RESEARCH ARTICLE

**Editorial Process:** Submission:10/21/2024 Acceptance:04/18/2025

# Scrutiny of the Co-Cytotoxic Impact of Metformin-Omeprazole on the Cervical Cancer Cell Line and Their Aptitude to Target Heat Shock 60

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## Abstract

**Objective:** This study aimed to assess the simultaneous effect of the metformin–omeprazole combination on inhibiting cervical cancer proliferation. Their ability to target heat shock protein 60. **Methods:** The anticancer properties of the metformin–omeprazole combination in cancer treatment were evaluated by employing a cervical cancer cell line (HeLa cell line). The assessment included two incubation periods: one of 24 hours and another of 72 hours. The concentrations of metformin, omeprazole, and their combination varied from 0.1 to 1000 µg/ml. The study encompassed an estimated combination index value to assess the potential synergistic effect of metronidazole and linagliptin. The study employs computational molecular docking simulation to determine the affinity of metformin and omeprazole for binding with heat shock protein 60. **Results:** The study concluded that the metformin-omeprazole combination significantly reduced the proliferation of cervical cancer cells. The inhibitory effect was demonstrated to depend on the mixture's concentration and the treatment duration. The combination index indicates that metformin and omeprazole synergistically interacted. furthermore, the computational molecular docking simulation indicated that metformin and omeprazole exhibited a propensity to associate with Hsp 60. The docking scores for metformin and omeprazole were measured at -7.3 kcal/mol and -6.2 kcal/mol, respectively. **Conclusion:** Study indicates that the simultaneous use of metformin and omeprazole synergistically suppresses the growth of cervical cancer cells via both cell cycle-specific and cell cycle-nonspecific pathways. The findings, corroborated by molecular docking studies, demonstrated that metformin and omeprazole can bind to Heat Shock Protein 60. Furthermore, the molecular docking data elucidate the synergistic interactions among the combination components since every drug occupies a distinct binding site on Hsp 60, indicating a complementary binding mode with Hsp 60. Regarding the expected adverse impact and the known pharmacokinetic profile of the mixture's components, the mixture offered an attractive alternative treatment for cervical cancer.

**Keywords:** Metformin- omeprazole- cervical cancer- Hela cell line- combination index- dose reduction index

*Asian Pac J Cancer Prev,* **26 (4)**, 1353-1363

## Introduction

Annually, more than 500,000 women receive a diagnosis of cervical cancer, leading to over 300,000 fatalities globally. The severity of the disease influences treatment decisions at diagnosis and the availability of local resources. A radical hysterectomy, chemotherapy, or a combination of both may be necessary [1]. Results from five randomized clinical trials indicate [2-7], that women with invasive cervical cancer eligible for radiotherapy should consider simultaneous cisplatin-based chemo-radiotherapy rather than radiotherapy alone. Multiple studies across 11 countries have demonstrated the positive impact of combined chemoradiation on prognosis. The analysis showed a 12% increase in overall survival and improved local and distant disease progression management [8-11]. Although chemoradiotherapy is commonly considered a fundamental treatment for cervical cancer, the adverse side effects of chemotherapy require the investigation of safer options. Multiple trials have been done to identify an efficacious treatment for cervical cancer by repurposing a medicine that is already used for another therapeutic purpose.

Numerous efforts have been undertaken to identify a more effective and safer alternative treatment for cancer, such as employing medications that are approved for

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other diseases in the treatment of cancer. Omeprazole is an example of a medication that shows a promising anticancer impact. Some studies suggest that the anti-cancer activity of omeprazole is related to the inhibition of vacuolar-type ATPase (V-ATPase) [12] And fatty acid synthase (FASN) [13-19]. Furthermore, Omeprazole was proposed to influence the cancer hallmarks of migration, invasion, and genomic instability [20, 21]. it 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 [22, 14, 20, 23] .

Omeprazole considerably impedes the invasion and migration of aggressive cancer cells associated with epithelial-mesenchymal transition (EMT), a critical phase in metastasis [24, 25] . Significant changes in the expression of E-cadherin and the mesenchymal markers vimentin, fibronectin, and N-cadherin characterize the epithelial-mesenchymal transition [26] .Omeprazole was shown to inhibit Snail expression, which may trigger epithelial-mesenchymal transition (EMT), without affecting the expression of other transcription factors related to EMT [27-29] . Furthermore, Omeprazole demonstrated a strong affinity for binding directly to the Snail protein by disrupting CREB-binding protein (CBP)/p300-mediated Snail acetylation, thereby facilitating Snail degradation [30-33]. Metformin is another drug with promising anticancer properties. Multiple studies have been conducted to assess and investigate its properties. Metformin influences cancer hallmarks by sustaining proliferative signaling and deregulating cellular energetics [34] metformin can activate AMPK by interacting with the lysosomal protein PEN2. Induced activation of AMPK has been demonstrated to inhibit mTOR, leading to cell cycle arrest in multiple myeloma cells [35-40]. Another study demonstrated that metformin exhibited an apoptotic effect and induced apoptosis in p53-deficient colon cancer cell lines [41].

