3) (Figure 3).

Additionally, the mixture's cytotoxicity towards the human fibroblast cell line was employed to assess the mixture toxicity on normal cells that may result from products that potentially result from pharmaceutical interaction between mixture constituents. The result demonstrated that the impact of the mixture was significantly greater on the HeLa cell line than the HFF cell line at both 24 and 72 hours of incubation (Tables 4, 5 and 6) (Figures 4, 5and 6).

The comparison of mixture cytotoxicity versus its constituents demonstrated mixture cytotoxicity exceeds that of their component's cytotoxicity, especially after 24 hours of incubation. (Supplementary Tables 1 and 2) (Supplementary Figures 1 and 2) .

Studying drug combinations

The subsequent results present the probe into the drug combinations of linagliptin and ciprofloxacin. Following a 24-hour incubation, the combination of linagliptin and ciprofloxacin at concentrations of 0.1 and 1 µg/ml exhibited very strong synergistic anticancer effects. A concentration of 10 and 100 µg/ml exhibited

Table 2. The impact of Ciprofloxacin on Cervical Cancer Viability at 24 and 72 hours

Concentration (µg/ml)	Inhibition of cellular proliferation (mean ± SE [a])		P- value
	24 hr.	72 hr.	
0.1	C 0.00±.000	D 4.00±2.000	0.026*
1	BC 2.00±1.000	D 11.00±3.000	0.008*
10	B 8.00±2.000	C 31.00±1.000	0.0001*
100	A 29.00±3.000	B 43.00±3.000	0.005*
1000	A 37.00±2.000	A 56.00±1.000	0.0001*
[b] LSD value	6.9	7.98	
IC_{50}	1370.4 µg/ml	786.8 µg/ml	

[a], standard error; [b], least significant difference; statistically significant differences are shown by variations in capital letters within the same column; *, significant at (P<0.05)

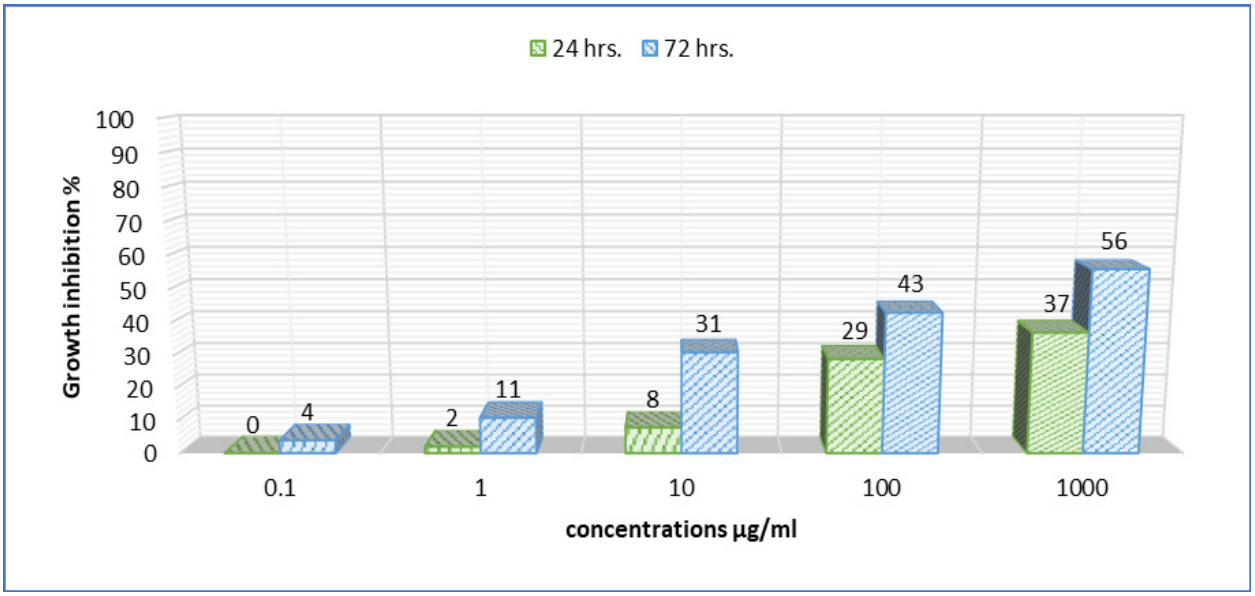

Figure 2. The Impact of Ciprofloxacin on Cervical Cancer Viability at 24 and 72 hours

