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Esomeprazole, Ciprofloxacin,Heat Shock Protein 70 Modulation

RESEARCH ARTICLE

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Impact of Esomeprazole, Ciprofloxacin and Their Combination on Cervical Cancer Cell Line Proliferation: A Focus on Heat Shock Protein 70 Modulation

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Abstract

Objective: This investigation aimed to evaluate the combined efficacy of ciprofloxacin and esomeprazole in inhibiting cervical cancer proliferation and their capacity to target heat shock protein 70 in vitro. **Methods:** The MTT assay was utilized to assess the anticancer properties of the ciprofloxacin-esomeprazole combination on the HeLa cervical cancer cell line. The human fibroblast cell line (HFF) is employed to assess the combination's safety. The assay was performed within 24 and 72 hours of incubation. Ciprofloxacin, esomeprazole, and their combination concentrations ranged from 0.1 to 1000 µg/ml, with the mixture containing 50% of the individual concentration of each medication when assessed individually. The study employed the selective toxicity index to assess the selectivity of the mixture for cancer cells. The combination index was utilized to evaluate the potential synergistic effects of ciprofloxacin and esomeprazole. This study utilizes computational molecular docking simulations to assess the binding affinity of Ciprofloxacin and esomeprazole to heat shock protein 70 (PDB code: 1hjo). **Results:** The MTT assay and selective toxicity index results indicated that the combination of ciprofloxacin and esomeprazole selectively inhibited the proliferation of cervical cancer cells. The inhibitory effect depended on the concentration of the mixture and the incubation duration, with a diminished impact on the viability of the HFF cell line. The combination index study indicates that the interaction between ciprofloxacin and esomeprazole shows a synergistic effect at each incubation period. The computational molecular docking simulation indicated that ciprofloxacin and esomeprazole target Hsp 70, with docking scores of -7.4 kcal/mol and -7.3 kcal/mol, respectively. **Conclusion:** Our findings from the MTT assay, selective toxicity index, combination index, and computational docking simulations suggest that the combination of ciprofloxacin and esomeprazole is a promising option for treating cervical cancer, given their set adverse effects and pharmacokinetic profiles.

Keywords: ciprofloxacin- esomeprazole- ciprofloxacin - esomeprazole mixture- Hela cell line- combination index

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Introduction

Annually, almost 500,000 women get a diagnosis of cervical cancer, leading to over 300,000 fatalities globally. Approximately 90% of cervical cancer cases arise in low- and middle-income countries. Organized screening programs have resulted in an estimated 50% decrease in the incidence and mortality rates of cervical cancer in high-income nations over the last thirty years. The severity of the disease influences treatment choices upon diagnosis and the availability of local resources. A radical hysterectomy, chemotherapy, or a combination of the two may be necessary [1]. Numerous randomized clinical trials indicate that women diagnosed with invasive cervical cancer who qualify for radiation treatment should choose concurrent cisplatin-based chemoradiotherapy over radiation therapy aloneb[2-7], A review of 18 studies conducted across 11 countries indicates that combination chemoradiation positively influences prognosis. The study demonstrated a 12% improvement in overall survival rates and progress in managing local and distant disease progression [8].

Chemoradiotherapy serves as a primary treatment for cervical cancer; nonetheless, the adverse effects associated with chemotherapy underscore the necessity for safer alternatives. Numerous trials have been conducted to determine an effective treatment for cervical cancer through the repurposing of a medication initially developed for a different therapeutic application [9-13].

Both ciprofloxacin and esomeprazole exhibited promising antitumor effects, as illustrated by numerous studies.

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Ciprofloxacin, administered at a non-antibacterial dose, can activate pro-apoptotic mechanisms and reduce the proliferation of specific cancer cells, including colorectal, bladder, and prostate neoplasms [14-16]. Ciprofloxacin has been investigated in vitro across multiple cell lines, indicating its potential use in cancer treatment via mechanisms that include apoptosis induction, cell cycle arrest, and disruption of mitochondrial membrane potential [17] .

Further study demonstrates that ciprofloxacin antibiotics can kill breast cancer cells via several mechanisms, including apoptosis induction, increased expression of p53, Bax, and Bcl-2 proteins, alterations in cell cycle distribution, DNA fragmentation, disruption of mitochondrial function via the Bax/Bcl-2 pathway, S-phase cell cycle arrest, and inhibition of topoisomerase II [18, 12]. Ciprofloxacin inhibits the proliferation of hepatocellular carcinoma cell lines by inducing DNA breaks and inhibiting topoisomerases [19].

Esomeprazole, among other ingredients in the mixture, has been extensively studied for its anticancer properties. Some studies indicate that esomeprazole anticancer activity is associated with the inhibition of vacuolar-type ATPase (V-ATPase) [20] and fatty acid synthase (FASN) [21-26]. Esomeprazole has been suggested to affect the cancer hallmarks of migration, invasion, and genomic instability [27, 28].

Esomeprazole induces apoptosis in cancer cells and enhances drug delivery by inhibiting V-ATPase and subsequent modulation of pH. V-ATPase is present in cancer cells and plays a role in regulating intra- and extracellular pH. The extracellular pH is characterised as acidic, whereas the intracellular pH is neutral to alkaline [29]. The external pH is acidic, while the intracellular pH is neutral to alkaline [29]. Esomeprazole effectively induced apoptosis in melanoma both in vitro and in vivo by inhibiting V-ATPase, which was associated with increased and reduced intracellular pH levels [29]. The modulation of pH can influence the delivery of chemotherapeutic agents. The low pH in the tumor microenvironment diminishes the efficacy of weakly essential chemotherapeutic agents [29].esomeprazole modulates extracellular pH by targeting V-ATPase in tumor cells, potentially reversing this effect [22]. Pre-treatment with omeprazole and esomeprazole has been demonstrated to enhance the drug response of weakly basic chemotherapeutics, including cisplatin, 5-fluorouracil, and vinblastine, in multidrug-resistant cells [22].

