Synergistic Antiproliferative Effect of Linagliptin-Metformin Combination on the Growth of Hela Cancer Cell Line

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Abstract: This *in-vitro* study explores the cytotoxic properties of the linagliptin-metformin combination on cervical cancer cells and examines the synergistic interaction between the two drugs. An MTT assay was used to explore the anti-cancer effects of the linagliptin-metformin mixture on a cervical cancer cell line (HeLa cell line) across 24 and 72-hour incubation periods. The concentrations of metformin, linagliptin, and their combination ranged from 0.1 to 1000 µg/ml. while the concentrations in the mixture were kept at fifty percentage of the individually used drug. The study included an estimated combination index value (CI) and the dosage reduction index (DRI) to ascertain the possibility of a synergistic effect between combined drugs and mixture safety. study finding exhibited that all studied drugs- metformin, linagliptin, and their combined mixture- inhibited the growth of cervical cancer cells with a superior efficacy of the mixture over individual drugs. Inhibition patterns of the drugs were directly proportional to the drug's concentration and the incubation time. The combination index finding revealed that the mixture's cytotoxic effect of metformin and linagliptin was synergistic. The dose reduction index value revealed that lower drug concentrations were required in the combination mixture than when used individually indicating a greater cytotoxic potential of the mixture. The study findings of MTT, CI, and DRI indicate that the mixture is an effective, safer, and promising anticancer therapy for cervical cancer.

Conclusion: This study explores the cytotoxic potential of metformin and linagliptin individually and in combination. The greater cytotoxic potential of the drugs in combination highlights their lower effective concentrations, paving the way for further research on using these drugs for effective cancer treatment.

Keywords: Cervical cancer cell line, Hela cell line, MTT assay, Metformin, Linagliptin, Combination index, Dose reduction index.

1. INTRODUCTION

Annually, a significant number of over 500,000 women globally are diagnosed with cervical cancer, leading to more than 300,000 mortalities worldwide due to this [1, 2]. Around 90% of global cervical cancer cases are prevalent in low-income and middle-income countries [3, 4]. The incidence and mortality rates of cervical cancer in high-income countries have reduced by more than 50% in the last three decades due to the implementation of formal screening programs. The severity of the disease at the time of diagnosis and locally available resources are two significant determinants of the choice of treatment that may include radical hysterectomy, chemo-radiation, or a combination of both [5-9]. Based on the outcomes of five randomized clinical trials [10-15]. National Cancer Institute recommendations regarding women with invasive cervical cancer who are eligible for radiotherapy to receive simultaneous cisplatin-based chemotherapy instead of radiotherapy alone [16, 17]. A comprehensive analysis consisting of 18 studies in 11 countries has proven the positive impact of simultaneous chemoradiation on the disease's prognosis. The analysis revealed an absolute improvement of 12% in overall survival and improved control of the disease's spread, both locally and distantly [18].

While chemoradiation is widely regarded as a fundamental treatment for cervical cancer, the adverse effects of chemotherapeutic agents necessitate the exploration of safer alternatives. Numerous trials have been conducted to identify an effective treatment for cervical cancer by repurposing medications already serving another therapeutic use.

A study has shown that esomeprazole, either alone or in conjunction with amygdaline, can eradicate cervical cancer cells. The efficacy of cell killing depends on the time of exposure and the dose used [19-21].

A recent study also demonstrated that ciprofloxacin is effective in inhibiting the proliferation of cervical cancer cells, depending on the length of time of administration and the concentration used [22].

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Multiple studies were performed to explore the anticancer properties of metformin. Regarding cervical cancer, one of these studies demonstrated that Metformin primarily modulates the insulin signaling pathway and enhances the expression of the tumor suppressor IGFBP7 to impede the invasion and proliferation of cervical cancer cells [23] while other ones revealed Metformin's efficacy in inhibiting the growth of mammary adenocarcinoma cells and producing a cytotoxic impact on ovarian cancer by particularly targeting SPHK1 [24, 25]. Metformin efficacy has also been studied in oesophagal cancer cells and melanoma cells [26, 27].

Multiple studies have demonstrated that linagliptin has anticancer properties. A recent study demonstrated that linagliptin inhibits the proliferation of cervical cancer cells in a time-dependent manner through a mechanism that includes the restriction of human Hsp90 [7]. Other studies have demonstrated the ability of linagliptin to inhibit the survival, growth, and migration of Glioblastoma multiforme cancer cells through various mechanisms [7, 28]. Linagliptin apoptosis activating efficacy has also been reported for osteosarcoma cancer cells [29].

Multiple strategies have been adopted to investigate a safer and more effective anticancer alternative. One trial concentrated on mixing marketing drugs that are already used for various diseases rather than cancer for cancer therapy and possess anticancer properties. In this context, several studies were conducted, including one that evaluated the efficacy of an esomeprazole-amygdalin mixture specifically in targeting cervical cancer cells [19, 30]. Similarly, the mixture of ciprofloxacin-laetrile has been shown to inhibit the growth of esophageal cancer cells effectively [22]. However, there remains a lack of studies on the inhibitory effects of linagliptin-metformin mixture on the growth of cervical cancer cells. Therefore, our study aims to evaluate the potential of the linagliptin-metformin mixture for inhibiting the proliferation of cervical cancer cells.