Table 3. The Influence of Linagliptin -Ciprofloxacin Combination on the Viability of HeLa Cancer Cells at 24 and 72 hours

Concentration (µg/ml)	Inhibition of cellular proliferation (mean ± SE [a])		P- value
	24 hr.	72 hr.	
0.1	D 7.00±2.000	D 14.00±4.000	0.053
1	C 19.00±2.000	CD 23.00±3.000	0.127
10	C 21.00±1.000	C 29.00±4.000	0.028*
100	B 34.00±4.000	B 44.00±4.000	0.038*
1000	A 47.00±3.000	A 62.00±2.000	0.002*
[b] LSD value	9.48	12.7	
IC_{50}	1060.4 µg/ml	640.6 µg/ml	

[a], standard error; [b], least significant difference; statistically significant differences are shown by variations in capital letters within the same column; *, significant at (P<0.05)

strong synergism, while 1,000 µg/ml displayed a slight synergistic pattern over the 24 hours. At a 72-hour incubation period, results indicated that 0.1 and 1 µg/ml concentrations exhibited strong synergistic anticancer

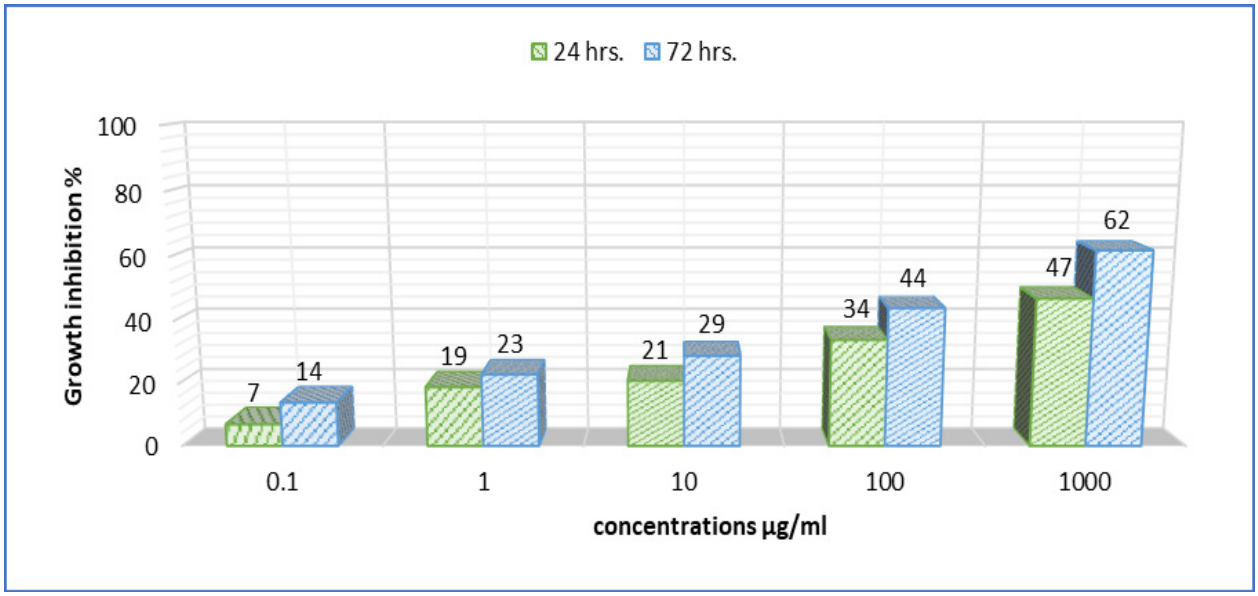

Figure 3. The Influence of Linagliptin -Ciprofloxacin Combination on the Viability of HeLa Cancer Cells at 24 and 72 hours