Various heat shock proteins, including Hsp 60, are critical in cancer development. Hsp 60 is integral to the transport and folding of mitochondrial proteins and has been linked to multiple cancer types [42]. Many studies suggest that HSP60 contributes to apoptosis by facilitating the activation of pro-caspase-3 via several caspases, including caspase-6. HSP60, situated in the cytosol, inhibits the translocation of the pro-apoptotic protein Bax into mitochondria, thus facilitating cell survival [43]. The prognostic association of HSP60 with cervical cancer has recently become a significant focus of research. In these investigations, the prognostic significance of HSP60 in cervical cancer was evaluated using 2-dimensional Electrophoresis (2-DE), semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR), and Western Blot (WB) analyses. The results suggest that HSP60 plays a critical role in the advancement of cervical cancer [44] .Recent studies demonstrate that HSP60 expression is elevated in prostate cancer tissues relative to normal prostatic tissue [45-48]

Combining current medicines for non-cancer therapeutic purposes is a viable approach to developing an effective, safer option for cancer treatment. Several studies were conducted on this subject, and one showed that the combination of amygdalin and esomeprazole had a specific ability to eradicate cervical cancer cells [49, 50] Others demonstrated that the combination of ciprofloxacin and laetrile successfully hinders the proliferation of esophageal cancer cells [51] . Despite these studies, they showed limitations in demonstrating the anticancer properties of the metformin–omeprazole combination. This study investigates the suppressive effect of metformin and omeprazole on the growth of cervical cancer cells and explores their ability to bind with Hsp 60.

## Materials and Methods

*Medications utilized in the study*

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

*Hela cell line*

The Hela cancer cell line, derived from a malignant cervical carcinoma, was first established in the tissue culture division of ICCMGR. The cells were cultured in 75 $cm^2$ tissue culture containers under controlled conditions, maintaining a relative humidity of 37°C and 5% CO2. The cells were incubated in RPMI-1640 medium (Sigma Chemicals, England) with 10% fetal calf serum (FBS) and 100 U/mL penicillin-streptomycin (100 µg/mL streptomycin) [52, 49].

*Cytotoxicity assay*

Metformin, omeprazole, and a combination were utilized to assess their efficacy in inhibiting cervical cancer cells cultured in a 96-well microtiter plate. Cancer cell proliferation demonstrated a gradual and consistent increase in the logarithmic growth phase. The toxicity of the evaluated medications was examined at two distinct incubation periods: 24 hours and 72 hours [53, 54].

Each well contains 10,000 cells. Seeding requires the use of a medium that contains 10% fetal bovine serum. The plates were incubated at 37°C for 24 hours to facilitate cell attachment. Serial dilutions were performed using a serum-free RPMI medium. Metformin, omeprazole, and a combination of both drugs were diluted in RPMI medium devoid of calf serum. A series of dilutions for each medication was produced, ranging from 0.1 to 1000 µg/ml [55, 51].

After 24 hours of cancer cell proliferation, the cells were divided into six identical samples, each receiving 200 µl of a medication. Each control was administered 200 microlitres of maintenance media, with exposure durations varying from 24 to 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 [56, 57].

A mathematical equation is used to determine the growth inhibition rate, and it is as follows: [57]

Growth inhibition %= (optical density of control wells-optical density of treated wells)/(optical density of control wells)*100%

*Study of drug combinations*

A comprehensive study of the combination of drugs in a mixture was conducted based on the investigation findings. After a treatment period of 24 and 72 hours, the concentration-effect curves were generated by plotting the percentage of cells that exhibited a reduction in growth against the concentration of the medication. The Compusyn computer software (Biosoft, Ferguson, Missouri, USA) was used to evaluate the synergy, additive effects, and antagonism to determine the pharmacological interaction. Through the process of calculating the combination index and dose reduction index values, the accomplishment was completed.

CI values below 1 suggest synergy, values above 1 indicate additivity and values exceeding 1 denote antagonism. The dose reduction index (DRI) measures the degree to which the concentration of each component in a combination can be lowered while maintaining equivalent efficacy to that of individual drug administration.

A DRI score of 1 indicates no decrease in concentration. A DRI number greater than 1 signifies a favorable reduction in concentration, whereas a DRI value less than 1 indicates an unfavorable decline in concentration [58, 59].

*Molecular docking*

The chemical structures of metformin and omeprazole were shown using ChemDraw software (Cambridge Soft, USA) and refined using the Chem3D version. The molecular configuration of the Hsp 60 chaperonins, known as the Heat shock protein, was acquired from the Protein Data Bank.