PPIs can significantly restrict the invasion and migration of aggressive cancer cells linked to epithelial-mesenchymal transition (EMT), an essential stage in metastasis [30, 31]. Changes in the expression of E-cadherin and mesenchymal markers such as vimentin, fibronectin, and N-cadherin are significant features of the epithelial-mesenchymal transition [32]. PPIs inhibit Snail expression, potentially triggering epithelial-mesenchymal transition (EMT) while not influencing the expression of other transcription factors associated with EMT [33-35, 12] PPIs exhibited a significant capacity to bind directly to the Snail protein by interfering with CREB-binding protein (CBP)/p300-mediated Snail acetylation, which promotes Snail degradation [36].

Proposed alternative mechanisms involve the capacity of PPIs to induce lysosomal membrane permeabilization, leading to increased lysosomal outflow from the cytoplasm, lysis of cellular components, and subsequent cell death. Lysosomal enzymes demonstrate hydrolytic activity and establish an acidic environment that facilitates the degradation of tumor cells [37-40] .

A comprehensive study was performed on multiple cancer targets, including the notable target Hsp70. Cells exhibit basal levels of Hsp70 expression in the absence of stress. The increased expression, a characteristic of malignant or stressed cells, promotes their survival. A clinical study indicates that Hsp70 is a reliable prognostic marker, as its elevated expression in malignant cells correlates with tumor progression in conditions such as endometrial malignancies, osteosarcomas, and renal cell tumors, compared to normal cells [41]. Heat shock protein 70 plays a crucial role in cervical cancer, facilitating cell survival, proliferation, and apoptosis resistance. The overexpression of Hsp70 correlates with unfavorable prognosis and chemoresistance, indicating its potential as a therapeutic target. Inhibiting Hsp70 has demonstrated the potential to enhance the sensitivity of cervical cancer cells to therapeutic interventions [42, 43].

Hsp70 and prostate-specific antigens function as biomarkers for identifying patients in the early stages of prostate cancer [44]. Additionally, Hsp70 expression increases with the progression of chronic myeloid leukemia [45]. Overexpression of Hsp70 in HL-60/BCR-ABL and K562 cells results in resistance to cell death induced by imatinib. Imatinib is a chemotherapeutic agent that inhibits the activity of Bcr-Abl tyrosine kinase [46]. Another study found that Hsp70 expression is elevated in gastric epithelial cells after infection with Helicobacter pylori. [47] Moreover, Hsp70.2, a member of the Hsp70 family, shows notable upregulation during spermatogenesis and the progression of breast cancer, leading to a delay in cellular senescence [48]. The presence of Hsp70 in the nucleus serves as a diagnostic marker for epithelial dysplasia, whereas antibodies targeting Hsp70 are detectable in individuals with hepatocellular Carcinoma [49, 50].

Combining current medications for non-cancer uses presents a valuable approach to creating effective cancer therapies that reduce side e effects and address resistance in cancer cells. A variety of investigations have delved into this topic, with one indicating that the combination of amygdalin and esomeprazole effectively eradicates cervical cancer cells. The effectiveness of this combination depended on the concentration of the medication and the duration of the incubation period [51, 38]. A separate study indicated that the combination of laetrile and vinblastine significantly inhibited the growth of esophageal cancer, showing a synergistic effect between the components of the mixture [52, 13]. A concurrent study indicated that combining ciprofloxacin and laetrile effectively inhibits the proliferation of esophageal cancer cells [53]. Numerous studies have examined this issue; however, they are limited in demonstrating the anticancer properties of the ciprofloxacin–esomeprazole combination and its

ability to target Hsp70 in cancer cells. This research examines the inhibitory effects of the ciprofloxacin-esomeprazole combination on the proliferation of cervical cancer cells and assesses the targeting of Hsp70.

Materials and Methods

Medications

The Samarra Pharmaceutical Factory supplied ciprofloxacin and esomeprazole as raw materials. The pharmaceuticals were diluted with RPMI medium to attain concentrations between 0.1 µg/ml and 1000 µg/ml.

cell lines

The HeLa cell line, sourced from human malignant cervical cancer, and the HFF cell line, obtained from human fibroblasts, were first developed at the tissue culture section of ICCMGR. The cells were cultivated in 75 cm² tissue culture flasks under regulated conditions, sustaining a relative humidity of 37°C and 5% CO2. The cells were incubated in RPMI-1640 media (Sigma Chemicals, England) supplemented with 10% fetal bovine serum (FBS) and 100 U/mL of penicillin-streptomycin (100 µg/mL streptomycin) [51, 54].

cytotoxic study

Ciprofloxacin, esomeprazole, and their combination were evaluated for their effectiveness in suppressing cervical cancer cells grown in a 96-well microtiter plate. The escalation in cancer cell proliferation was steady and incremental throughout the logarithmic growth phase. The toxicity of the assessed drugs was studied during two separate incubation durations: 24 hours and 72 hours [55].

Each well comprises 10,000 cells. Seeding necessitates a medium with 10% fetal bovine serum. The plates were incubated for 24 hours at 37°C to promote cell attachment. Serial dilutions were conducted utilizing a serum-free RPMI medium. Ciprofloxacin, esomeprazole, and their combination were diluted in RPMI medium lacking calf serum. A series of dilutions for each medication was prepared, spanning concentrations from 0.1 to 1000 µg/ml [53, 56].