2. MATERIAL AND METHODS

2.1. Research Medications

Samarra Pharmaceutical Factory supplied linagliptin and metformin, which are used as raw materials. By diluting the drugs with RPMI medium, a broad spectrum of concentrations was achieved, ranging from 0.1 µg/ml to 1000 µg/ml for both linagliptin and metformin when tested alone. Their concentrations in the mixture were reduced to half of their concentrations when used alone.

2.2. Human Cervical Cancer Cell Culture

The Hela cancer cell line, derived from a malignant cervical carcinoma, was first developed in the tissue culture section of ICCMGR. The cells were grown in 75 cm^2 tissue culture containers under controlled circumstances with a relative humidity of 37°C and 5% CO_2. The cells were cultured in a seeding solution containing RPMI-1640 media (Sigma Chemicals, England) with 10% fetal calf serum (FBS) and 100 U/mL penicillin-streptomycin(100 µg/mL streptomycin) [19, 31].

2.3. Cytotoxicity Study

Metformin, linagliptin, and both drugs were used to treat cervical cancer cells cultivated in a 96-well microtiter plate. Mixtures are made by combining equal quantities of each medication. During the logarithmic growth phase, the number of cancer cells gradually increased. The toxicity of the examined drugs was evaluated at different incubation periods, precisely at 24 and 72 hours [32, 33].

Each well consisted of 10,000 cells. Seeding entailed using a medium that contained 10% fetal bovine serum. The plates were incubated at 37°C for 24 hours to facilitate cell adhesion. RPMI medium without serum was used to perform serial dilutions. Metformin, linagliptin, and a mixture of these drugs were diluted in this RPMI medium without calf serum, resulting in a range of dilutions, from 0.1 to 1000 µg/ml, for each treatment [22, 34].

After 24 hours of allowing the cancer cells to grow, they were divided into six identical samples, with the same number for control groups. with each sample given 200 µl of a diverse concentration of each medication diluted with free serum RPMI media. Each control (negative control) was treated well with a 200-microliter maintenance medium, with exposure durations varying from 24 to 72 hours. The plates were reinserted into the incubator after sealing by a self-adhesive material. Afterwards, the cells were treated with MTT dye. MTT dye, or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, is a yellow tetrazolium salt that is reduced to purple formazan by metabolically active cells. This reduction serves as an indicator of cell viability and proliferation, as it reflects mitochondrial activity. The decrease in formazan production indicates potential cytotoxicity, cell cycle arrest, or apoptosis [35].

A microtiter plate reader (ELISA reader) was employed to measure the optical density of each well at a transmission wavelength of 550 nm [36, 37].

The growth inhibition rate is determined by employing the subsequent mathematical equation [37].

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

2.4. Drug Combination Profiling

An analysis was undertaken to examine the anti-proliferative effects of the metformin-linagliptin combination mixture. The concentration-effect curves were generated by plotting the percentage of cells exhibiting reduced growth versus the drug concentration following 24 and 72 hours of treatment. The pharmacological interaction that may occur between each linagliptin and metformin was evaluated by assessing the synergy, additive effects, and antagonism using the Compusyn computer program (Biosoft, Ferguson, MO, USA). It was achieved by computing the combination index (CI) and dose reduction index values.

The combination index was used to determine the interaction patterns between linagliptin and metformin, assessing whether the relationship was synergistic, antagonistic, or additive. This approach was applied to evaluate the pharmacological efficacy of the combination.

CI values below 1 suggest synergy, values over 1 indicate additivity, while values exceeding 1 indicate antagonism. The dose reduction index (DRI) measures the degree to which the concentration of each component in a combination can be decreased while maintaining the same level of effectiveness as when each medication is applied individually. A DRI score of 1 indicates that there is no decrease in the concentration. On the other hand, a DRI value larger than 1 implies a favourable reduction (an effective decline) in concentration, while a DRI value less than 1 denotes an unfavorable decrease (negligible decline) in concentration [38, 39].

2.5. Research Ethics

The study does not involve human or animal study.

2.6. Statistical Analysis

With six replicates, data from the MTT test are presented as mean ± standard error (SE). The one-way ANOVA test was used to describe the differences among study groups. The tuky and LSD test was used to compare groups. The investigation was conducted using SPSS statistical analysis software, version 20, with a significance level of $p < 0.05$ [40].

3. RESULTS

3.1. Cytotoxicity Assay:

3.1.1. Metformin Cytotoxicity

The study findings demonstrated the efficacy of metformin in reducing the proliferation of cervical cancer cells. the results indicated that the extent of growth inhibition by metformin was primarily dependent on time because time had a superior impact than concentration. Additionally, there was a notable disparity in growth inhibition between lower and higher metformin concentrations (Table **1** Figure **1**).

Table 1: The Impact of Metformin on the Growth of Hela Cancer Cells at 24 and 72 Hours

Concentration (µg/ml)	Inhibition of Cellular Viability (Mean ± SE a)		P- value
	24 hr.	72 hr.	
0.1	C 1.00 ± 0.577	D 2.00 ± 0.577	0.288
1	C 2.00 ± 0.577	C 15.00 ± 1.732	0.002*
10	C 6.00 ± 1.155	BC 25.00 ± 2.887	0.004*
100	B 20.00 ± 1.155	B 28.00 ± 2.309	0.036*
1000	A 35.00 ± 1.155	A 49.00 ± 1.732	0.003*
b LSD value	6.08	12.6	-
IC 50	1466 µg/ml	1002 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).