Protein structures were optimized and adjusted using AutoDock Tools. The optimal conformation of the ligands was determined using AutoDock Tools, followed by the ligands' generation of a PDBQT file.

After optimization, each ligand's structures (metformin and omeprazole) and the human Hsp 60 chaperone protein were inputted into AutoDock-Tools. Subsequently, the docking procedure was performed utilizing the identical program. The docking energy scores and binding interactions were comprehensively analyzed using PLIP and BIOVIA Discovery Studio [60, 61].

*Ethical approval*

This research did not include any human subjects.

*Statistical Analysis*

The MTT test results are presented as the mean ± standard deviation (SD) based on six replicates. A one-way analysis of variance (ANOVA) test was utilized. The LSD test was employed to compare various groups. The study used statistical software version 20, with a significance threshold set at $p < 0.05$ [62].

## Results

*Cancer cell line cytotoxicity study*

*Metformin cytotoxicity*

Study results demonstrated that metformin can reduce the proliferation of cervical cancer cells. The pattern of growth inhibition was contingent upon the concentration, notably evident at the highest concentration. Additionally, the pattern of growth inhibition was affected by the incubation period, particularly at doses of 1, 10, and 1000 µg/ml. The IC50 values substantiate this observation, falling from 1582.19 µg/ml to 1225.89 µg/ml, revealing the incubation period's impact on cellular viability: Table (1) and Figure (1).

*Omeprazole cytotoxicity*

The study results indicate that omeprazole has a growth inhibition ability regarding cervical cancer, primarily following a concentration-dependent pattern. The impact of time was less than the concentration impact, except at 100 µg/ml. Further, there was a lowered decline in IC 50 between the two incubation periods, suggesting a less time effect on cellular growth inhibition: Table (2), Figure (2).

*(metformin -omeprazole) combination cytotoxicity*

The study's findings indicated that the combination of metformin and omeprazole reduces the viability of cancer cells. The aptitude is contingent upon the concentration of the combination. This impact is elucidated through significant differences in growth inhibition observed between the highest and lowest concentrations.

Furthermore, the pattern of growth inhibition was dependent on the incubation duration, particularly at

Table 1. The Impact of Metformin 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 | C 1.00 ± 1.000 | D 3.00 ± 2.000 | 0.196 |
| 1 | C 3.00 ± 1.000 | C 17.00 ± 2.000 | 0.0001* |
| 10 | C 7.00 ± 3.000 | BC 23.00 ± 3.000 | 0.003* |
| 100 | B 21.00 ± 4.000 | B 27.00 ± 1.000 | 0.065 |
| 1000 | A 33.00 ± 4.000 | A 43.00 ± 3.000 | 0.026* |
| [b] LSD value | 10.68 | 8.46 | - |
| $IC_{50}$ | 1582.19 µg/ml | 1225.89 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)

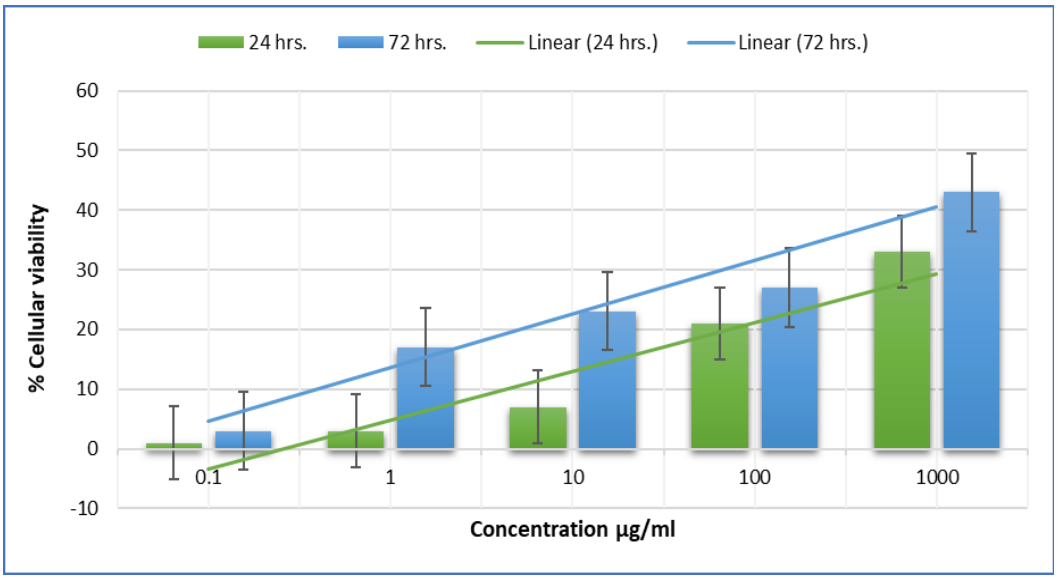


figure 1. The Impact of Metformin on the Viability of Hela Cancer Cells at 24 and 72 hours