Following 24 hours of cancer cell proliferation, each treatment concentration was allocated to six wells, each receiving 200 µl of RPMI media containing the drug. Control wells were administered 200 microlitres of maintenance media, with exposure durations ranging from 24 to 72 hours. The plates were reinserted into the incubator after being securely affixed with a self-adhesive substance. The cells were then treated with MTT dye.

A microtiter plate reader (ELISA reader) was employed to measure the optical density of each well at a transmission wavelength of 550 nm [57, 58] .

The equation used to calculate the growth inhibition rate is: [58]

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

The IC_{50} values for ciprofloxacin, esomeprazole, and a combination of them have been estimated for each incubation duration employed. GraphPad Prism, version 9.5.0, (2022) [59].

Selective toxicity index

A study investigated the selective toxicity of the ciprofloxacin esomeprazole combination toward cancer cells at each incubation period (24 and 72) hrs. After estimating the IC_{50} level for the combination by employing a cell proliferation curve for each HeLa and HFF cell line, the selective cytotoxicity index was calculated according to the given formula [60]:

$$\textbf{Selective toxicity Index } (\boldsymbol{SI}) = \frac{\boldsymbol{IC}\ 50\ \boldsymbol{of normal\ cell\ lines}}{\boldsymbol{IC}\ 50\ \boldsymbol{of cancer\ cell\ lines}} \times 100$$

A favourable SI > 1.0 indicates a drug with greater efficacy against tumor cells than toxicity against normal cells.

Drug combinations assessment

A study was conducted to examine the integration of mixture components. The evaluation entailed creating concentration-effect curves, illustrating the percentage of cells exhibiting diminished growth concerning drug concentration following 24 and 72 hours of treatment. The interaction of medications was evaluated for synergy, additive effects, and antagonism using Compusyn software (Biosoft, Ferguson, MO, USA), which computed the combination index and dose reduction index values.

CI values below 1 indicate synergy, values above 1 denote additivity and values exceeding 1 reflect antagonism. The dose reduction index (DRI) measures the degree to which the concentration of individual components in a mixture can be decreased while maintaining equivalent efficacy relative to each medication. A DRI greater than 1 indicates a favourable reduction in concentration, whereas a DRI less than 1 denotes an unfavorable decrease in dosage [61, 62].

Molecular docking

The chemical structures of ciprofloxacin and esomeprazole were illustrated using ChemDraw software (Cambridge Soft, USA), which was later enhanced with the Chem3D version. The molecular structure of Hsp 70 chaperonins (Heat Shock Protein 70) was obtained from the Protein Data Bank, code (PDB: 1hjo).

The application of AutoDock Tools optimized and modified protein structures established the optimal conformation of the ligands and generated a PDBQT file.

Following optimization, the structures of the ligand's ciprofloxacin, esomeprazole, and the human Hsp 70 chaperone protein were entered into AutoDock-Tools. The docking procedure was then carried out using the same program. The docking energy scores and binding interactions were analyzed using PLIP and BIOVIA Discovery Studio [63, 64].

Ethical approval

This research excludes human beings from its scope.

Statistical Analysis

The MTT test findings are presented as the mean and

standard deviation (SD) calculated from six repetitions. A one-way analysis of variance (ANOVA) was used. A paired t-test and LSD tests were used to analyze the differences among the groups. The study used SPSS version 20 for statistical analysis, setting the significance level at $p < 0.05$ [65].

Results

Cytotoxicity study:

Ciprofloxacin cytotoxicity

The results of ciprofloxacin cytotoxicity on cervical cancer demonstrated its ability to inhibit cancer cell growth, particularly with increasing concentrations of ciprofloxacin. This inhibition occurred in a concentration-dependent manner, as evidenced by significant variations in cytotoxicity across different ciprofloxacin concentrations during each incubation period. The incubation time significantly influenced the cytotoxicity of ciprofloxacin, as evidenced by notable variations in cytotoxicity across different incubation periods at all ciprofloxacin concentrations. The decline in IC_{50} levels after 72 hours of incubation, compared to 24 hours of incubation, is evident. Table (1) and Figure (1,2).

Esomeprazole cytotoxicity

The outcomes of esomeprazole cytotoxicity on cervical cancer indicate its capacity to reduce cancer cell growth, particularly as esomeprazole concentration increases, leading to a concentration-dependent inhibition. This is evidenced by significant variations in cytotoxicity across various esomeprazole concentrations during each incubation period. In contrast, the incubation time had a minimal effect on esomeprazole cytotoxicity, as indicated by the lack of significant variation in cytotoxicity across the two incubation periods at all esomeprazole concentrations. This fact is supported by a lesser decline in the IC_{50} level after 72 hours of incubation compared to 24 hours. Table (2), Figure (3,4).

(ciprofloxacin- esomeprazole) combination cytotoxicity

The combination of ciprofloxacin and esomeprazole demonstrated an ability to inhibit the growth of cervical cancer cells, with increased concentrations of the mixture leading to a concentration-dependent inhibition. This was evidenced by significant variations in cytotoxicity across different incubation periods for the various concentrations of the mixture. The incubation time significantly influenced the cytotoxicity of the mix, as evidenced by notable variations in cytotoxicity across

