RESEARCH ARTICLE

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The Cytotoxic Effect of Ciprofloxacin Laetrile Combination on Esophageal Cancer Cell Line

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Abstract

Objective: aim of this study was to examine the synergistic effect between the antibacterial drug ciprofloxacin and the natural compound laetrile on esophageal cancer cells, specifically focusing on their combined cytotoxic effect. **Methods:** The combined cytotoxic effects of two alternative incubation durations (24 and 72 hours) were studied using an esophageal cancer cell line. Ciprofloxacin, laetrile, and their combinations were tested at concentrations ranging from 1 to 1000 micrograms/milliliter, to enhance the safety of the combination, the concentrations of the combination constituents were reduced by half compared to when they are used individually, the combination index was then calculated to estimate the components' possible synergistic effects. **Result:** The results indicate that the combined cytotoxicity of ciprofloxacin and laetrile was greater than the cytotoxicity of either ciprofloxacin or laetrile alone, the combination cytotoxicity increased with higher concentrations and longer incubation periods, in other words, the cytotoxicity pattern of the combination was time-dependent (cell-cycle specific), and concentration dependent, (cell-cycle non-specific). **Conclusion:** The study found that the combination of ciprofloxacin and laetrile had a greater inhibitory effect on the growth of esophageal cancer cells compared to ciprofloxacin or laetrile alone. This suggests a synergistic effect between the components of the mixture, which can be attributed to a complementary mechanism between the ingredients in the combination.

Keywords: Esophageal cancer cell line- ciprofloxacin cytotoxicity- laetrile cytotoxicity

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Introduction

As adenocarcinoma of the distal esophagus and gastroesophageal junction (squamous cell carcinomas or adenomas) account for more than 90% of esophageal neoplasm, their prevalence is on the rise [1], Tobacco Smoking is linked to an increase in the occurrence of esophageal cancer [2, 3]. A minimum of 50% of patients exhibit metastatic cancer, and the majority of patients with localized illness will eventually acquire metastases. Therefore, most individuals diagnosed with esophageal cancer will be eligible for palliative chemotherapy; several Historically, chemotherapy has shown effectiveness against esophageal cancer. The usual treatment for both squamous cell carcinoma and adenocarcinoma is a combination of cisplatin and continuous-infusion 5-fluorouracil. This treatment has shown a response rate of 25% to 35% in cases of metastatic illness [4].

Several subsequent methods were used to elucidate the deleterious effects of conventional cancer treatments, including the utilization of substances derived from natural sources such as plant extracts, which have shown potential anticancer properties. Investigating the efficacy of certain medications used to treat non-malignant illnesses plays a significant role in cancer therapy [5], as aspirin, which exhibits preventive efficacy against colorectal cancer through a mechanism involving inhibition of platelet aggregation [6],A recent study demonstrates that acetaminophen, when administered at the therapeutic level, significantly decreases the viability of liver cancer cells via stimulating the apoptotic pathway [7]. Esomeprazole exhibits a cytotoxic impact on uterine cervical neoplasia cells, where cell death is directly proportional to the dose of esomeprazole and the length of incubation [5, 8].

Noteworthy ciprofloxacin at dose rather than antibacterial dose show capability for lower the duplication of distinctive kind of cancer cell [9] as (colorectal, bladder & prostate) neoplasm, ciprofloxacin demonstrates a capability to operate the (pro apoptotic) mechanism [10, 11]. on other side laetrile anticancer ability occur after hydrolysis of laetrile under influence of (glucosidase) enzyme inside the cancer cells, liberated cyanide and benzaldehyde [12-14].

previous study shows missing in testing the anticancer ability of (laetrile, ciprofloxacin combination), based on

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the mechanism of action for laetrile and ciprofloxacin we hypothesis, a combination between them may show a synergism anticancer ability toward cancer cells, for this hypothesis we used esophageal cancer cell line for testing the cytotoxicity of the combination

Materials and Methods

An evaluation was conducted to assess the anticancer efficacy of the combination of ciprofloxacin and laetrile on esophageal cancer. The cytotoxic activity of the combination on an esophageal cancer cell line was determined. The study involved using a serial dilution of the combination, ranging from 1 to 1000 micrograms per millilitre. The cancer cells were treated and incubated for either 24 or 72 hours.