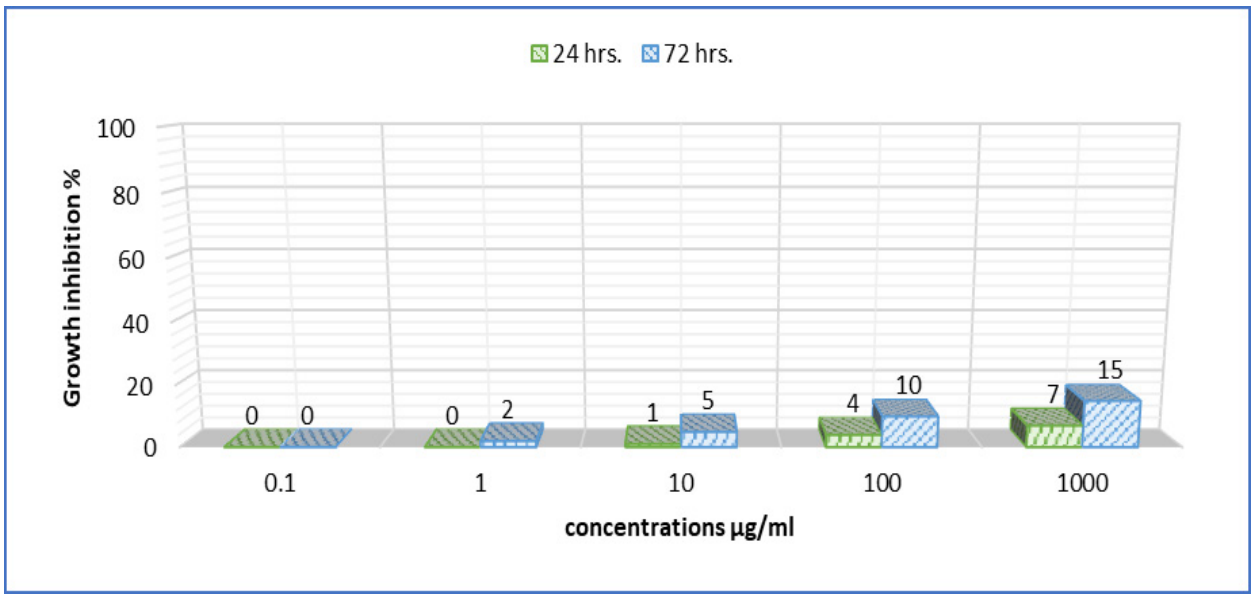

Figure 4. The Influence of Linagliptin -Ciprofloxacin Combination on the Viability of HFF Cell Line at 24 and 72 hours

Table 4. The Influence of Linagliptin -Ciprofloxacin Combination on the Viability of HFF Cell Line at 24 and 72 hours

Concentration (µg/ml)	Inhibition of cellular proliferation (mean ± SE [a])		P- value
	24 hr.	72 hr.	
0.1	B 0.00±.000	C 0.00±.000	N.S
1	B 0.00±.000	C 2.00±1.000	0.026
10	A 1.00±1.000	BC 5.00±2.000	0.036
100	A 4.00±2.000	AB 10.00±2.000	0.021
1000	A 7.00±3.000	A 15.00±2.000	0.018
[b] LSD value	6.08	5.86	
IC_{50}	7900.1 µg/ml	3949 µg/ml	

[a], standard error; [b], least significant difference; statistically significant differences are shown by variations in capital letters within the same column; *, significant at (P<0.05)

effects. A 10 µg/ml concentration exhibited synergism, while 100 and 1,000 µg/ml concentrations displayed a moderate synergistic pattern.