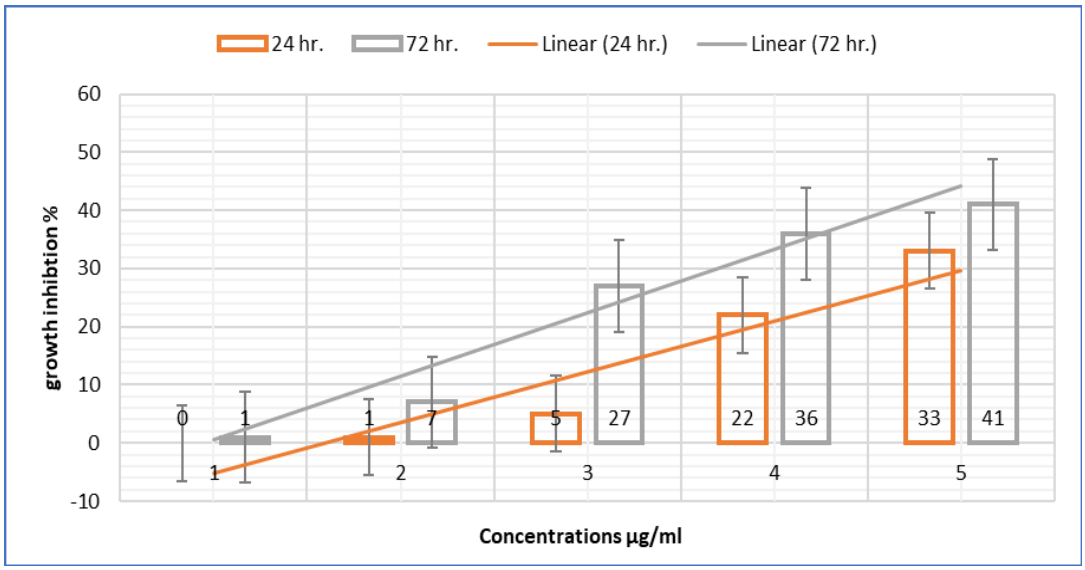

Figure 1. The Impact of Ciprofloxacin on Cervical Cancer Viability at 24 and 72 Hours

Table 1. The Impact of Ciprofloxacin on Cervical Cancer Viability at 24 and 72 hours

Concentration (µg/ml)	Inhibition of cellular proliferation (mean ± SD[a])		P- value
	24 hr.	72 hr.	
0.1	C 0.00 ± 0.000	C 1.00 ± 1.000	0.158
1	C 1.00 ± 1.000	C 7.00 ± 3.000	0.030*
10	C 5.00 ± 2.000	B 27.00 ± 4.000	0.001*
100	B 22.00 ± 2.000	A 36.00 ± 3.000	0.003*
1000	A 33.00 ± 3.000	A 41.00 ± 1.000	0.012*
[b] LSD value	6.9	9.76	-
IC_{50}	1553.4 µg/ml	1275.5 µg/ml	-

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

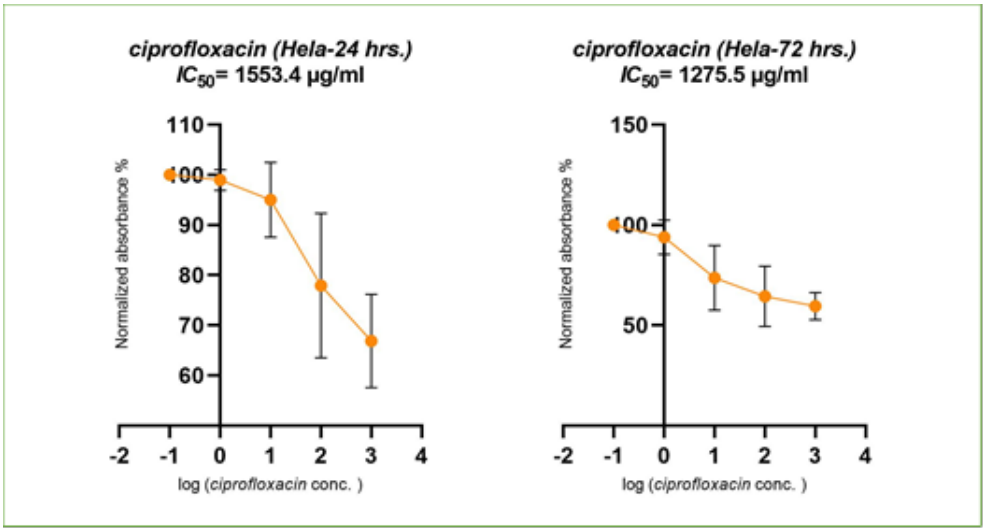

Figure 2. Log Dose-Response Curve for the Estimate of IC_{50} Regarding the Cytotoxicity of Ciprofloxacin on the Hela Cell Line at 24 and 72 hrs. Incubation.

Table 2. The Impact of Esomeprazole on Cervical Cancer Viability at 24 and 72 Hours

Concentration (µg/ml)	Inhibition of cellular proliferation (mean ± SD [a])		P-value
	24 hr.	72 hr.	
0.1	C 0.00 ± 0.000	D 1.00 ± 1.000	0.158
1	C 2.00 ± 2.000	CD 5.00 ± 2.000	0.14
10	BC 7.00 ± 1.000	C 10.00 ± 2.000	0.081
100	B 14.00 ± 4.000	B 18.00 ± 2.000	0.196
1000	A 38.00 ± 3.000	A 42.00 ± 1.000	0.176
[b] LSD value	8.92	6.08	
IC_{50}	1333.7 µg/ml	1239.5 µg/ml	

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

Table 3. Ciprofloxacin-Esomeprazole Combination Impacts HeLa Cancer Cell Line Viability at 24 and 72 Hours

Concentration (µg/ml)	Inhibition of cellular proliferation (mean ± SD [a])		P- value
	24 hr.	72 hr.	
0.1	D 6.00 ± 2.000	C 10.00 ± 5.000	0.268
1	D 11.00 ± 1.000	C 15.00 ± 2.000	0.036*
10	C 20.00 ± 2.000	B 28.00 ± 2.000	0.008*
100	B 32.00 ± 2.000	A 47.00 ± 3.000	0.002*
1000	A 41.00 ± 1.000	A 67.00 ± 2.000	0.0001*
[b] LSD value	6.08	11.04	
IC_{50}	1290.6 µg/ml	582.07 µg/ml	

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

the two incubation periods at all mixture concentrations except for the lowest concentration. The decline in IC_{50} levels after 72 hours of incubation, compared to 24 hours, further indicates a time-dependent manner of growth inhibition (Table 3) (Figure 5,Supplementary Figure 1).