Study tools
(laetrile)

Laetrile was purchased from Santa Cruz, California, and was used in a variety of concentrations, from 1 microgram per milliliter (g/ml) to 1000 g/ml, with each concentration obtained by diluting RPMI without serum.

Ciprofloxacin

Ciprofloxacin 200 mg/100 ml intravenous injectable form was employed at various concentrations ranging from (1 g/ml to 1000 g/ml) after being diluted with RPMI-free serum medium.

Cell culture

An esophageal cancer cell line was obtained from the tissue culture unit at the Iraqi Centre for Cancer and Medical Genetics Research (ICCMGR). The cells were cultured in 75 cm^2 flasks under humidified conditions with 5% CO_2 at 37°C using RPMI-1640 medium (Sigma chemicals, England) supplemented with 10% calf serum and 1% penicillin-streptomycin antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin) from Lilly, Italy [15].

Cytotoxicity Assay

The cultured neoplastic cells were exposed to varying concentrations of ciprofloxacin, laetrile, and a combination of ciprofloxacin and laetrile. Each well exhibited an increase in the population of neoplastic cells throughout the growth log phase. The effectiveness of the tested agents in killing the cells was measured after multiple incubation periods. Each well contained approximately 7,000 neoplastic cells, and a medium with serum was used to support their growth. The neoplastic cells were seeded onto a microtiter plate, which was then placed in an incubator for 24 hours to allow the cells to attach to the wells. Subsequently, a maintenance medium was used to prepare five dilutions of the tested agents, ranging from 1 to 1000 µg/ml for each laetrile and ciprofloxacin, and from 0.5 to 500 µg/ml for the combination of ciprofloxacin with laetrile [15].

After a 24-hour incubation period, neoplastic cells were treated with six replicates of each tested agent. Each hole was treated with 0.2 ml of the agent, while the control holes were treated with 0.2 ml of maintenance media. The plate was wrapped with isolated self-attachment film and placed in the incubator for 24 and 72 hours. MTT stain was used to label neoplastic cells.

The optical density of each well was measured using a micro-ELISA reader at a wavelength of 550 nm, according to the following equation [15-17].

inhibitor rate %= (the optical density of control-optical density of test)/(optical density of the control)*100

Statistical Analysis

The Statistical Analysis System (SAS) [18] was employed to determine various components in the experimental parameters. The Least Significant Difference (LSD) test was utilized to determine if there were statistically significant differences between the means of the experiment and the influence of concentration and time on the rate of cancer cell growth.

Results

The cytotoxic effect of laetrile

The results of the laetrile cytotoxic effect on the esophageal cancer cell line demonstrated an increase in growth inhibition occurs with an increase in its concentration (concentration depends) (Table 1). Statistically, there is no difference between the growth inhibition in each incubation period for all concentrations except (1000) µg/ml (Figure 1).

The cytotoxic effect of ciprofloxacin

The results of ciprofloxacin cytotoxic show an increase in growth inhibition occurs with an increase in its concentration (concentration depends) and incubation periods (time depends) (Table 2); results also show a significant difference between the growth inhibition of each incubation period for all concentrations and among all concentration in each incubation periods, (Figure 2).

The cytotoxic effect of (half concentrations of laetrile and half concentrations of ciprofloxacin) combination

The results of mixture growth inhibition on esophageal cancer cell line show an elevate in growth inhibition occur with elevation in its concentration (concentration depends) and with its incubation periods (time depends); the result shows a significant difference between the growth inhibition of each incubation periods for all concentrations and among all concentration in each incubation periods (Figure 3).