Table 2. The Impact of Omeprazole 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 | C 1.00±1.000 | D 2.00±1.000 | 0.288 |
| 1 | C 3.00±2.000 | CD 7.00±3.000 | 0.127 |
| 10 | B 11.00±1.000 | BC 13.00±5.000 | 0.534 |
| 100 | B 17.00±2.000 | B 21.00±1.000 | 0.036* |
| 1000 | A 48.00±3.000 | A 53.00±3.000 | 0.111 |
| [b] LSD value | 7.1 | 10.92 | - |
| $IC_{50}$ | 1031.61 µg/ml | 916.03 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 0.1, 10, and 100 µg/ml. The impact of incubation time on growth inhibition is evidenced by the decrease in the IC 50 level, which declined from 1112.07 µg/ml to 602.52 µg/ml across the two incubation periods. (Table 3) (Figure 3).

Moreover, the inhibitory impact of the mixture on cervical cancer growth exceeded that of any individual component at every incubation period. (Table 4,5)

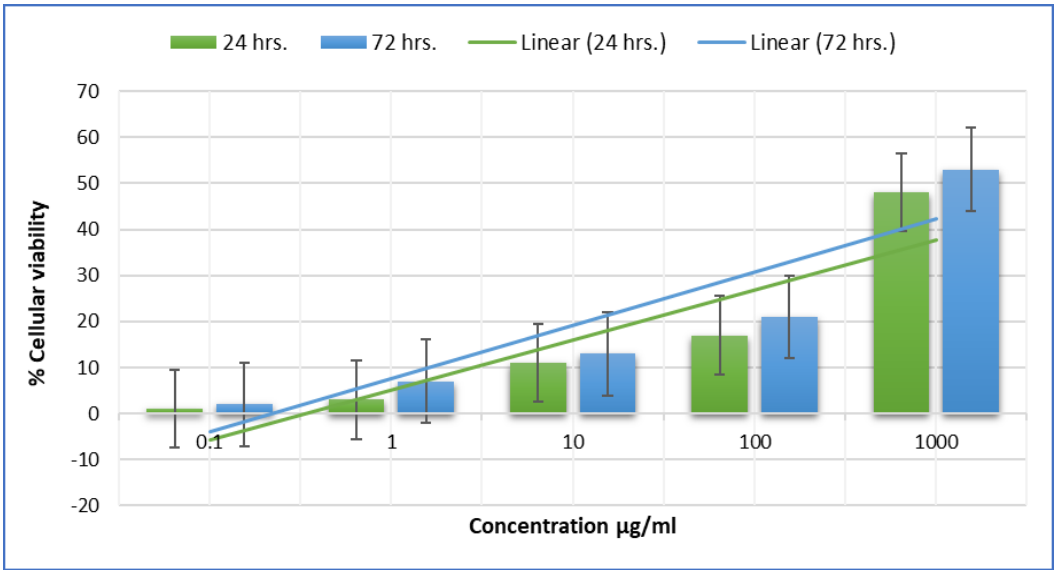


Figure 2. The Impact of Omeprazole on the Viability of Hela Cancer Cells at 24 and 72 Hours

Table 3. The Impact of the Metformin-Omeprazole Combination on HeLa Cancer Cell 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 2.00 ± 2.000 | D 8.00 ± 3.000 | 0.045* |
| 1 | C 11.00 ± 1.000 | D 17.00 ± 4.000 | 0.065 |
| 10 | B 23.00 ± 3.000 | C 27.00 ± 2.000 | 0.127 |
| 100 | A 36.00 ± 3.000 | B 42.00 ± 2.000 | 0.045* |
| 1000 | A 45.00 ± 5.000 | A 67.00 ± 1.000 | 0.002* |
| [b] LSD value | 11.28 | 9.48 | - |
| $IC_{50}$ | 1112.07 µg/ml | 602.52 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)