The dose reduction index findings indicated that the

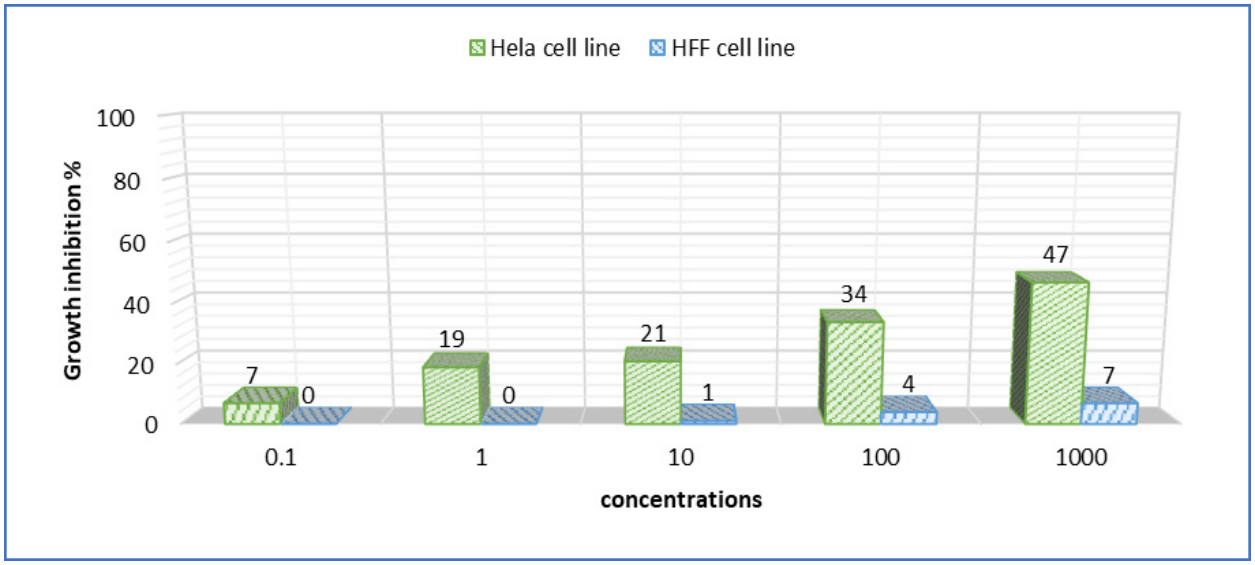

Figure 5. Comparison of the 24-hour Growth Inhibition of Linagliptin -Ciprofloxacin Combination between Hela and HFF Cell Lines.