Comparison among the growth inhibition of laetrile, ciprofloxacin and combination between themes

The results of comparison among the growth inhibition of laetrile, ciprofloxacin and combination between themes on esophageal cancer cell line show, at all concentration in 24 and 72 hrs (Table 3,4). Incubation period, the inhibition of the neoplastic cells growth by the combination was significantly more than the growth inhibition of laetrile and ciprofloxacin each one alone. (Figure 4, 6), by depending on the value of the combination index the result showed a

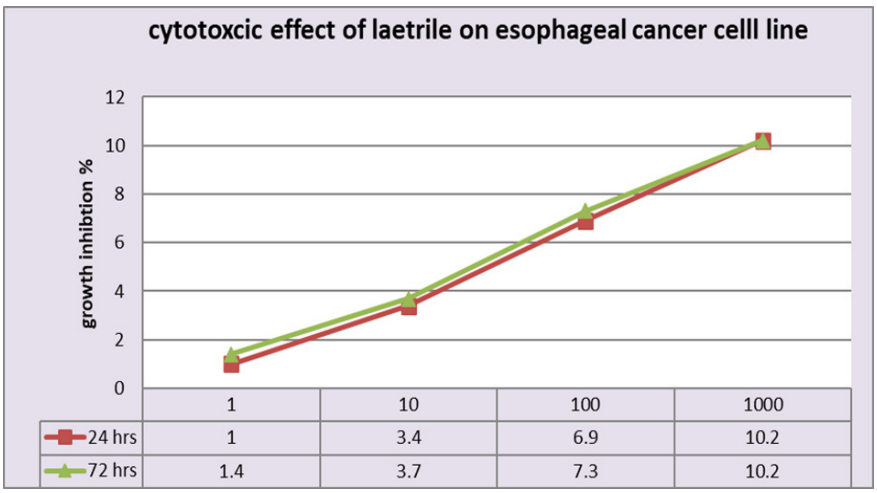

Figure 1. Laetrile's Growth Inhibitory Effects on an Esophageal Cancer Cell Line 24- and 72-hours Periods of Incubation

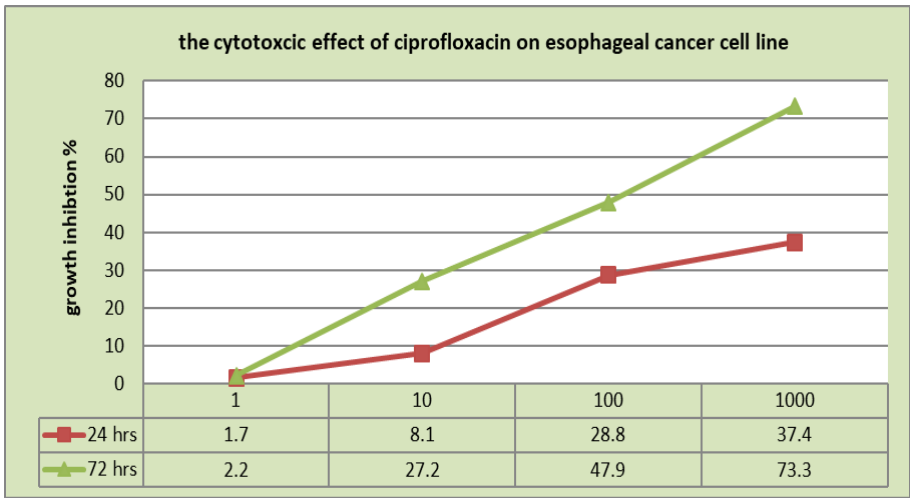

Figure 2. Ciprofloxacin's Growth Inhibitory Effects on an Esophageal Cancer Cell Line at 24- and 72-hour Periods of Incubation Period

Table 1. The Inhibitory Effect of Laetrile on the Development of Esophageal Cancer Cells was Seen after 24 and 72 hours of Incubation.

	24hrs.	72 hrs.	signify
1	C 1	B 1.4	0.374 NS.
10	C 3.4	B 3.7	1.000 NS.
100	B 6.9	A 7.3	0.643 NS.
1000	A 10.2	A 10.2	0.886 NS.
LSD	2.55	3.16	-

*Mean significance (P <0.05)

Table 2. The Inhibitory Effect of Ciprofloxacin on the Development of Esophageal Cancer Cells after 24 and 72 Hours of Incubation.