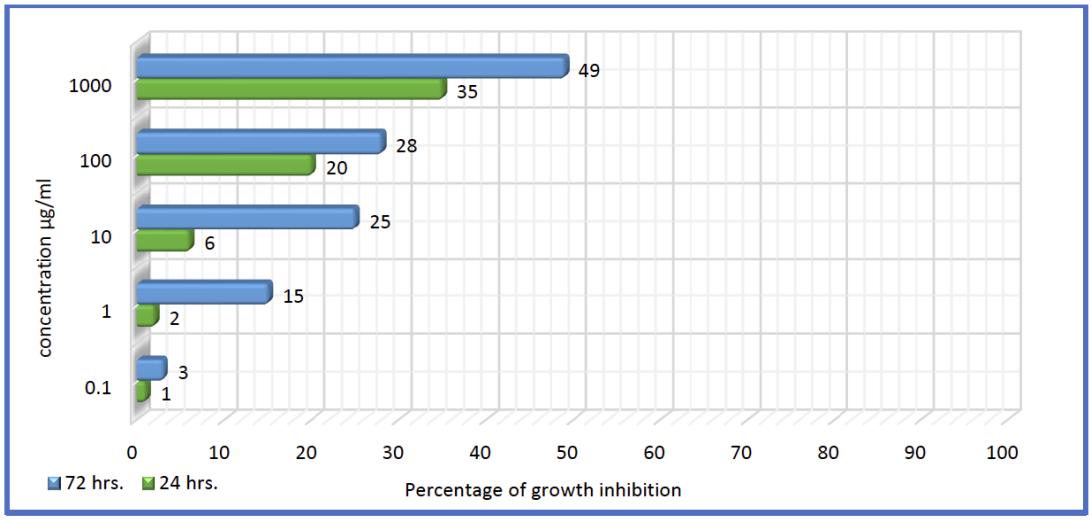

Figure 1: The impact of metformin on the growth of Hela cancer cells at 24 and 72 hours.

3.1.2. linagliptin Cytotoxicity

Linagliptin exhibited inhibitory effects on the proliferation of cervical cancer cells. The growth inhibition behaviour of linagliptin was dependent on time. Furthermore, variability in the degree of growth suppression was observed at different concentrations during each incubation phase, suggesting the concentration influences the cytotoxic effect of linagliptin (Table **2**, Figure **2**).

3.1.3. Metformin-Linagliptin Combination Mixture Cytotoxicity

The study demonstrated that the combination of metformin and linagliptin had a considerable antiproliferative effect. The extent of growth inhibition was influenced by both the duration of incubation and the concentration of the mixture (Table **3**, Figure **3**).

Furthermore, the mixture's cytotoxicity demonstrated superiority over the cytotoxicity of both linagliptin and metformin at each incubation period (Table **5**,**6** Figure **5**,**6**,**7**).

3.2. Studying Drugs-Combination Effects

The study of combined metformin-linagliptin yielded that after 24 hours of incubation, the combination mixture at concentrations of 0.1, 1, 10, 100, and 1000 µg/ml (concentrations half of the individual drugs) showed a strong synergistic anticancer effect. Furthermore, at the 72nd hour of incubation, all concentrations exhibited stronger synergistic effects compared to their effect measured at 24 hours.

The dose reduction index findings revealed that concentrations of individual components of the combination mixture needed to induce cytotoxicity were lower at all time intervals (24 and 72 hours of incubation) for all concentrations of metformin and linagliptin than when these drugs were used individually.

Table 2: The Impact of Linagliptin on the Growth of Hela Cancer Cells at 24 and 72 Hours

Concentration (µg/ml)	Inhibition of Cellular Viability (Mean ± SE a)		*P- value*
	24 hr.	72 hr.	
0.1	D 2.00 ± .577	C 31.00 ± .577	0.0001*
1	CD 7.00 ± 1.155	BC 38.00 ± 1.732	0.0001*
10	BC 19.00 ± 2.309	BC 40.00 ± 1.155	0.001*
100	AB 31.00 ± .577	AB 49.00 ± 1.155	0.0001*
1000	A 36.00 ± 1.732	A 52.00 ± 1.155	0.002*
b LSD value	13.38	11.28	-
IC 50	1525 µg/ml	793 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).