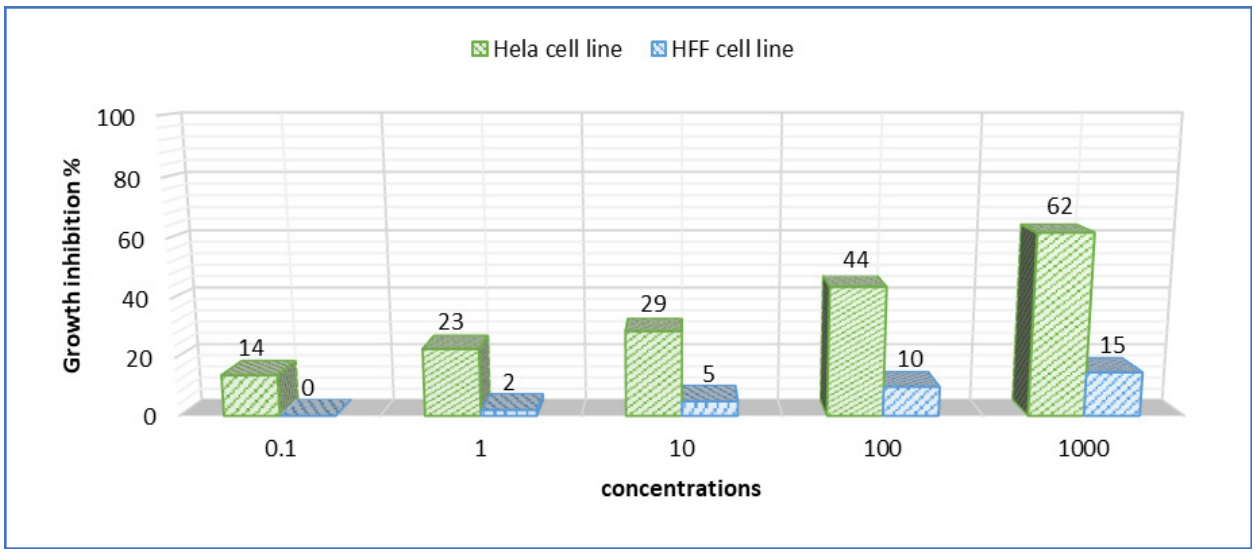

Figure 6. Comparison of the 72-hour Growth Inhibition of Linagliptin -Ciprofloxacin Combination between Hela and HFF Cell Lines

Table 5. Comparison of the 24-hour Growth Inhibition of Linagliptin -Ciprofloxacin Combination between Hela and HFF Cell Lines

Concentration (µg/ml)	Inhibition of cellular proliferation (mean ± SE [a])		P- value
	Hela	HDF	
0.1	7.00±2.000	0.00±.000	0.004*
1	19.00±2.000	0.00±.000	0.0001*
10	21.00±1.000	1.00±1.000	0.0001*
100	34.00±4.000	4.00±2.000	0.0001*
1000	47.00±3.000	7.00±3.000	0.0001*
[b] LSD value	9.48	6.08	
IC_{50}	1060.4 µg/ml	7900.1 µg/ml	

[a], standard error; [b], least significant difference; statistically significant differences are shown by variations in capital letters within the same column; *, significant at (P<0.05)

Table 6. Comparison of the 72-hour Growth Inhibition of Linagliptin -Ciprofloxacin Combination between Hela and HFF Cell Lines

Concentration (µg/ml)	Inhibition of cellular proliferation (mean ± SE [a])		P- value
	Hela	HFF	
0.1	14.00±4.000	0.00±.000	0.004*
1	23.00±3.000	2.00±1.000	0.0001*
10	29.00±4.000	5.00±2.000	0.0001*
100	44.00±4.000	10.00±2.000	0.0001*
1000	62.00±2.000	15.00±2.000	0.0001*
b LSD value	12.7	5.86	
IC_{50}	640.6 µg/ml	3949 µg/ml	

[a], standard error; [b], least significant difference; statistically significant differences are shown by variations in capital letters within the same column; *, significant at (P<0.05)

concentrations of the mixture ingredients required to induce cytotoxicity decreased at all time intervals (24 and 72 hours of incubation) for all concentrations of linagliptin and ciprofloxacin, suggesting a favorable reduction in the effective concentration of the mixture components. Supplementary Tables (3 and 4) Supplementary Figures (3 and 4).

Molecular docking studies

Molecular docking modeling investigated the interaction between each linagliptin and ciprofloxacin with human Hsp 60 (PDB code: 4pj1) as a basis. The study utilized AutoDock tools version 1.5.7 and BIOVIA Discovery Studio [55].

Our molecular docking studies results demonstrated

that the molecular docking score of binding linagliptin with Hsp 60 was (-7.6) kcal/mol. Molecular docking analysis was presented. Three carbon-hydrogen bonds formed with the GLY A:154 amino acid residues at 3.68 Å distance, LYS A:492 amino acid residues at 3.68 Å distance, and ASP A:480 amino acid residues at 2.28 Å distance. Five Pi-alkyl bound tow bonds formed with the PRO A:33 amino acid residues at a 4.43 Å and 3.99 Å distance, tow bonds formed with ILE A:150 amino acid residues at a 5.31 Å and 5.10 Å distance, and MET A:482 amino acid residues at a 5.03 Å distance. Tow alkyl bond formed with the ILE A:150 amino acid residues at 4.86 Å and 3.97 Å distances. One pi-cation bond formed with the LYS A:156 amino acid residues at 4.83 Å distances. And finally, one pi-sigma bond formed with the ILE A:494 amino acid residues at 3.801 Å distances (Supplementary Figure 5).