Concentration	24hrs.	72 hrs.	sign
1	D 1.7	D 2.2	0.364 NS.
10	C 8.1	C 27.2	0.002* S
100	B 28.8	B 47.9	0.005* S
1000	A 37.4	A 73.3	0.000*S
LSD	5.545	6.835	-

*Mean significance (P <0.05)

Table 3. The Combination of Laetrile and Ciprofloxacin Demonstrated Growth Suppression on an Esophageal Cancer Cell Line after 24 and 72 hours of Incubation.

Concentration	24hrs.	72 hrs.	sign
1	C 5	C 8.4	0.034*
10	B 15.6	B 39.8	0.002*
100	A 45	A 63	0.008*
1000	A 48	A 86.1	0.000*
LSD	7.55	6.2	-

*Mean significance (P <0.05)

Table 4. Comparison of Growth Inhibition between Laetrile, Ciprofloxacin, and Their Combination after 24 hours.

Concentration	laetrile	ciprofloxacin	combination	LSD
1	1.7 b	1 b	5:00 AM	1.33
10	8.1 b	3.4 b	15.6 a	5.37
100	28.8 b	6.9 c	45 a	8.425
1000	37.4 b	10.2 c	48 a	7.045
LSD	5.545	2.55	7.55	-

*Mean significance (P <0.05)

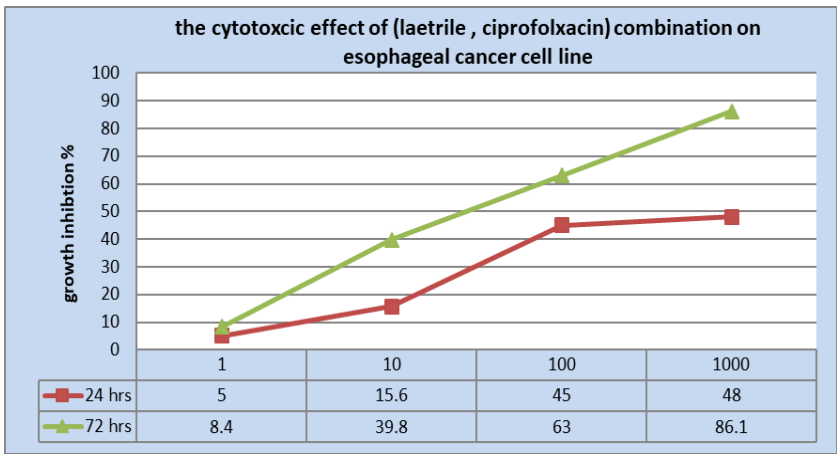

Figure 3. The Growth Inhibition of (Laetrile, Ciprofloxacin) Combination on Esophageal Cancer Cell Line at 24 and 72 hrs. incubation periods

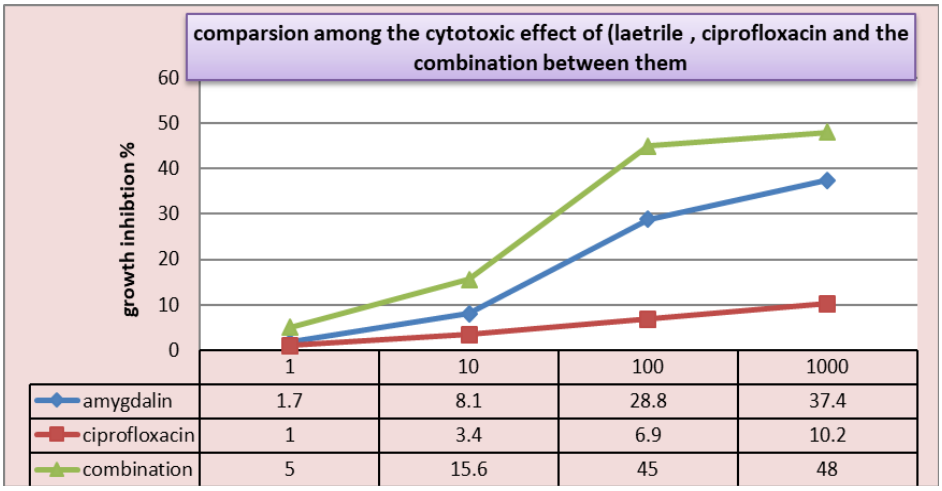

Figure 4. Comparative of Growth Inhibition by Laetrile, Ciprofloxacin, and Their Combination after 24 hours.