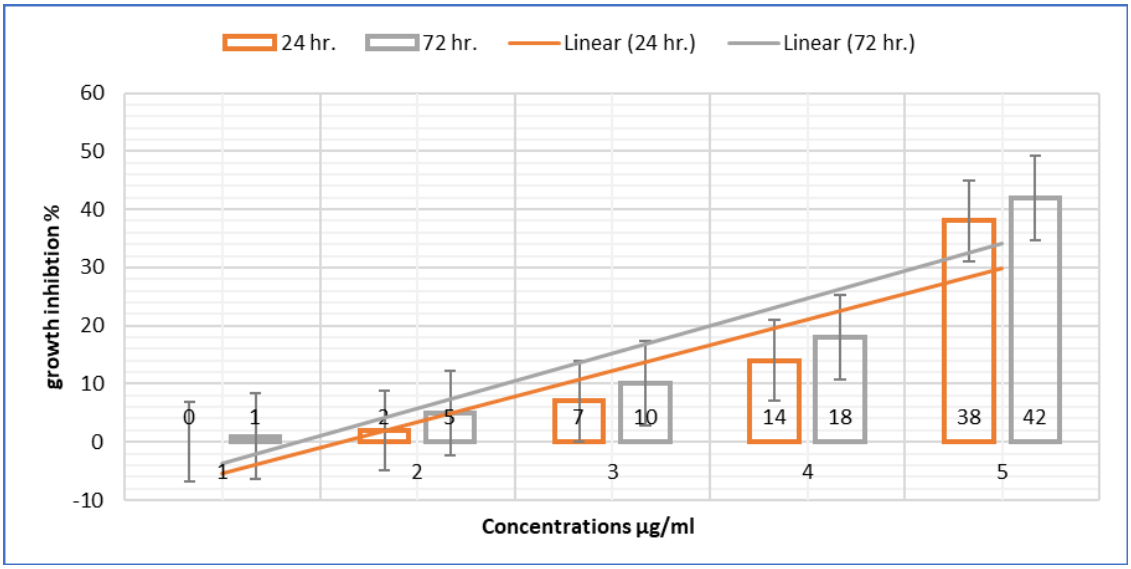

Figure 3. The Impact of Esomeprazole on Cervical Cancer Viability at 24 and 72 Hours

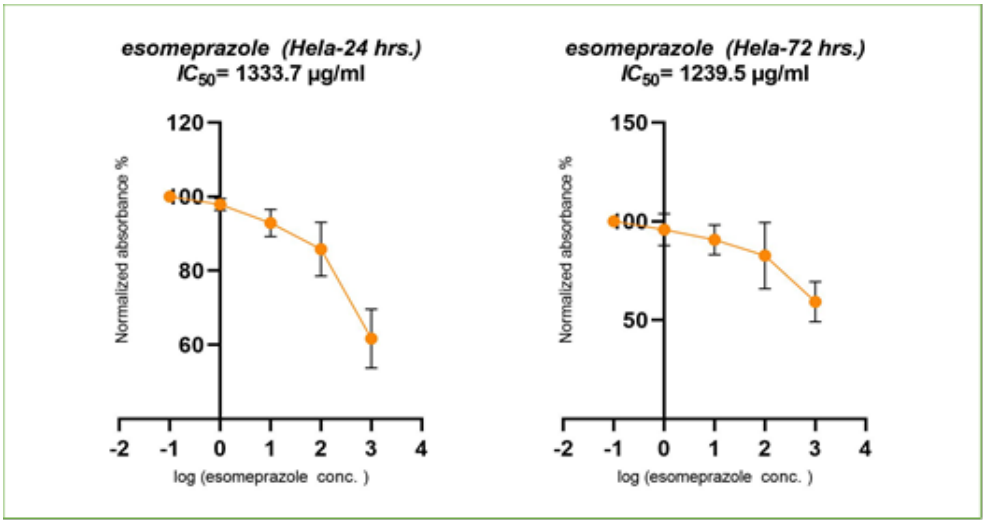

Figure 4. Log Dose-Response Curve for the Estimate of IC_{50} Regarding the Cytotoxicity of Esomeprazole on the Hela Cell Line at 24 and 72 hrs. Incubation

Table 4. Ciprofloxacin-Esomeprazole Combination Impacts HFF Cell Line Viability at 24 and 72 Hours.

Concentration (µg/ml)	Inhibition of cellular proliferation (mean ± SD [a])		P-value
	24 hr.	72 hr.	
0.1	C 0.00 ± 0.000	C 0.00 ± 0.000	N.S
1	C 0.00 ± 0.000	BC 2.00 ± 2.000	0.158
10	BC 3.00 ± 2.000	ABC 9.00 ± 4.000	0.081
100	AB 7.00 ± 2.000	AB 12.00 ± 2.000	0.038*
1000	A 10.00 ± 2.000	A 14.00 ± 4.000	0.196
[b] LSD value	5.64	10.3	-
IC_{50}	5832.9 µg/ml	4801.9 µg/ml	