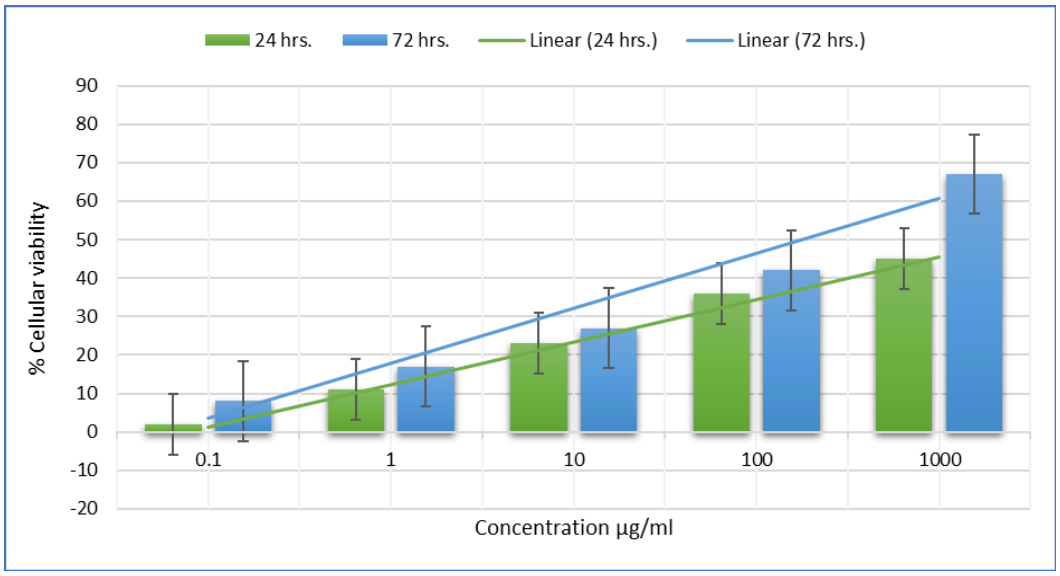


Figure 3. The Impact of the Metformin-Omeprazole Combination on HeLa Cancer Cell Viability at 24 and 72 Hours

(Figure 4,5, Supplementary Figures 4,5,6).

*Studying drug combinations*

The study of drug combinations encompassing metformin and omeprazole produces the following results. Following a 24-hour incubation, the combination of metformin and omeprazole at 0.1, 1, 10, and 100 µg/ml exhibited a synergistic anticancer effect. In contrast, A concentration of 1000 µg/ml exhibited antagonism. At 72 hours, all concentrations demonstrated a synergistic combination effect.

The findings of the dose reduction index indicated that the concentrations of the combined ingredients required to induce cytotoxicity were decreased at all time intervals (24 and 72 hours of incubation) for all concentrations of metformin and omeprazole, except the higher concentration of omeprazole at 24 incubation periods.

The reduction in effective concentration was notable for metformin and omeprazole, indicating a favourable drop in the effective concentration of the mixture

Table 4. A 24-hour Growth Inhibition Comparison of Metformin, Omeprazole, and a Combination

| Concentration (µg/ml) | Growth inhibition (mean ± SE [a]) | | | [b] LSD value |
|---|---|---|---|---|
| | Metformin | Omeprazole | mix | |
| 0.1 | C 1.00 ± 1.000 | C 1.00±1.000 | C 2.00 ± 2.000 | N. S |
| 1 | C 3.00 ± 1.000 | C 3.00±2.000 | C 11.00 ± 1.000 | 5.66* |
| 10 | C 7.00 ± 3.000 | B 11.00±1.000 | B 23.00 ± 3.000 | 10.06* |
| 100 | B 21.00 ± 4.000 | B 17.00±2.000 | A 36.00 ± 3.000 | 12.42* |
| 1000 | A 33.00 ± 4.000 | A 48.00±3.000 | A 45.00 ± 5.000 | 16.32 |
| b LSD value | 10.68 | 7.1 | 11.28 | - |
| $IC_{50}$ | 1582.19 µg/ml | 1031.61 µg/ml | 1112.07 µg/ml | - |

[a], standard error; [b], least significant difference. Capital letters within the same column indicate statistically significant differences, while variations in lowercase letters within the same rows also signify statistically significant differences.: significant at (P<0.05)

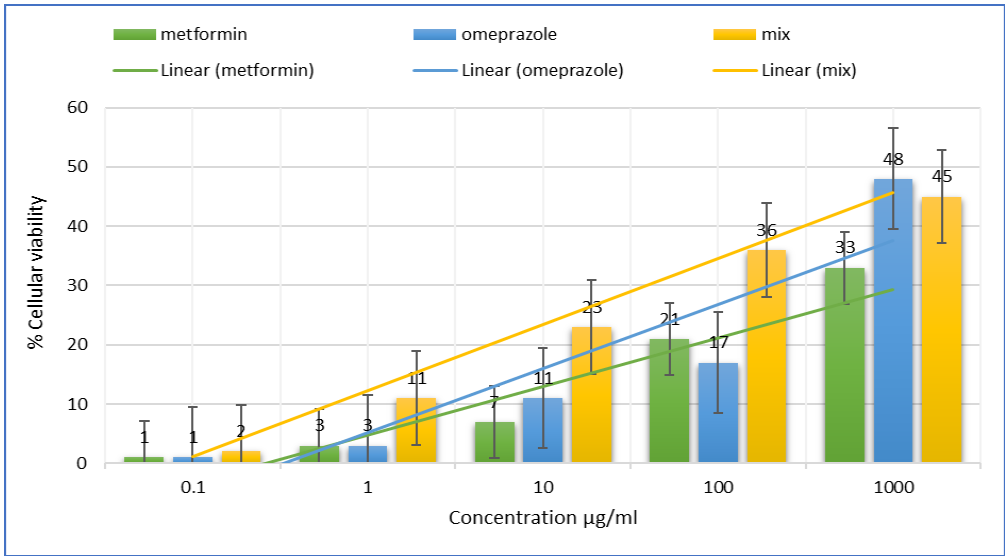


Figure 4. A 24-hour Growth Inhibition Comparison of Metformin, Omeprazole, and a Combination.