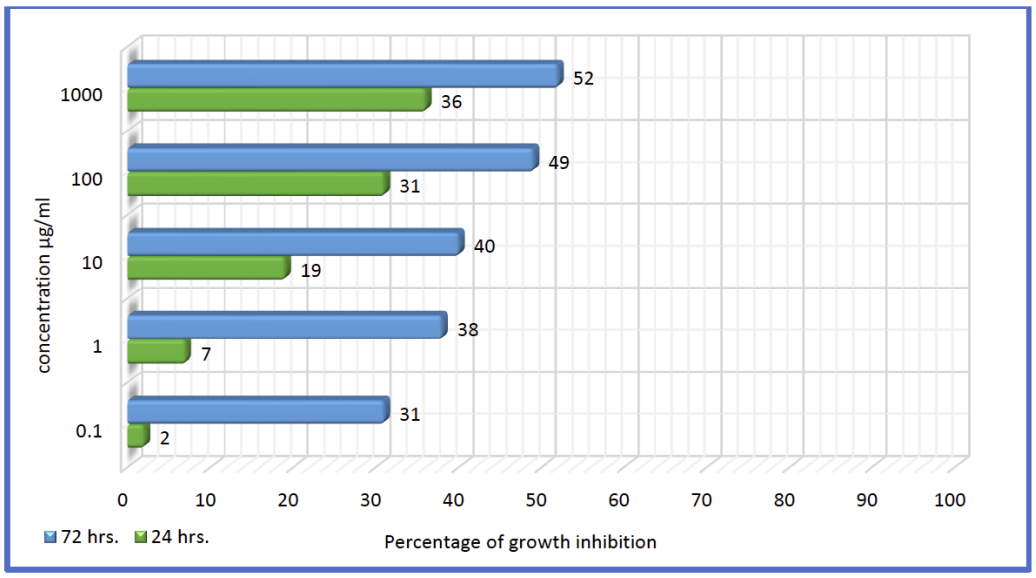

Figure 2: The impact of linagliptin on the growth of Hela cancer cells at 24 and 72 hours.

Table 3: The Impact of Linagliptin and Metformin Combination on the Growth of Hela Cancer Cells at 24 and 72 Hours

Concentration (µg/ml)	Inhibition of Cellular Viability (Mean ± SE a)		*P- value*
	24 hr.	72 hr.	
0.1	C 27.00 ± 1.155	B 37.67 ± 2.028	0.010*
1	C 32.00 ± 1.155	B 43.00 ± 1.732	0.006*
10	B 47.00 ± 1.155	A 61.00 ± .577	0.0001*
100	AB 53.00 ± 1.732	A 63.00 ± 1.732	0.015*
1000	A 55.00 ± .577	A 68.00 ± 1.732	0.002*
b LSD value	7.64	10.33	-
IC 50	23.281 µg/ml	5.937 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).

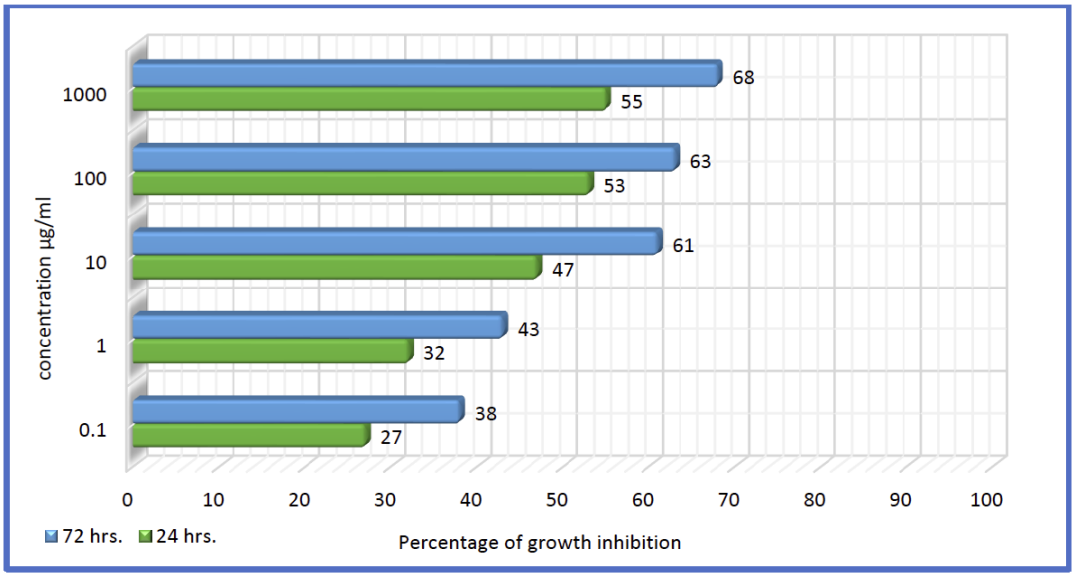

Figure 3: The impact of linagliptin and metformin combination on the growth of Hela cancer cells at 24 and 72.

Table 4: The Combination Pattern of Metformin and Linagliptin on Hela Cancer Cells after 24 Hours of Incubation

Concentration µg/ml		Con. Ratio	CI Value	Combination pattern	Dose Reduction Index Value	
Met	Lina				Met	Lina
0.5 µg/ml	0.5 µg/ml	1:1	0.00069	**Very Strong Synergism**	7634.40	2137.02
5 µg/ml	5 µg/ml		0.00319	**Very Strong Synergism**	1297.43	412.926
50 µg/ml	50 µg/ml		0.00619	**Very Strong Synergism**	523.577	233.585
500 µg/ml	500 µg/ml		0.03344	**Very Strong Synergism**	88.8696	45.0652
5000 µg/ml	5000 µg/ml		0.27228	**Strong Synergism**	10.6110	5.61673

The CI (Combination Index) and DRI (Dose Reduction Index) values were assessed using Compusyn software. A CI number greater than 1 signifies antagonism, a CI value of 1 implies an additive effect, and a CI value less than 1 indicates synergism. A dose reduction index (DRI) greater than one is associated with decreased toxicity.**(Chou 2018, 2006)**. **Met**: metformin, **Lina**: linagliptin.