Table 5. The Combination Index Value for the Mixture after a 24-hour Incubation Period

Total mix dose	Value of combination index	Combination pattern
1 µg/ml	0.16439	Strong Synergism
10 µg/ml	0.044	Very Strong Synergism
100 µg/ml	0.07381	Very Strong Synergism
1000 µg/ml	0.52633	Synergism

different level of synergism effect between ciprofloxacin and laetrile (Figure 5,7) , The histopathological finding of cancer cells that were treated with ciprofloxacin, laetrile

Table 6. Comparison among Growth Inhibition of Laetrile, Ciprofloxacin and Combination between Themes at 72 hrs.

Concentration	laetrile	ciprofloxacin	combination	LSD
1	2.2 b	1.4 b	8.4 a	2
10	27.2 b	3.7 c	39.8 a	6.695
100	47.9 b	7.3 c	63 a	6.14
1000	73.3 b	10.2 c	86.1 a	7.145
LSD	6.835	3.16	6.2	-

*Mean significance (P <0.05)

Table 7. The Combination Index Value for the Mixture after 72 hours of IncubationTime

Total mix dose	Value of combination index	Combination pattern
1 µg/ml	0.11379	Strong synergism
10 µg/ml	0.0141	Very Strong Synergism
100 µg/ml	0.18702	Strong Synergism
1000 µg/ml	0.11786	Strong Synergism

and the combination between showed in Figures 8, (Tables 5,6,7) [13] .

Discussion

The cytotoxicity of laetrile is mostly attributed to the release of hydrocyanic acid and benzaldehyde within cancer cells due to the action of the glucosidase enzyme [19], Hydrocyanic acid acts as an antineoplastic agent by blocking cytochrome C oxidase in the mitochondrial respiratory electron transport chain. This inhibits both oxidative metabolism and the oxidative phosphorylation pathway, ultimately leading to energy deficiency [20], The cytotoxicity of benzaldehyde is attributable to its ability to induce apoptosis by activating caspases 3, 8, and 9 [21].

