RESEARCH ARTICLE

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# Effect of Laetrile Vinblastine Combination on the Proliferation of the Hela Cancer Cell Line

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## Abstract

**aim:** This study aimed to evaluate the inhibitory effect of laetrile, vinblastine, and their mixture on cervical cancer cells and probe potential synergistic consequences. **Method:** The study scrutinized the inhibitory impact of laetrile vinblastine and their mixture on the growth of human cervical cancer cells (Hela cancer cell line). The cells were incubated for 24, 48, and 72 hours with concentrations varying from 1 microgram to 10,000 micrograms of each substance. **Result:** study results showed, the combination of vinblastine and laetrile effectively reduced the viability of human cervical cancer cells. This effect was stronger than the individual cytotoxic effects of each compound. The results suggest that the cytotoxicity of the vinblastine and laetrile combination increases with higher concentrations of the compounds. Additionally, the study revealed a synergistic effect between the mixture ingredients, particularly at the lowest and highest concentrations during the 24 and 72-hour incubation periods. **Conclusion:** The antiproliferative effect of (the combination of laetrile and vinblastine) was greater than the antiproliferative effect of either compound used alone, suggesting a synergistic relationship between the two.

**Keywords:** laetrile- vinblastine- Hela cancer cell line

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## Introduction

Throughout the course of history, a multitude of medicinal plants have been acknowledged and utilized due to their healing attributes, Plants possess the capacity to engage in the synthesis of diverse chemical compounds that serve crucial roles in performing essential biological processes, at least 12,000 distinct compounds have been identified and isolated (Said et al., 2022; Ueda et al., 2022).

There have been numerous recent inquiries about medical matters. Efforts have been made to utilize plants to advance in treating and managing cancer progression, the primary drawbacks of synthetic medications pertain to the accompanying adverse effects; Natural therapies, such as plants or plant-derived natural compounds, have effectively eradicated cancer cells, Prior to this, the investigation into botanical compounds as potential anti-cancer treatments had already commenced, Examination and advancement of vinca alkaloids, specifically vinblastine and vincristine, along with prunus armeniaca kernels, often known as apricot kernels, have demonstrated potential as effective agents against cancer. Numerous studies have been conducted to assess their anticancer properties, one of these results indicates that the main cause of apricot kernels' ability to fight cancer is the presence of laetrile (Ayiomamitis et al., 2019).

Laetrile, a glycoside compound, is derived from of prunus dulcis kernels, commonly referred to as (bitter almond). The Prunus genus encompasses diverse species, including Prunus armeniaca (apricot) and Prunus serotina (black cherry). These species have been shown to contain laetrile, a compound investigated for its potential to selectively kill cancer cells without causing systemic damage. Additionally, laetrile has been studied for its analgesic properties (Barakat et al., 2022; Elimam et al., 2022; Jumaa and Hussein, 2015; Sajid et al., 2022).

The Vinca alkaloids have been extracted from the plant known as Vinca Rosea Linn. The primary mechanism by which Vinblastine exerts its anticancer activity is its strong binding affinity to the fundamental protein subunit of microtubules, known as tubulin. The interaction between specific components interferes with the proper functioning of the mitotic spindle apparatus, resulting in the subsequent halting of the cell cycle during the metaphase stage.

Vinblastine has been found to possess an additional anticancer mechanism that induces lysosomal membrane permeabilization. This process results in an increased

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release of lysosomal contents into the cytoplasm, Ultimately, this process results in the breakdown of cellular components and consequent demise of malignant cells. Furthermore, in addition to their hydrolytic activity, lysosomal enzymes also contribute to creating an acidic environment, The aforementioned factor plays a substantial role in the elimination of neoplastic cells, The efficacy of vinblastine to induce apoptosis in cancer cells through the permeabilization of lysosomal membranes represents a significant achievement in the domain of cancer therapy, (Domagala et al., 2018; Serrano-Puebla and Boya, 2016). The treatment has received significant attention for its efficacy in managing many neoplastic conditions, such as non-Hodgkin's and Hodgkin's lymphomas, acute lymphoblastic leukemia, breast carcinoma, Wilm's tumor, and neuroblastoma (Hammouda et al., 2022; Sekino and Teishima, 2020; Uscanga-Palomeque et al., 2019).