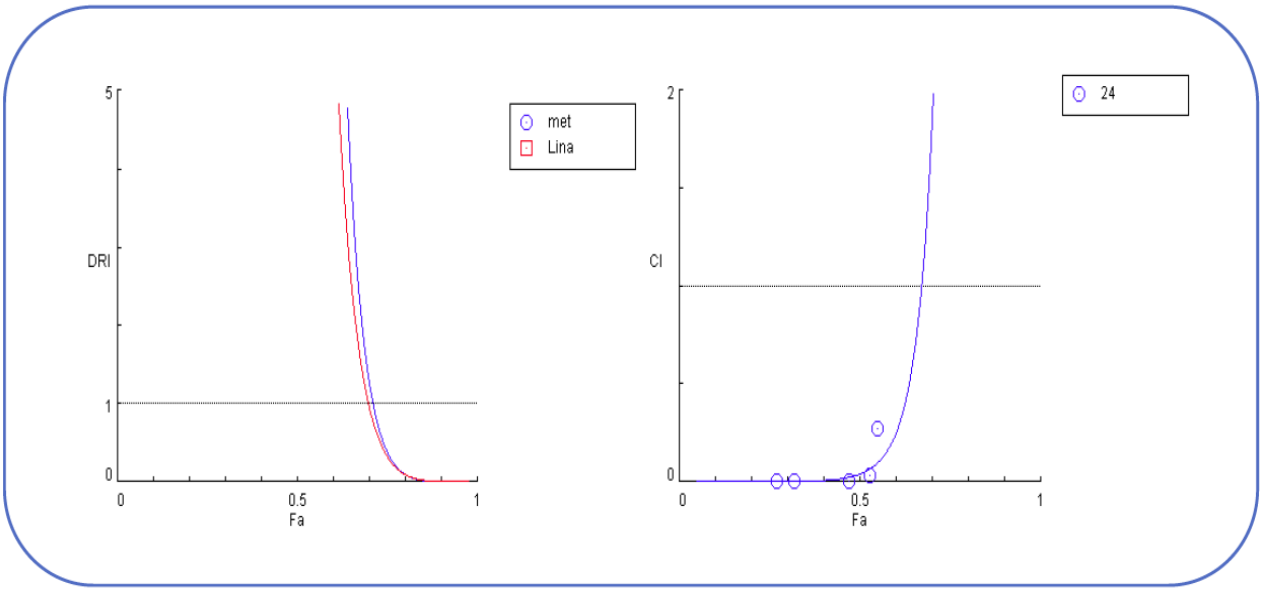

Figure 4: combination index curve (right) and dose reduction index curve (left) for the mixture at 24 hrs., Met: metformin, Lina: linagliptin.

Table 5: The Combination Pattern of Metformin and Linagliptin on Hela Cancer Cells after 72 Hours of Incubation

Concentration µg/ml		Con. Ratio	CI value	Combination Pattern	Dose Reduction Index Value	
Met	Lina				Met	Lina
0.5 µg/ml	0.5 µg/ml		0.02635	**Very Strong Synergism**	38.3407	3680.70
5 µg/ml	5 µg/ml	1:1	0.03141	**Very Strong Synergism**	33.3828	687.057
50 µg/ml	50 µg/ml		0.00178	**Very Strong Synergism**	6654.31	614.630
500 µg/ml	500 µg/ml		0.01323	**Very Strong Synergism**	1611.80	79.3272
5000 µg/ml	5000 µg/ml		0.06540	**Very Strong Synergism**	15.4373	1621.37

The CI (Combination Index) and DRI (Dose Reduction Index) values were assessed using Compusyn software. A CI number greater than 1 signifies antagonism, a CI value of 1 implies an additive effect, and a CI value less than 1 indicates synergism. A dose reduction index (DRI) greater than one is associated with decreased toxicity. **(Chou 2018, 2006).** Met: metformin, Lina: linagliptin.

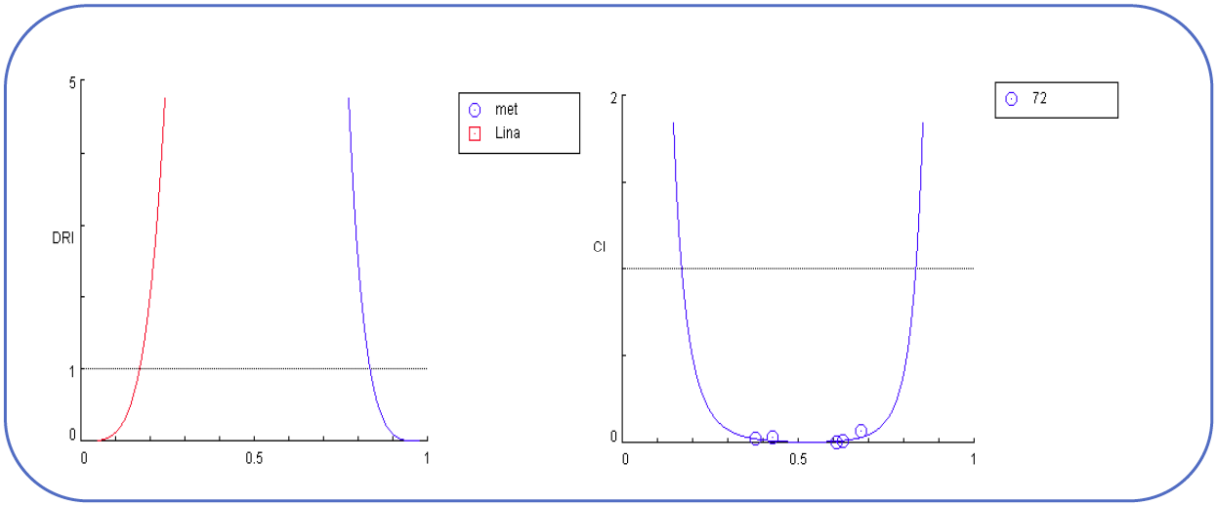

Figure 5: combination index curve (right) and dose reduction index curve (left) for the mixture at 72 hrs. Met: metformin, Lina: linagliptin.