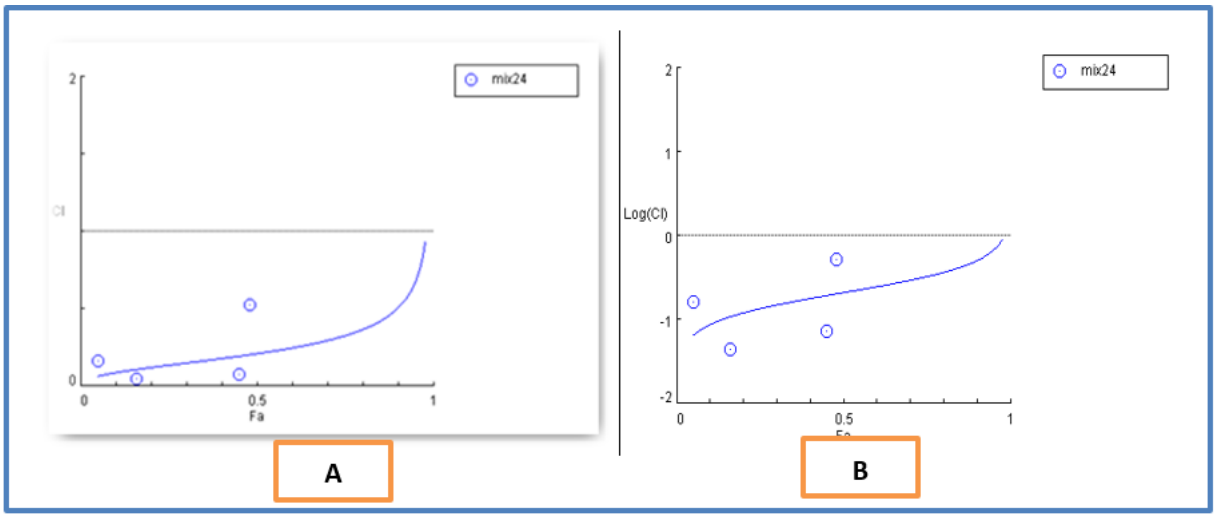

Figure 5. A Combination Index Graph; B - 24 hour mixed logarithmic combination index plot ;(CI < 1 means Synergism, CI = 1 additive impact, CI > 1 Antagonism impact) (Ting- Chao & Martin , 2004).(CI = combination index) , (Fa = factor activity).

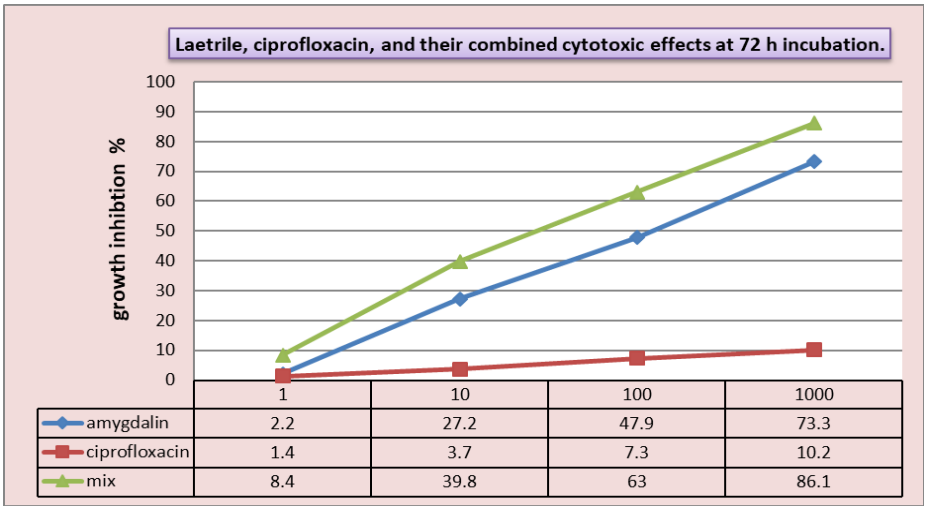

Figure 6. Comparison of Growth Inhibition between Laetrile, Ciprofloxacin, and the Combination of Both Compounds at the 72-hours

Our findings are consistent with another study, which demonstrated that ciprofloxacin has a significant impact on the growth of transitional cell carcinoma cells [4]. In contrast, other studies have shown that Fluoroquinolone antibiotics cause cell death in breast cancer cells in a manner that depends on the dosage and duration of treatment, This cell death is achieved through various mechanisms, including the induction of apoptosis, increased expression of p53, Bax, and Bcl-2 proteins, alterations in cell cycle distribution and DNA fragmentation, disruption of mitochondrial function through the Bax/Bcl-2 pathway, arrest of the cell cycle at the S-phase, and inhibition of topoisomerase II. Additionally, there is evidence of oligonucleosomal DNA fragmentation and an increase in p53 expression [22].

Other research has shown that ciprofloxacin can slow the growth of hepatocellular carcinoma cell lines; its mechanisms of action include DNA breaks, and the inhibition of topoisomerases. Furthermore, ciprofloxacin has a synergistic effect when combined with cisplatin in this regard [23].

A combination of ciprofloxacin and laetrile was used to reduce the growth of cancer cells. The killing ability of laetrile is related to the levels of cyanide and benzaldehyde

Table 8. Procedure for Determining the Pattern of Synergism and Antagonism Using Combination Index analysis. (Ting- Chao & Martin, 2004).