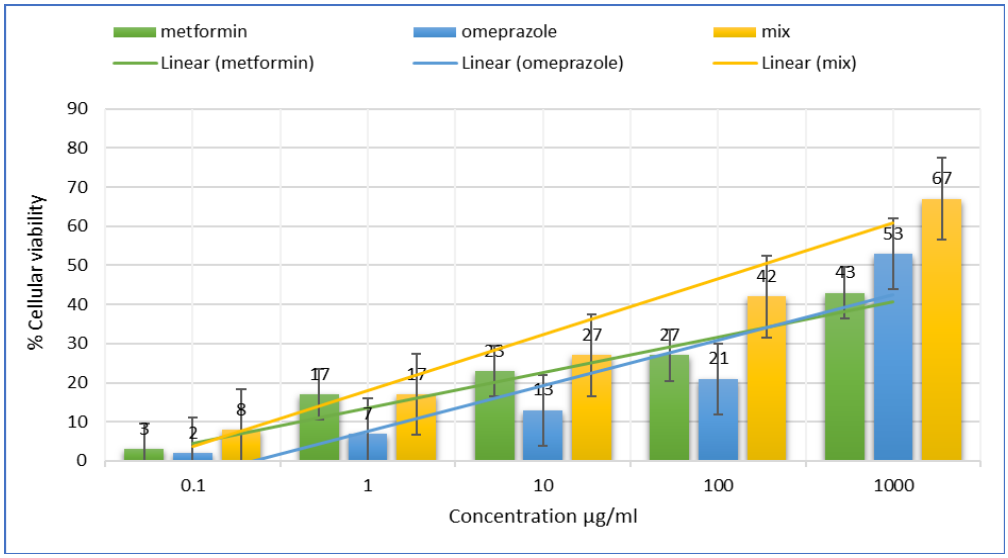


Figure 5. A 72-hour Growth Inhibition Comparison of Metformin, Omeprazole, and a Combination

components relative to the individual components. Tables (6,7) Supplementary Figures (1,2)

*Molecular docking studies*

Molecular docking modeling explored the interaction between metformin and omeprazole with human Hsp 60. The investigation employed AutoDock tools 1.5.7 and BIOVIA Discovery Studio [63].

Our molecular docking studies results demonstrated that the molecular docking score of binding omeprazole with Hsp 60 was (-7.3) kcal/mol. Molecular docking analysis was presented. One Conventional hydrogen bond

Table 5. A 72-hour Growth Inhibition Comparison of Metformin, Omeprazole, and a Combination.

| Concentration (µg/ml) | Growth inhibition (mean ± SE[a]) | | | [b] LSD value |
|---|---|---|---|---|
| | Metformin | Omeprazole | mix | |
| 0.1 | D 3.00 ± 2.000 | D 2.00±1.000 | D 8.00 ± 3.000 | N. S |
| 1 | C 17.00 ± 2.000 | CD 7.00±3.000 | D 17.00 ± 4.000 | 12.42 |
| 10 | BC 23.00 ± 3.000 | BC 13.00±5.000 | C 27.00 ± 2.000 | 14.22 |
| 100 | B 27.00 ± 1.000 | B 21.00±1.000 | B 42.00 ± 2.000 | 12.17 |
| 1000 | A 43.00 ± 3.000 | A 53.00±3.000 | A 67.00 ± 1.000 | 10.06 |
| [b] LSD value | 8.46 | 10.92 | 9.48 | - |
| $IC_{50}$ | 1225.89 µg/ml | 916.03 µg/ml | 602.52 µg/ml | - |

[a], standard error; [b], least significant difference. Capital letters within the same column indicate statistically significant differences, while variations in lowercase letters within the same rows also signify statistically significant differences.: significant at (P<0.05)

Table 6. The Suppressive Impact of the Metformin-Omeprazole Combination on HeLa Cancer Cell Line Proliferation after 24 hours of Incubation

| concentration µg/ml | | Con. ratio | CI value | Combination behaviour | DRI value | |
|---|---|---|---|---|---|---|
| Metformin | Omeprazole | 1:01 | | | Metformin | Omeprazole |
| 0.05 µg/ml | 0.05 µg/ml | | 0.48094 | Synergism | 4.32674 | 4.0029 |
| 0.5 µg/ml | 0.5 µg/ml | | 0.09144 | Very Strong Synergism | 28.2385 | 17.8493 |
| 5 µg/ml | 5 µg/ml | | 0.13288 | Strong Synergism | 21.8744 | 11.4725 |
| 50 µg/ml | 50 µg/ml | | 0.33476 | Synergism | 9.49869 | 4.35761 |
| 500 µg/ml | 500 µg/ml | | 1.48285 | antagonism | 2.26562 | 0.96018 |