Table 6: Mixture, Metformin, and Linagliptin 24-Hour Growth Inhibition Comparison

Concentration (µg/ml)	Growth Inhibition (Mean ± SE [a])			[b]*LSD value*
	Metformin	**Linagliptin**	**Mix**	
0.1	C 1.00 ± 0.577 b	D 2.00 ± .577 b	C 27.00 ± 1.155 a	5.66
1	C 2.00 ± 0.577 b	CD 7.00 ± 1.155 b	C 32.00 ± 1.155 a	6.92
10	C 6.00 ± 1.155 c	BC 19.00 ± 2.309 b	B 47.00 ± 1.155 a	11.3
100	B 20.00 ± 1.155 c	AB 31.00 ± .577 b	AB 53.00 ± 1.732 a	8.64
1000	A 35.00 ± 1.155 b	A 36.00 ± 1.732 b	A 55.00 ± .577 a	8.59
[b]LSD value	6.08	13.38	7.64	
IC 50	1466 µg/ml	1525 µg/ml	23.281 µg/ml	

a: standard error, b: least significant difference. Statistically significant differences are shown by capital letters within the same column, whereas variations in lowercase letters within the same rows also indicate statistically significant differences: significant at (P<0.05).

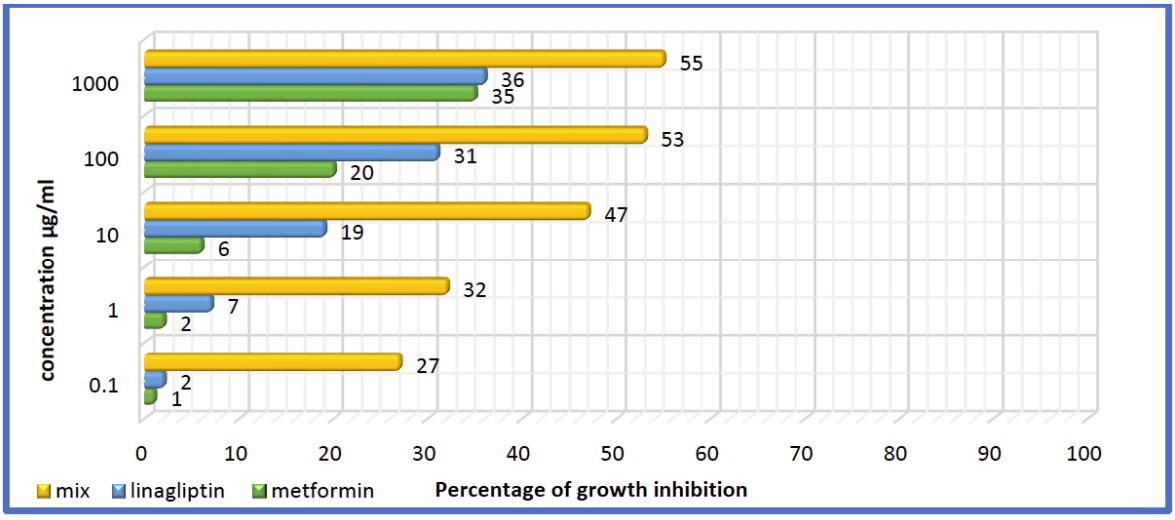

Figure 6: Mixture, metformin, and linagliptin 24-hour growth inhibition comparison.

Table 7: Mixture, Metformin, and Linagliptin 72-Hour Growth Inhibition Comparison

Concentration (µg/ml)	Growth Inhibition (Mean ± SE [a])			[b]LSD value
	Metformin	Linagliptin	Mix	
0.1	D 2.00 ± 0.577 b	C 31.00 ± .577 a	B 37.67 ± 2.028 a	8.73
1	C 15.00 ± 1.732 b	BC 38.00 ± 1.732 b	B 43.00 ± 1.732 a	11.98
10	BC 25.00 ± 2.887 c	BC 40.00 ± 1.155 b	A 61.00 ± .577 a	12.64
100	B 28.00 ± 2.309 c	AB 49.00 ± 1.155 b	A 63.00 ± 1.732 a	12.42
1000	A 49.00 ± 1.732 b	A 52.00 ± 1.155 b	A 68.00 ± 1.732 a	10.82
[b]LSD value	12.6	11.28	10.33	-
IC 50	1466 µg/ml	1002 µg/ml	5.937 µg/ml	-

a: standard error, b: least significant difference. Statistically significant differences are shown by capital letters within the same column, whereas variations in lowercase letters within the same rows also indicate statistically significant differences: significant at (P<0.05).