Pattern of combination	Combination index
Indicate Very Strong Synergism pattern	Less than 0.1
Indicate Strong Synergism pattern	Between 0.1 to 0.3
Indicate Synergism pattern	Between 0.3 to 0.7
Indicate Moderate Synergism pattern	Between 0.7 to 0.85
Indicate Slight Synergism pattern	Between 0.85 to 0.90
Indicate Nearly Additive pattern	Between 0.90 to 1.10
Indicate Slight Antagonism pattern	Between 1.10 to 1.20
Indicate Moderate Antagonism pattern	Between 1.20 to 1.45
Indicate Antagonism pattern	Between 1.45 to 3.3
Indicate Strong Antagonism pattern	Between 3.3 to 10
Indicate Very Strong Antagonism pattern	More than 10

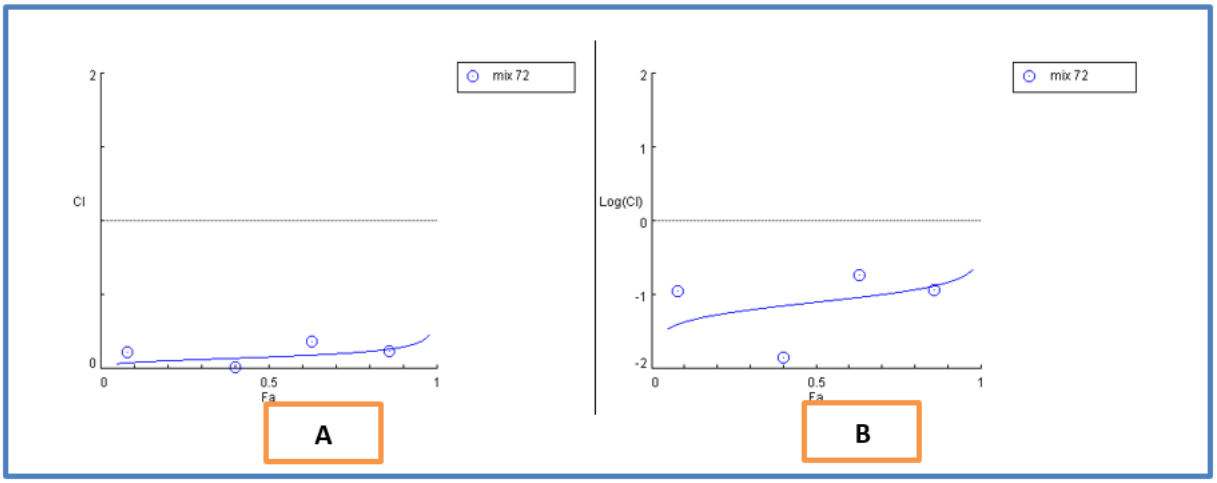

Figure 7. A combination index graph; B - 24 hour mixed logarithmic combination index plot ;(CI < 1 means Synergism, CI = 1 additive impact, CI > 1 Antagonism impact) (Ting- Chao & Martin , 2004).(CI = combination index) , (Fa = factor activity).