The CI (Combination Index) and DRI (Dose Reduction Index) Values were evaluated utilizing Compusyn software. A CI number exceeding 1 indicates antagonism, a CI value of 1 denotes an additive effect, and a CI value below 1 suggests synergism. A dose reduction index (DRI) exceeding one correlates with reduced toxicity. (Chou, 2006, Chou, 2018)

Table 7. The Suppressive Impact of the Metformin-Omeprazole Combination on HeLa Cancer Cell Line Proliferation after 72 hours of Incubation

| concentration µg/ml | | Con. ratio | CI value | Combination behaviour | DRI value | |
|---|---|---|---|---|---|---|
| metformin | omeprazole | 1:01 | | | metformin | omeprazole |
| 0.05 µg/ml | 0.05 µg/ml | | 0.25399 | Strong Synergism | 4.6043 | 27.1693 |
| 0.5 µg/ml | 0.5 µg/ml | | 0.17262 | Strong Synergism | 7.77399 | 22.7354 |
| 5 µg/ml | 5 µg/ml | | 0.28464 | Strong Synergism | 5.46281 | 9.84397 |
| 50 µg/ml | 50 µg/ml | | 0.39152 | Synergism | 5.0121 | 5.20837 |
| 500 µg/ml | 500 µg/ml | | 0.21545 | Strong Synergism | 15.0364 | 6.71378 |

The CI (Combination Index) and DRI (Dose Reduction Index) Values were evaluated using Compusyn software. A CI number exceeding 1 indicates antagonism, a CI value of 1 denotes an additive effect, and a CI value below 1 suggests synergism. A dose reduction index (DRI) exceeding one correlates with reduced toxicity. (Chou, 2006, Chou, 2018).

formed with the ASN A:287 amino acid residues at 2.21 Å distance. One carbon-hydrogen bound formed with the ASN A:284 amino acid residues at 3.35 Å distance. One pi-anion formed with the GLU A:364 amino acid residues at 4.74 Å distance. Four pi-pi-stacked formed with the PHE A:281, TYR A:361, PHE A:281, and TYR A:361 amino acid residues at 5.19 Å, 3.74 Å, 5.94, and 3.91 Å distances, respectively. Three pi-alkyl formed with the TYR A:361, LYS A:363, and LYS A:363 amino acid residues at 5.09 Å, 5.09 Å, and 5.46 Å distance, respectively (Supplementary Figure 1).

furthermore, molecular docking study data of metformin with Hsp 60 revealed a total docking score of (-6.2) kcal/mol. Molecular docking analysis was presented. Three conventional hydrogen-bound with each ASP A:399, ASP A:52, and SER A:151 amino acid residues at 2.88 Å, 2.70 Å, and 2.97 Å of distance, respectively (Supplementary Figure 2).

For comparison purposes, the molecular docking study of Epolactaene (the standard Hsp 60 inhibitor) revealed a total docking score of (-6.7) kcal/mol. Molecular docking analysis was presented. Four conventional hydrogen-bound with SER A:228 amino acid residue at 4.08 Å, SER A:229 amino acid residue at 1.89 Å, SER A:229 amino acid residue at 2.20 Å and GLN A:231 amino acid residue at 2.39 Å. Two alkyls bound also found with LYS A:225 amino acid residue at 5.39 Å and LEU A:310 amino acid residue at 4.74 Å (Supplementary Figure 3).

## Discussion

The study on cytotoxicity and combination index revealed that the metformin–omeprazole combination exhibited synergistic antiproliferative effects on cervical cancer cells, characterized by both cell cycle-specific and non-specific inhibition patterns. The results of the computational molecular docking simulations indicate that both drugs demonstrate a novel mechanism targeting Hsp 60. Each exhibited a distinct binding site on Hsp60, clarifying the synergistic interactions between the drugs in the mixture.

Prior studies have shown several other suggested mechanisms for each drug. metformin has been verified to be effective in reducing the incidence of specific malignancies, including pancreatic cancer [64, 65]. A recent study has evidenced that metformin effectively reduces the likelihood of colon cancer development and the associated mortality rate [66, 67]. Metformin has demonstrated efficacy in decreasing the formation of adenomas and polyps in patients undergoing polypectomy [68]. It lowers the death risk among diabetics who get a colon cancer diagnosis [69, 70]. Metformin has been shown in yet another investigation to decrease the risk of developing prostate cancer and liver cancer, as well as the mortality rate associated with these two varieties of cancer [70-74]. Multiple proposed mechanisms have been studied to explore metformin's anticancer effects. Metformin activation of AMPK in rat hepatoma H4IIE cells reduces pS6 phosphorylation [75]. A distinct in vitro study concluded that metformin directly inhibited AMP deaminase, leading to elevated AMP levels and the subsequent activation of AMPK [76, 41, 77]. Moreover, Metformin can eliminate active K-ras from the cellular membrane via a PKC-dependent mechanism [78].