*Aim of study*

The objective of this study was to evaluate the inhibitory effect of a combination treatment involving vinblastine and laetrile on the proliferation of Hela cancer cells in an in vitro model.

## Materials and Methods

*laetrile*

Laetrile was obtained from (Santa Cruz, , California, United States). and utilized at 1 to 10,000 µg/ml concentrations. The concentrations, as mentioned earlier, were achieved through the process of diluting laetrile with a medium that is devoid of serum.

Chemotherapeutics agent; The Vinblastine sulfate 1 mg/ml Injection manufactured by Hospira UK was utilized at various concentrations ranging from 1 to 10000 µg/ml. The concentrations, as mentioned earlier, were attained through the process of diluting the injection with a media that is devoid of serum.

*Cell culture*

The Iraqi Centre for Cancer and Medical Genetics Research (ICCMGR) tissue culture unit provided the Hela cell line. The cells were grown in tissue culture flasks with a surface area of 75 cm2, while being subjected to a controlled environment characterized by a humid atmosphere comprising 5% CO2 and a temperature of 37°C, the culture medium employed in this study was RPMI-1640, procured from Sigma Chemicals in USA, Penicillin-streptomycin (100 U/mL penicillin and 100 g/mL streptomycin) and bovine calf serum (FBS) (10%) from (ICCMGR) were added as supplements.

*Cytotoxicity Assay*

The experiment involved the cultivation of cells in a microtiter plate with 96 wells, these cells were exposed to various quantities of vinblastine, laetrile, and a combination of laetrile and vinblastine, In the logarithmic phase of growth, Over the course of several incubation periods, the percentage of cancer cells in every single well will go up and the cytotoxic effect of the drugs under study will be assessed, the present investigation aims to examine each well contained 7X10^3 cells. Cancer cells were seeded using a 10% serum calf medium. The plates were then incubated at 37°C for 24 hours to allow the cancer cells to attach; subsequently, a maintenance medium was used to prepare fivefold serial dilution for each laetrile and vinblastine, ranging from 1-10000 µg/ml. additionally, a combination of vinblastine and laetrile was prepared with dilutions ranging from 0.5-5000 µg/ml.

Following a 24-hour incubation period, the cells were subjected to exposure. Specifically, six replicates were treated with 200µl of each tested concentration. In the control group, A 200 µl of maintenance medium was introduced into each well. There were three different durations of exposure: 24, 48, and 72 hours. After being covered with a self-adhesive substance and then being sealed, the plates were then placed back into the incubator. After that, the cells were stained with MTT dye to see how active they were.

Using an ELISA reader with a transmitting wavelength of 550 nm (De Sousa et al., 2023; Ueda et al., 2022). each well's optical density can be measured quantitatively.

A mathematical equation is used to arrive at the inhibitor rate, which is the result of the equation (Ayiomamitis et al., 2019).

*Statistical Analysis*

To determine the impact of various factors on research parameters, the Statistical Analysis System (SAS) was used (Kadhim and Mohamad, 2023). To identify significant differences between means, the Least Significant Difference (LSD) test was used in this study.

## Results

*Antiproliferative effect of vinblastine*

The proliferation of Hela cells was seen to be inhibited upon treatment with vinblastine exhibits a dose-dependent increase for each incubation period at 1, 10, 100, 1000, and 10000 µg/ml concentrations. (Table 1) depict the growth inhibition of human cervical Hela cells following treatment with vinblastine for 24, 48, and 72 hours during the incubation period, The observed growth suppression also demonstrates a temporal pattern, notably at the 72-hour time. The incubation periods yielded more growth inhibition of vinblastine at doses of 10, 100, 1000, and 10000 µg/ml compared to the growth suppression seen after 24 and 48 hours for the identical concentrations.