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

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

Concentration (µg/ml)	Inhibition of cellular proliferation (mean ± SD [a])		P-value
	Hela	HDF	
0.1	D 6.00 ± 2.000	C 0.00 ± 0.000	0.007*
1	D 11.00 ± 1.000	C 0.00 ± 0.000	0.0001*
10	C 20.00 ± 2.000	BC 3.00 ± 2.000	0.0001*
100	B 32.00 ± 2.000	AB 7.00 ± 2.000	0.0001*
1000	A 41.00 ± 1.000	A 10.00 ± 2.000	0.0001*
[b] LSD value	6.08	5.64	-
IC_{50}	1290.6 µg/ml		

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

Additionally, the cytotoxicity of the mixture towards the human fibroblast cell line was utilized to evaluate the toxicity on healthy cells, which may arise from products resulting from pharmaceutical interactions among the

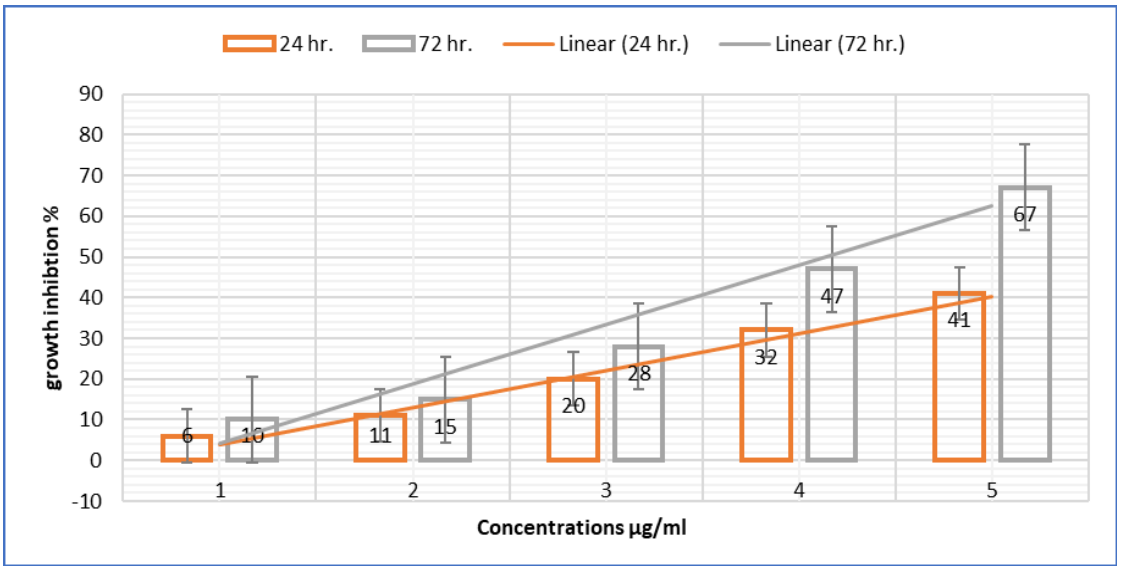

Figure 5. Ciprofloxacin-Esomeprazole Combination Impacts HeLa Cancer Cell Line Viability at 24 and 72 Hours.

mixture's constituents. The findings indicated that the mixture had a significantly greater impact on the HeLa cell line than the HFF cell line at 24 and 72 hours of incubation (Table 4,5 and Supplementary Table 1) (Supplementary Figure 2,3,4,5).

The comparison of mixture cytotoxicity to its constituents revealed that the cytotoxicity of the mixture significantly exceeded that of its components across all incubation periods. Additionally, the IC_{50} levels in cells treated with the mixture were lower than those treated with ciprofloxacin or esomeprazole alone (Supplementary Table 2,3) (Supplementary Figure 6,7, 14)

Selective toxicity index assessment

The selective toxicity index score of the ciprofloxacin–esomeprazole combination was 4.519 and 8.249 for 24 and 72 hours, respectively. This suggests that the combination selectively targets cervical cancer cells over normal healthy cells, with an increase in the selectivity index corresponding to longer incubation times Supplementary Figure (8).

Studying drug combinations

The combination index finding for prob the pattern of Ciprofloxacin and esomeprazole combinations was as follows. After a 24-hour incubation at 0.1, 1, and 10 µg/ml concentrations, the combination pattern had very strong synergistic anticancer effects. While 100 µg/ml showed strong synergism, 1000 µg/ml showed moderate antagonism.

Results indicated that 0.1, 1, and 10 µg/ml showed very strong synergistic anticancer behaviour at a 72-hour incubation period. A 100 µg/ml concentration exhibited strong synergism, while 1000 µg/ml displayed a synergistic pattern.

The dose reduction index findings demonstrated that the concentrations of the mixture ingredients necessary to induce cytotoxicity dropped at all time intervals (24 and 72 hours of incubation) for all concentrations of ciprofloxacin and esomeprazole, indicating that the mixture results in a favourable reduction in the effective concentration of its components, demonstrating raised combination safety and reduced drug side effects Supplementary Table (4,5) Supplementary Figure (9,10).

Molecular docking studies

Molecular docking modelling examined the interactions of ciprofloxacin and esomeprazole with human Hsp 70 (PDB code: 1hjo) as a basis. The study employed AutoDock tools version 1.5.7 and BIOVIA Discovery Studio [66].

molecular docking studies found that the molecular docking score of binding ciprofloxacin with Hsp 70 was (-7.4) kcal/mol. Molecular docking analysis was presented. One halogen (fluorine) bond formed with the GLU A:231 amino acid residues at a 3.69 Å distance. One Pi-cation bond formed with the LYS A:56 amino acid residues at a 3.88 Å distance, and one Pi-anion bond formed with ASP A:234 amino acid residues at a 4.16 Å distance. Finally, one alkyl bond formed with the ARG A:264 amino acid residues at 4.58 Å distances (Supplementary Figure 11).