Conversely, study outcomes revealed that omeprazole could minimize the growth of cervical cancer with cytotoxic behavior, including concentration-dependent. On the same topic, multiple studies exhibited the ability of omeprazole to reduce the growth of several types of cancer, such as gastric cancer [79]. Pancreatic cancer [12]. Human B-cell malignancies [80]. And glioblastoma [81]. And its ability to minimize the invasion of breast cancer and pancreatic cancer [82-84].

Several suggested mechanisms clarify omeprazole's anticancer properties. For example, Omeprazole can significantly hinder the invasion and migration of aggressive cancer cells related to epithelial-mesenchymal transition (EMT), an essential process during metastasis [24, 25]. The epithelial-mesenchymal transition is marked by dramatically altered expression of E-cadherin and the mesenchymal markers vimentin, fibronectin, and N-cadherin [26] .Omeprazole was found to suppress Snail expression, which can induce epithelial-mesenchymal transition (EMT) without impacting the expression of other transcription factors associated with EMT [27-29] . Furthermore, omeprazole exhibited a high affinity for directly and physically binding to the Snail protein via disrupted CREB-binding protein (CBP)/p300-mediated Snail acetylation, which promotes Snail degradation [30].

A molecular docking study was performed to clarify the novel anticancer mechanisms of each drug in the mixture. We focus 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 [42] . and it promotes apoptosis by activating pro-caspase-3 via caspases like caspase-6 [43] .

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. findings strongly indicate that HSP60 is integral to the progression of cervical cancer [44-47] .

Based on the factors above, Hsp 60 has been elected due to its key function in cancer. Our molecular docking study results indicate that each drug interacts with Hsp60 at varying levels of binding affinity. The results of the molecular docking study suggest that omeprazole exhibits a more significant antiproliferative effect than metformin, as evidenced by the higher molecular docking score associated with omeprazole. Molecular docking studies indicate the synergistic effect of omeprazole and metformin. Each drug interacts with the Hsp 60 protein at a specific site, leading to a complementary and synergistic effect when combined.

The dose reduction index result reveals that the cytotoxic concentration of each medication in the combination was inferior to that of each drug individually, implying a less likelihood of adverse effects from the mixture compared to the individual drugs. A limitation of the study was the absence of restrictions on the drug concentration ranges. We utilized various concentrations to determine the optimal doses for omeprazole and metformin.

In conclusion, our study's findings indicate that the metformin–omeprazole combination effectively inhibits the proliferation of cervical cancer cells. Inhibition occurs through both cell cycle-specific and cell cycle-nonspecific mechanisms. Results demonstrated that the combination of these components shows synergistic cytotoxicity, as evaluated by the combination index value.

Computational docking simulations indicated that metformin and omeprazole target Heat Shock Protein 60. These findings clarify the synergistic pattern among mixture ingredients, as each drug has a specific binding site on Hsp60, indicating a complementary binding mechanism with Hsp60. Furthermore, the dose reduction index value indicates that the concentration of constituents in the mixture required to achieve significant cytotoxicity is lower than that of each ingredient, indicating that the combination is safer than each component alone.

## Author Contribution Statement

Design and development: Rasha Kareem, Azal Hamoody, Yahiya Ibrahim, Aqeela Hayder. Gathering and organizing data: Rasha Kareem, Yahiya Ibrahim, Youssef Shakuri. Data analysis/interpretation: Youssef Shakuri, Aqeela Hayder, Rasha Kareem. Article composition: Azal Hamoody, Rasha Kareem, Aqeela Hayder, Youssef Shakuri. Critique the essay for significant ideas: Rasha Kareem, Yahiya Ibrahim, Azal Hamoody. Statistical analysis expertise: Youssef Shakori, Yahiya Ibrahim, Rasha Kareem, Aqeela Hayder. Ultimate article endorsement and guarantee: Rasha Kareem, Aqeela Hayder, Yahiya Ibrahim.

## Acknowledgements

The research team is grateful to the researchers and instructional staff at al-Mustansiriyah University and ICMGR in Baghdad, Iraq, for their invaluable support during the study. I also want to thank the Samarra Pharmaceutical Factory's quality control department for supplying the medication employed in this study.

*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.

*Conflicts of interest*

The authors maintain that there is no conflict-of-interest present.

*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

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