*Antiproliferative effect of laetrile*

The findings indicate that Hela cells treated with laetrile growth inhibition exhibited a dose-dependent pattern across all incubation periods. Additionally, the growth inhibition of Hela cells showed a time-dependent pattern, particularly at the 72-hour incubation period, as compared to the 24-hour and 48-hour incubation periods, across all concentrations of laetrile (Table 2).

The cytotoxicity of Laetrile towards the Hela cancer cell line is observed to increase with higher concentrations of Laetrile and longer exposure durations. The extent of this cytotoxicity is dependent on the specific concentration and duration employed.

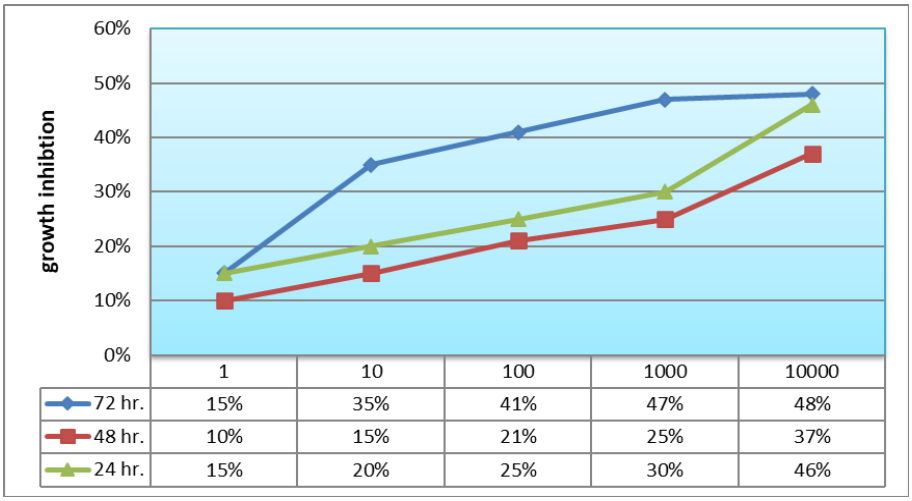


Figure 1. Illustrates the Inhibitory Impact of Vinblastine on the Proliferation of the Hela Neoplastic Cell Line.

Table 1. The Impact of Concentration and Time on the Rate of Growth Inhibition of the Hela Cancer Cell Line by Vinblastine

| LSD value | Growth suppression | | | Concentration (µg/ml) |
|---|---|---|---|---|
| | 24 hr. | 48 hr. | 72 hr. | |
| N.S | C 15 a | B 10 a | B 15 a | 1 |
| 13.44* | BC 20 b | B 15 b | A 35 a | 10 |
| 16.3* | BC 25 bc | B 21 c | A 41 a | 100 |
| 10.98* | B 30 b | AB 25 b | A 47 a | 1000 |
| N.S | A 46 a | A 37 a | A 48 a | 10000 |
| ---- | 11.62 * | 15.64 * | 13.8 * | LSD value |

* (P<0.05); NS, Non-significant; Different capital letters indicate significant differences (P< 0.05) between the means of the respective columns. Significant differences (P< 0.05) between the means of each row are denoted by different lowercase letters.

Table 2. Impact of Concentration and duration on the Growth Suppression Rate for Laetrile in the Hela Neoplastic Cell Line

| Concentration (µg/ml) | Growth suppression | | | LSD value |
|---|---|---|---|---|
| | 72 hr. | 48 hr. | 24 hr. | |
| 1 | B 30 a | B 20 a | D 5 b | 10.14* |
| 10 | AB 33 a | AB 30 ab | C 15 b | 12.78* |
| 100 | AB 37 a | AB 34 a | BC 20 b | 7.76* |
| 1,000 | AB 39 a | A 36 a | AB 27 b | 8.2* |
| 10,000 | A 44 a | A 39 ab | A 35 b | 8.62* |
| LSD value | 13.32* | 14.88* | 9.86* | ---- |

* (P<0.05); NS, Non-significant; Different capital letters indicate significant differences (P< 0.05) between the means of the respective columns. Significant differences (P< 0.05) between the means of each row are denoted by different lowercase letters.