Furthermore, molecular docking study data of esomeprazole with Hsp 70 revealed a total docking score of (-7.3) kcal/mol. Molecular docking analysis was presented. One conventional hydrogen-bound with TYR A: 41 amino acid residues at 2.77 Å of distance. Three carbon-hydrogen bound with LYS A:56, GLY A:202 and GLU A:268 amino acid residue at 3.68 Å, 3.26 Å and 3.55 Å of distances, subsequently. Finally, four Pi-cation bonds with two LYS A:56, one ARG A:264, one GLU A:231 and one GLY A: 230 amino acid residues at 3.51 Å, 3.83 Å, 4.79 Å,4.73 Å and 5.62 Å of distances subsequently (Supplementary Figure 12)

For comparison, Findings from the molecular docking study of 2-Phenylethynesulfonamide, an inhibitor of Hsp 70, are presented.[68-70]. a total docking score of (-6.4) kcal/mol. Presented six Conventional hydrogen bonds with the one THR A:13, two THR A: 14, THR A:15, GLY A:202, and GLY A:203 amino acid residues at 2.67 Å, 2.03 Å, 2.21 Å, 2.07 Å, 1.83 Å and 2.79 Å of distance, respectively. finally, with one Pi-Pi stacked bond with TYR A:15 at 4.24 Å of distance (Supplementary Figure 13).

Comparison of the docking score among the three medications as shown in Supplementary Table (6).

Discussion

The study inspected the combined anticancer effectiveness of ciprofloxacin and esomeprazole on HeLa cancer cell viability and examined the mixture's ability to target the Hsp70 chaperone protein. The study results indicated that the combination of ciprofloxacin and esomeprazole effectively inhibits the proliferation of cervical cancer cells in a concentration- and time-dependent manner, indicating both cell cycle-specific and cell cycle-nonspecific cytotoxic behaviour. The combination index results indicated that the mixture demonstrated synergistic behaviour across all concentrations and incubation periods. The dose reduction index study finding revealed a significant decline in the effective cytotoxic concentration of the mixture's components compared to the effective cytotoxic concentration of each ingredient, suggesting enhanced safety and minimized adverse effects.

Furthermore, the combination demonstrated selective toxicity towards cancer cells relative to normal cells, as shown by a selectivity index score over one, suggesting a favourable selectivity index.

The chemical docking analysis demonstrated that each constituent of the combination is bound with Hsp70 at varying affinities and binding locations, elucidating the mixture's anticancer mechanism and the synergistic interactions among its components. The cytotoxicity results of the mix on the viability of the HFF cell line indicate that the combination selectively targets cancer cells, given that both malignant and healthy cells express Hsp70. This feature elucidates the preferential toxicity of the combination towards neoplastic cells. Moreover, the findings concerning the mixture's cytotoxicity on the HFF cell line indicate a lack of pharmaceutical interaction potential among the constituents of the mix.

Multiple previous studies have shown that each mixture

constituent possesses anticancer properties. One study regarding ciprofloxacin demonstrated that it significantly inhibits the growth of transitional cell carcinoma cells [70]. Another study indicates that Fluoroquinolone antibiotics induce cell death in breast cancer cells, depending on the treatment dosage and duration. Cell death can occur through various mechanisms, including the induction of apoptosis, increased expression of p53, Bax, and Bcl-2 proteins, alterations in cell cycle distribution, DNA fragmentation, disruption of mitochondrial function via the Bax/Bcl-2 pathway, S-phase cell cycle arrest, and inhibition of topoisomerase II. Additionally, evidence suggests oligonucleosomal DNA fragmentation accompanied by increased p53 expression [18, 12]. Ciprofloxacin can inhibit the proliferation of hepatocellular carcinoma cell lines by inducing DNA breaks and inhibiting topoisomerases. It demonstrates a synergistic effect when used alongside cisplatin [19].

Additionally, various proposed mechanisms elucidate the anticancer properties of esomeprazole. One demonstrated that esomeprazole can inhibit the proliferation of gastric cancer cells and significantly improve their chemosensitivity, as evidenced by MTT assays. Flow cytometry analysis indicated that esomeprazole induced apoptosis and resulted in cell cycle arrest during the S and G2/M phases [71]. A subsequent study demonstrated that proton pump inhibitors, specifically esomeprazole, may significantly impede the invasion and migration of aggressive cancer cells linked to epithelial-mesenchymal transition (EMT), an essential stage in metastasis [30, 31, 72]. E-cadherin, vimentin, fibronectin, and N-cadherin expression significantly changes during the epithelial-mesenchymal transition [32]. Esomeprazole was found to inhibit Snail expression, a factor that can induce epithelial-mesenchymal transition (EMT), while not influencing the expression of other transcription factors associated with EMT [33-35, 12] . Furthermore, Esomeprazole exhibited a significant capacity to bind directly to the Snail protein by inhibiting CREB-binding protein (CBP)/p300-mediated Snail acetylation, which promotes Snail degradation [36].

Furthermore, other Proposed mechanisms include esomeprazole's ability to induce lysosomal membrane permeabilization, leading to increased lysosomal outflow into the cytoplasm, lysis of cellular components, and subsequent cell death. Lysosomal enzymes demonstrate hydrolytic activity and establish an acidic environment that aids in eliminating tumor cells [37-39]..

In contrast to the proposed mechanisms of ciprofloxacin and esomeprazole identified in previous studies, our study presents a novel anticancer mechanism for these compounds, emphasizing their ability to target Hsp 70.