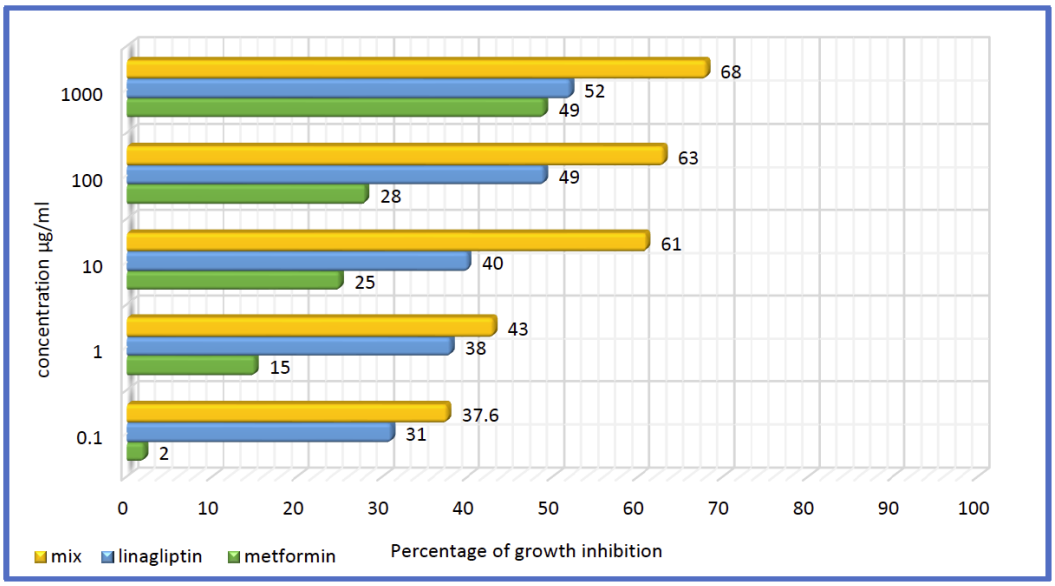

Figure 7: Mixture, metformin, and linagliptin 72-hour growth inhibition comparison.

The decrease in cytotoxic concentration was remarkable for metformin and linagliptin, indicating a favourable reduction in concentration (Table **4**,**5** Figure **4**,**5**).

4. DISCUSSION

Therefore, as our study yielded results that were time- and concentration-dependent, it may be proposed that the combination used in our study exerted both cell cycle-specific and cell cycle non-specific antiproliferative effects on cervical cancer cells.

The results of our study showed that the (linagliptin – metformin) mixture cytotoxicity was time- and concentration-dependent; it may be proposed that the mixture used in our study exerted both cell cycle-specific and cell cycle non-specific antiproliferative effects. This behaviour may be elucidated by the ability of some chemotherapies to kill a cell at any stage of the cell cycle. The cytotoxicity of these compounds is contingent upon their concentration. These are referred to as cell-cycle nonspecific agents. Other chemotherapies eliminate cancer cells only during a specific phase and are ineffective during rest. The cytotoxicity of these compounds is contingent upon the time duration of their presence in the cellular target. These are referred to as cell-cycle-specific agents [41].

Furthermore, the combined treatment had a much higher impact on cell growth inhibition than metformin or linagliptin alone.

The combined index indicated that the observed effect was synergistic across all incubation periods. This index showed a very strong synergistic impact between metformin and linagliptin at all mixture concentrations. The synergistic effect arises from the diverse modes of action exhibited by the ingredients in the mixture, which mutually enhance each other.

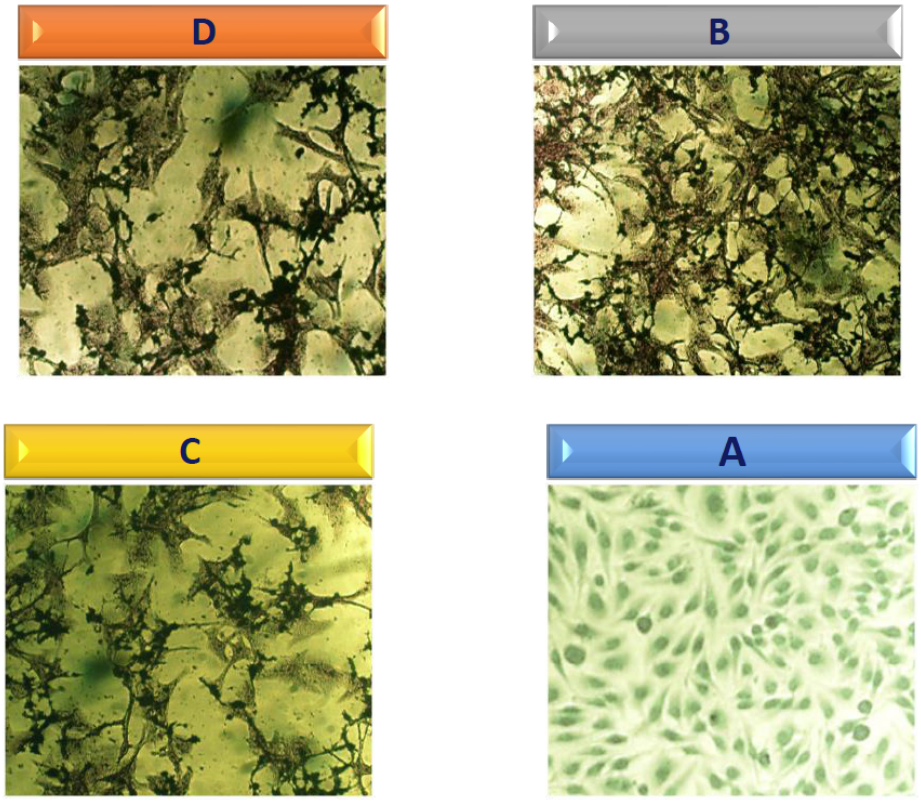

Figure (8): morphology of Hela cancer cells. (**B**) Cancer cells were subjected to a 1000 µg/ml concentration of metformin for 72 hours. (**C**) Cancer cells were subjected to a 1000 µg/ml concentration of linagliptin for 72 hours. (**D**) Cancer cells were treated with 1000 µg/ml of metformin-linagliptin combination for 72 hours. (**A**) Cervical cancer cells were not treated and served as the control group.