Furthermore, molecular docking study data of ciprofloxacin with Hsp 60 revealed a total docking score of (-8.1) kcal/mol. Molecular docking analysis was presented. Three conventional hydrogen-bound with each GLY A: 416, ALA A: 481, and UNK amino acid residue at 2.76 Å, 2.44 Å, and 1.86 Å of distance subsequently. .one carbon-hydrogen bound with ASP A:480 amino acid residue at 3.46 Å distances.

Tow halogen (fluorine) bonds with ASP A:480 amino acid residues at 3.33 Å and 3.18 Å distances. Tow alkyl bonds with PRO A:33 and ILE A:150 amino acid residues at 3.91 Å and 4.72 Å distances. Finally, six pi-alkyl bonds with two PRO A: 33, two ILE A: 150 and two ILE A: 494 amino acid residues at 3.72 Å, 4.52 Å, 4.96 Å, 5.11 Å, 4.46 Å and 4.67 Å distances subsequently (Supplementary Figure 6).

For comparison purposes, molecular docking study data of mizoribine (Hsp 60 inhibitor) [56, 57]. Revealed a total docking score of (-7.6) kcal/mol. It formed six Conventional hydrogen bonds with the LYS A:51, GLY A: 53, THR A:89, SER A:151, UNK A:28 and ASP A:496 amino acid residues at 3.01 Å, 2.01 Å, and 2.06 Å, 2.19 Å, 2.67 Å and 2.19 Å of distance, respectively. Two carbon-hydrogen bonds with ASP A:87 and ASP A:399 amino acid residues at 3.47 Å and 3.46 Å of distance, respectively. Finally, one pi-sulfur bond with MET A:31 amino acid residues at 5.67 Å of distance (Supplementary Figure 7, 8).

Discussion

The study assessed the paired anticancer efficacy of linagliptin and ciprofloxacin on cervical cancer cell survival and investigated the mixture's aptitude to target the Hsp60 chaperone protein. The study results demonstrated that the linagliptin-ciprofloxacin combination effectively inhibits the proliferation of cervical cancer cells in a concentration- and time-dependent manner, exhibiting both cell cycle-specific and cell cycle-nonspecific impacts. The combination index results showed the mixture exhibited synergistic behavior at all concentrations, especially after 24 hours of incubation. The dosage reduction index demonstrated a favorable decrease in the effective cytotoxic concentration of the mixture's elements compared to the effective cytotoxic concentration of each ingredient, indicating improved safety and reduced adverse effects.

The chemical docking assessment revealed that each component of the mixture can bind to Hsp60 with differing affinities and binding sites, providing insights into the mixture's anticancer mechanism and synergistic interactions among the mixture's components. The cytotoxicity findings of the mixture on the viability of the HFF cell line suggest that the mixture specifically targets cancer cells, as both cancerous and healthy cells express Hsp60. This fact clarifies the selective toxicity of the mixture toward cancer cells. Furthermore, the results of the mixture cytotoxicity on the HFF cell line indicate no pharmaceutical interaction between the mixture components.

The findings related to the combination index and the targeting of Hsp 60 indicate a synergistic interaction among the mixture's components, and further evidence supports this synergistic mechanism. Each ingredient has a distinct anticancer mechanism; collectively, they act in a complementary mechanism.