We focus on heat shock protein 70 (HSP70) due to its essential role in the pathway that facilitates the production of Cellular FLICE (FADD-like IL-1beta-converting enzyme)-inhibitory protein (c-FLIP). C-FLIP plays a crucial role in resistance and serves as a key regulator that inhibits apoptosis triggered by tumor necrosis factor-alpha (TNF-alpha), Fas-L, and TNF-related apoptosis-inducing ligand (TRAIL), in addition to chemotherapy-induced apoptosis in cancer cells [73]. Furthermore, Malignant cells demonstrate elevated levels of Hsp70 compared to normal cells. Elevated levels of Hsp70 are associated with a tumorigenic phenotype, frequently resulting in resistance to chemotherapy and apoptosis [74, 75].

Hsp70 interferes with various stages of apoptotic pathways, preventing inappropriate initiation of cellular death under stress conditions. In addition to regulating apoptosis, Hsp70 can modulate the immune response and facilitate antigen delivery alongside the MHC-I molecule. It activates innate and adaptive immune systems and is a potent immunomodulator [76], The expression of Hsp70, a protein conserved through evolution and involved in apoptotic signaling, enhances cell viability under stress conditions. Cells exhibiting Hsp70 knockdown demonstrate increased susceptibility to apoptosis [77], and the overexpression of Hsp70 inhibits apoptosis upstream or downstream of mitochondria [78].

Moreover, Hsp70 engages with nerve growth factor and platelet-derived growth factor, facilitating cell survival by activating the PI3K signaling pathway. The activation of PI3K leads to the activation of serine/threonine kinases (Akt/PKB), producing a survival signal mediated by growth factors. Substrates of Akt kinase: Both Bad and caspase-9 participate in programmed cell death, called apoptosis, as components of a sequence of events [79, 80] The Hsp70 protein increases the stability of the Akt/PKB complex in K562 cells [81].

Additionally, another study exhibited that HspA12B, a member of the Hsp70 family, is essential for blood vessel development in zebrafish. It facilitates endothelial cell migration and tube formation by maintaining Akt activation [82]. The Hsp70 family regulates cell survival and differentiation. Hsp70 plays a role in protein prephosphorylation and stability by facilitating the activation of unphosphorylated protein kinases [83]. Hsp70 functions as a suppressor of apoptosis signal-regulating kinase-1 in NIH3T3 cells, a kinase activated by stress. Down-regulating Hsp70 results in the production of H2O2 and the activation of ASK-1, which ultimately induces apoptosis [84].

Due to their importance in cancer pathogenesis, several studies focusing on Hsp 70 as a cancer target suggested several agents as Hsp 70 inhibitors, such as (2-phenyl ethyne sulfonamide), a Phenylethylsulfonamide-derived [85, 86]. (Apoptozole and Az-TPP-O3) an Imidazole-derived [87-89]. (YM-1 and JG-83) a Rhodocyanine-derived. [90, 91]. (Epigallocatechin-3-gallate (EGCG), Quercetin, Kahweol, Cantharidin, and Veratridine) a Natural compound [92-98].

Based on the factors mentioned earlier, Hsp 70 has been selected due to its crucial function in cancer and its potential as a target for cancer therapy. Our molecular docking study results demonstrate that each drug interacts with Hsp70 with different binding sites and affinities. Targeting Hsp70 may represent a key anticancer mechanism concerning the similarity in docking scores of ciprofloxacin and esomeprazole, potentially elucidating the similarities in their cytotoxic effects.

In addition, the combination index study findings. the molecular docking studies elucidate the synergistic effect of ciprofloxacin and esomeprazole. Each drug interacts

with the Hsp 70 protein at a specific location, leading to a complementary and synergistic effect when the two drugs are combined.

The lack of restrictions on drug concentration ranges limited the study. A variety of concentrations were employed to identify the optimal effective concentration for ciprofloxacin and esomeprazole.

In conclusion, the findings of the present study demonstrate that the combination of ciprofloxacin and esomeprazole significantly reduces the growth of the Hela cancer cell line. The inhibition behaviour exhibited both cell cycle-specific and cell cycle-nonspecific characteristics. The results indicate that each drug displayed a specific level of cytotoxicity, whereas the combination exhibited synergistic cytotoxicity, as evaluated by the combination index value.

Computational docking simulations suggested a novel anticancer mechanism of each ciprofloxacin and esomeprazole via their interaction with Heat Shock Protein 70. The findings clarify the synergistic interactions among mixed ingredients, with each drug targeting a specific binding site on Hsp 70, suggesting a complementary targeting mechanism with Hsp 70.

Additionally, we proposed that the mixture exhibited selective toxicity toward cancer cells rather than in healthy cells, as indicated by the selectivity index score. The dose reduction index findings indicate that the concentration of medications required in the mixture to attain significant cytotoxicity is lower than the cytotoxicity concentration of each medication when employed individually. These findings, along with well-known pharmacokinetics and adverse effect profiles, indicate that the combination of ciprofloxacin and esomeprazole offers an effective, safe option for cervical cancer.

Author Contribution Statement

Design and development: Kawakeb N Abdulla, Solafa Rabi Salih, Azal Hamoody. Gathering and organizing data: Anas K. Awn, Kawakeb N Abdulla, Youssef Shakuri. Data analysis/interpretation: Anas K. Awn, Azal Hamoody, Solafa Rabi Salih. Article composition: Kawakeb N Abdulla, Anas K. Awn, Solafa Rabi Salih. Critique the essay for significant ideas: Anas K. Awn, Kawakeb N Abdulla, Solafa Rabi Salih. Statistical analysis expertise: Youssef Shakuri, Kawakeb N Abdulla, Anas K. Awn , Azal Hamoody. Ultimate article endorsement and guarantee: Solafa Rabi Salih, Anas K. Awn , Kawakeb N Abdulla

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Conflicts of interest

No conflict of interest is declared by the authors.

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

The authors confirm that this work does not utilize generative AI or AI-assisted technologies.

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

SI: Selective toxicity Index

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