The metformin cytotoxicity results demonstrated its capacity to inhibit the growth of cervical cancer cells in a time-dependent manner. Previous studies have confirmed that metformin, a frequently prescribed medicine for diabetes, is also efficacious in reducing the occurrence of certain malignancies, such as pancreatic cancer [42, 43]. Recent studies indicate that metformin effectively decreases the likelihood of developing colon cancer and also reduces the chances of mortality associated with the disease [44, 45]. with reducing the formation of adenomas and polyps [46] and decreasing the mortality rate in individuals with diabetes who are diagnosed with colon cancer [47, 48]. It has shown an ability to lower the occurrence and mortality rate linked to prostate and hepatic carcinomas [48-52]. Various hypothesized mechanisms have been examined to investigate the anticancer properties of metformin. One process involves the activation of AMPK in rat hepatoma H4IIE cells, resulting in a decrease in pS6 phosphorylation [53]. A separate in vitro study demonstrated that metformin directly impeded AMP deaminase, resulting in increased levels of AMP and the subsequent activation of AMPK [54]. Moreover, evidence suggests that drugs that hinder the operation of mitochondrial complex 1 in the respiratory system can increase the levels of AMP and initiate the activation of AMPK. Consequently, this results in the inhibition of mTOR and the subsequent activation of signalling pathways that enhance cell survival [55, 56]. In addition, Metformin demonstrated the capacity to remove active K-ras (K-ras contributes to intrinsic pro-tumorigenesis and is crucial in establishing a suppressive tumor immunological milieu by activating downstream effectors and secreting diverse immune-suppressive cytokines and chemokines) [57].

Our research revealed that linagliptin can effectively inhibit the proliferation of cervical cancer cells through both cell-cycle-specific and cell-cycle nonspecific mechanisms. This conclusion aligns with previous research indicating that treatment using linagliptin significantly reduced the survival rate of Saos-2 cells (a kind of human bone cancer cell) and hFOB1.19 cells (a type of human fetal bone cell) [15, 29]. A separate study has shown that Linagliptin can suppress the viability, proliferation, and migration of Glioblastoma cancer cells [28]. In addition, linagliptin demonstrates the ability to inhibit the proliferation of HCT116 cells, which are a type of human colorectal cancer cell [58]. The growth inhibition pattern primarily relied on the

incubation duration, with a more considerable dependence on time than concentration. Given this finding, we postulated that the mechanism responsible for the anticancer effects of linagliptin is related to its impact on specific phases of the cell cycle. Several research have shown data supporting the adoption of this concept. According to a study, linagliptin can potentially induce cell cycle arrest at the G2/M phase when given in small doses and at both the G2/M and S phases when given in high doses [58]. Another proposed mechanism corroborating our findings is that Linagliptin can substantially interact with Cyclin-Dependent Kinase 1 (CDK1), a protein essential for regulating the cell cycle. CDK1 plays a role in adding phosphate groups to multiple substrate proteins, including histones H1, laminin, and Rbis, the function of CDK1.

Linagliptin can strongly inhibit cell multiplication and tumor proliferation by specifically targeting Aurora kinase B and CDK1 and decreasing the phosphorylation of Rb and the synthesis of Bcl 2. Pro-caspase3 [58]. Another suggested mechanism revealed that linagliptin can target Aurora kinase B. This kinase is a strongly preserved serine-threonine protein kinase that belongs to the Aurora family and plays a crucial role in regulating mitosis [59].

Our study indicates that the combination of metformin and linagliptin generates synergistic anticancer effects. As evidenced by the dose reduction index value, the effective concentration of each medication in the mixture is lower than its concentrations when used separately; this reduces the likelihood of adverse effects from the mixture, suggesting that it is safer than its constituents.

However, our study has some limitations, including lacking a specific concentration range for the different study treatments. Instead, a wide range of concentrations between (0.1-1000) micrograms/ml was employed to determine the most effective cytotoxic concentration for each treatment. We recommend exploring the mixture's anticancer abilities on further cancer cell lines.

5. CONCLUSION

The findings of our investigation indicate that the combination of metformin and linagliptin can suppress the growth of cervical cancer cells. The results also demonstrate that the combination of these components exhibits synergistic cytotoxicity as assessed by the combination index value. Moreover, analysis of the dose reduction index value reveals that the concentration of constituents in the mixture required to cause substantial cytotoxicity is lower than that of each element used alone, which indicates that the combination may possess a greater degree of safety compared to each component alone. Future research may be carried out on the efficacy of this drug combination on other cancer cell lines.

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
AMPK	=	5' Adenosine monophosphate-activated protein kinase
pS6	=	Ribosomal protein S6
mTOR	=	Mammalian target of rapamycin
PKC	=	Protein kinase C
PP2A	=	Protein phosphatase 2

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