Several studies were conducted on the same issue as our study's finding of linagliptin cytotoxicity. Linagliptin demonstrated a significant capacity to reduce the viability of Saos-2 cells, a human bone cancer cell line, and hFOB1.19 cells, a human fetal bone cell line [19]. Linagliptin has been shown to inhibit Glioblastoma cancer cells' survival, growth, and invasion [58], Linagliptin has been shown to inhibit the survival, growth, and invasion of Glioblastoma cancer cells [59]. The pattern of growth inhibition was primarily influenced by the incubation duration, indicating a greater dependence on time than concentration. This finding suggests that linagliptin's anticancer properties are linked to its effects on specific cell cycle stages.

Numerous studies have shown various anticancer mechanisms of linagliptin. Linagliptin has been shown to induce cell cycle arrest in the G2/M phase at low doses and both the G2/M and S phases at high concentrations. [59]Another proposed mechanism is that linagliptin strongly interacts with Cyclin-Dependent Kinase 1 (CDK1), an essential protein in cell cycle control. CDK1 phosphorylates many substrate proteins, including histones H1, laminin, and Rbis. Furthermore, Linagliptin demonstrates a significant inhibitory effect on cell proliferation and tumor growth through the selective targeting of Aurora kinase B and CDK1, resulting in diminished phosphorylation of Rb and a reduction in Bcl 2 production. Pro-caspase 3 is a protein [59]. Also, Linagliptin demonstrated the ability to target Aurora kinase B selectively. This kinase is a conserved serine-threonine protein kinase within the Aurora family, essential for regulating cell division [60], Increased expression of Aurora kinase B has been observed in pleomorphic gliomas, malignant mesothelioma, and hematological malignancies. This gene shows notable overexpression in colorectal, liver, and breast cancers [61]

In contrast, several studies were performed to assess ciprofloxacin's anticancer properties. One demonstrated that ciprofloxacin significantly inhibits the growth of transitional cell carcinoma cells [62]. Conversely,

additional study indicates that Fluoroquinolone antibiotics induce cell death in breast cancer cells, contingent upon the dosage and duration of treatment. Cell death occurs via multiple mechanisms, such as apoptosis induction, elevated expression of p53, Bax, and Bcl-2 proteins, changes in cell cycle distribution and DNA fragmentation, mitochondrial function disruption through the Bax/Bcl-2 pathway, S-phase cell cycle arrest, and topoisomerase II inhibition. Furthermore, evidence indicates oligonucleosomal DNA fragmentation alongside an elevation in p53 expression [63, 16]. Another study indicates that ciprofloxacin can inhibit the proliferation of hepatocellular carcinoma cell lines by inducing DNA breaks and inhibiting topoisomerases. Ciprofloxacin exhibits a synergistic effect when administered in conjunction with cisplatin [64]

In addition to all these anticancer mechanisms of each linagliptin and ciprofloxacin, the present study suggested a novel anticancer mechanism of each linagliptin and ciprofloxacin, represented by their ability to target Hsp 60.

The present study focuses on heat shock protein 60 (Hsp 60) due to its essential role in the transport and folding of mitochondrial proteins and its reported association with various cancer types [32] .HSP60 plays a pro-apoptotic role by promoting the activation pro-caspase-3 through various caspases, such as caspase-6. HSP60, located in the cytosol, inhibits the translocation of the pro-apoptotic protein Bax into mitochondria, thereby promoting cell survival [33].

Recently, the prognostic association of HSP60 with cervical cancer has emerged as a significant area of investigation. The predictive value of HSP60 in cervical cancer was assessed through 2-dimensional Electrophoresis (2-DE), semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR), and Western Blot (WB) analyses in these studies. The findings strongly indicate that HSP60 is integral to the progression of cervical cancer [34].Data from patients with advanced prostate cancer revealed a significant correlation between HSP60 expression and tumor progression. The expression of HSP 60 has been shown to have a strong correlation with androgen independence in cases of locally advanced prostate cancer. The intensity and extent of HSP60 immunoreactivity predicted biochemical recurrence in prostate cancer patients. This study found that patients exhibiting intense HSP60 staining in biopsy samples had shorter recurrence-free survival than those with weak HSP60 expression. A study conducted on patients with prostate cancer indicated that HSP60 expression is elevated in prostate cancer tissues relative to normal prostatic tissue [35-37].