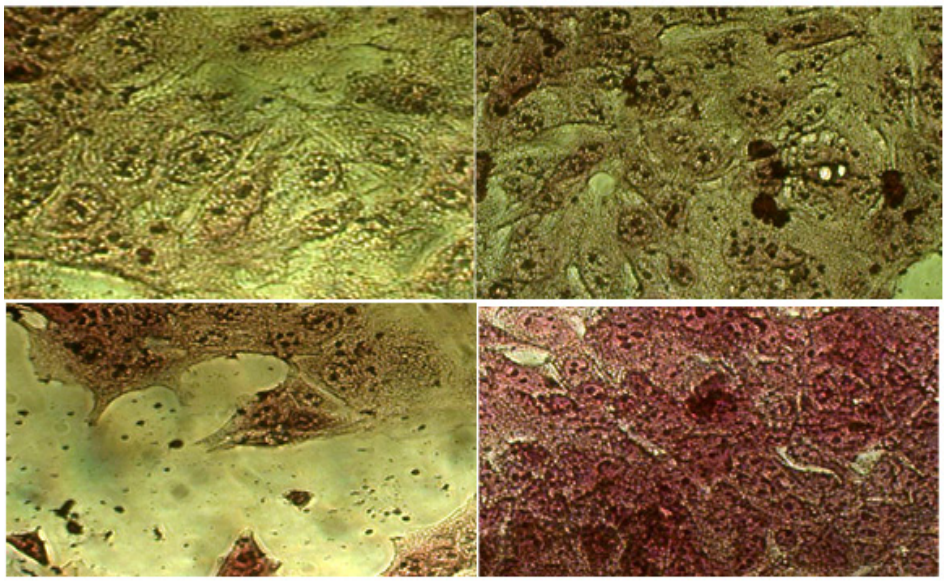

Figure 8. Esophageal Cancer Cell Morphology. The image in the upper right corner displays cancer cells following a 72-hour treatment with 1000 (µg/ml) of ciprofloxacin. The first image in the top left corner shows cancer cells exposed to a concentration of 1000 (µg/ml) of laetrile for 72 hours. The second image in the bottom left corner shows cancer cells treated with 500 (µg/ml) of ciprofloxacin and 500 (µg/ml) of laetrile for 72 hours. The third image in the bottom right corner depicts cancer cells not receiving treatment, serving as the control group.

in the cytoplasm, which are produced when laetrile is broken down by the enzyme glucosidase. Therefore, an agent that can increase the levels of glucosidase enzyme would enhance the cytotoxicity of laetrile. Ciprofloxacin is able to increase the levels of intracellular glucosidase enzyme by penetrating the membrane of lysosomal bodies [12-14].

Another possible mechanism that explains the synergy between the mixture is the ability of released cyanide to reduce the resistance of neoplastic cells to the killing effects of ciprofloxacin. This suggested mechanism occurs through the cyanide's capability to induce energy deprivation, resulting in a decrease in ATP levels that are necessary for the operation of the P-glycoprotein transport system responsible for removing ciprofloxacin from neoplastic cells [24].

On the other hand, cyanide has the capacity to decrease cellular energy, resulting in reduced growth of neoplastic cell resistance to perforations of the lysosomal membrane induced by ciprofloxacin. This is achieved by lowering the synthesis of the Hsp70 family of chaperone proteins [25, 26].

Reducing the expression of the Hsp70 family of chaperone proteins would deactivate resistance, resulting in a decrease in the ability of the lysosomal membrane to withstand acidic conditions. This would increase the permeability of the lysosomal membrane, causing leakage of lysosomal enzymes into the cytoplasm of the cancerous cell [27, 28, 21].

The mixture has the ability to induce apoptosis through

several mechanisms. One of these pathways involves the activation of three caspase enzymes (3, 8, and 9) by benzaldehyde [29], Another proposed pathway involves the capacity of ciprofloxacin to transport lysosomal enzymes, such as protease enzymes (cathepsin B, CD, and cathepsin L), from the lysosome to the cytoplasm. These protease enzymes, similar to caspase, initiate a sequence of events that ultimately lead to the activation of the apoptotic mechanism, resulting in the destruction of neoplastic cells [12].

In conclusion, the combination of ciprofloxacin and laetrile has been found to effectively kill esophageal cancer cells. The cytotoxicity of the mixture was greater than that of ciprofloxacin and laetrile when used individually, indicating a synergistic effect between them as anticancer agents. This synergistic behavior is supported by the combination index result. Furthermore, the combination was found to be safer than each component alone, as the concentration of each member in the combination was half the concentration of each constituent when used alone.

Author Contribution Statement

Conception and design: Azal hamoody jumaa; Collection and assembly of data: Azal hamoody jumaa, Youssef Shakori Yassin; Analysis and interpretation of data: Azal hamoody jumaa; Drafting of the article: Azal hamoody jumaa, Youssef Shakori Yassin; Critical revision of article for important intellectual content: Azal hamoody jumaa, Ali muafaq said; Statistical expertise: Azal hamoody jumaa; Final approval and guarantor of the article: Azal hamoody jumaa, Youssef Shakori Yassin.

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Research ethics

This article lacks any research involving human participants.

Conflicts of interest

There are no conflicts of interest.

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