Based on the factors above, Hsp 60 has been selected due to its significant role in cancer. Our molecular docking study results demonstrate that each drug in the mixture exhibits different levels of binding affinity with Hsp60. The molecular docking study results indicate that ciprofloxacin demonstrates a more antiproliferative effect than linagliptin, which may result in a higher molecular docking score for ciprofloxacin. Molecular docking studies demonstrate the synergistic effect of ciprofloxacin and linagliptin, as each drug interacts with the Hsp 60 protein at distinct sites, resulting in a complementary and synergistic effect when administered together.

Abnormality in the expression level of Hsp 60 has been detected in different diseases, including inflammatory diseases and various cancers. Therefore, there is a strong interest in investigating molecules that can modulate Hsp60. Most of the reported inhibitors were discovered through multiple chemoproteomics strategies. Such as mizoribine, Epolactaene, myrtucommulone, stephacidin B, and avrainvillamide. The potential applications of these inhibitors include anti-cancer, anti-inflammatory diseases, and anti-autoimmune diseases [65, 66].

The study's limitation was the absence of restrictions on drug concentration ranges. A wide range of concentrations was utilized to determine the optimal effective concentration for linagliptin and ciprofloxacin.

In conclusion, our study's findings indicate that the combination of linagliptin and ciprofloxacin effectively inhibits the proliferation of cervical cancer cells. Inhibition patterns are exhibited through both cell cycle-specific and cell cycle-nonspecific mechanisms. The findings demonstrate that the combination of these medications shows synergistic cytotoxicity, as assessed by the combination index value.

Computational docking simulations indicated that linagliptin and ciprofloxacin interact with Heat Shock Protein 60. These findings elucidate the synergistic interactions among mixed medications, as each drug targets a specific binding site on Hsp60, indicating a complementary binding mechanism with Hsp60.

Furthermore, we suggested the mixture demonstrated selective toxicity by preferentially targeting Hsp60 in cancer cells over normal cells, as evidenced by the significantly reduced proliferation observed in the Hela cell line compared to the HFF cell line. The dose reduction index results demonstrate that the concentration of medications in the mixture needed to achieve significant cytotoxicity is less than that of each medication administered separately. Based on these findings, the combination of linagliptin and ciprofloxacin presents an effective, safe, and potent treatment option for cervical cancer.

Abbreviations

(ICCMGR): The Iraqi Centre for Cancer and Medical Genetics Research.

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide stain

RPMI: Roswell Park Memorial Institute medium

SAS: Statistical Analysis System

LSD: Least Significant Difference

DRI: dose reduction index

CI: combination index

Hsp 60: heat shock protein 60

HFF cell line: human fibroblast cell line

PPIs: proton pump inhibitors

Author Contribution Statement

Design and development: Tiba Th. Al-Mahdwi, Azal Hamoody, Ali Muafaq Said; Gathering and organizing

data: Azal Hamoody, Istikrar M.Hade, Youssef Shakuri; Data analysis/interpretation: Istikrar M.Hade, Azal Hamoody, Tiba Th. Al-Mahdwi; Article composition: Youssef Shakuri , Tiba Th. Al-Mahdwi, Ali Muafaq Said; Critique the essay for significant ideas: Azal Hamoody, Youssef Shakuri; Statistical analysis expertise: Azal Hamoody,Istikrar M.Hade, Ali Muafaq Said; Ultimate article endorsement and guarantee: Azal Hamoody, Istikrar M.Hade, Ali Muafaq Said.

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Declaration of Generative AI and AI-assisted technologies in the writing process

The authors affirm that this work does not employ generative AI or AI-assisted technologies.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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