*Antiproliferative effect of (vinblastine, laetrile combination)*

The observed growth inhibition exhibited by the combination exhibited a proliferation inhibition pattern on Hela cells that is similar to that of vinblastine and laetrile, the observed similarity can be attributed to their ability to elicit growth inhibition in a manner dependent on both concentration and time. The findings indicate that the growth inhibition of the combination exhibits an escalating trend as the concentration of the mixture increases within the range of 1-10,000 µg/ml. Furthermore, the growth inhibition increases with longer incubation periods,

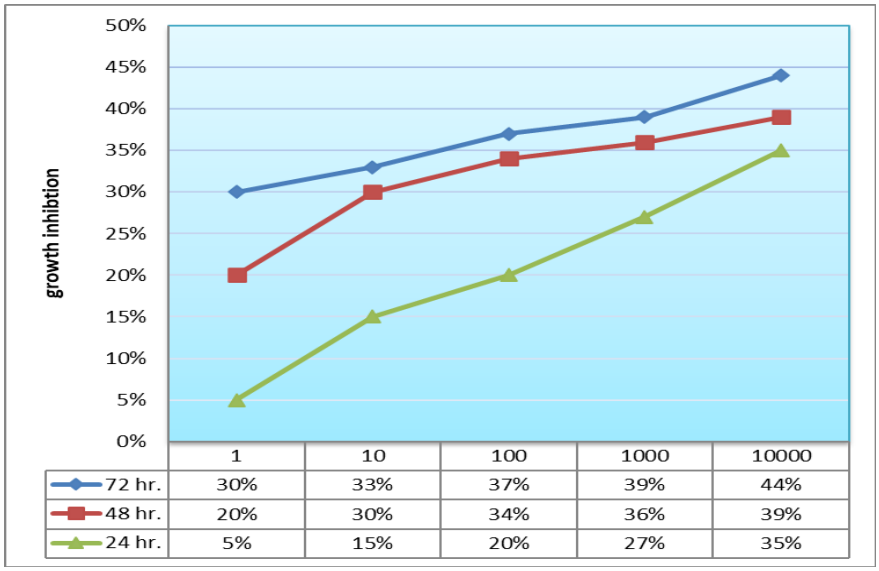


Figure 2. Illustrates the Growth Suppression Impact of Laetrile on the Hela Neoplastic Cell Line

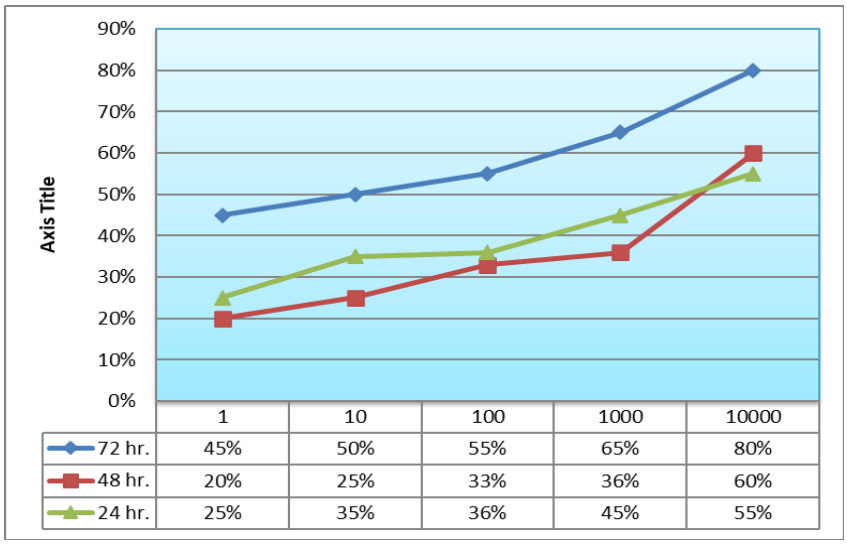


Figure 3. Illustrates the Inhibitory Effect of the Mixture on the Hela Neoplastic Cell Line's Proliferation.

particularly at 72 hours. The observed results indicate a clear synergistic effect of employing the combination approach on the various components of the mixture, particularly during incubation periods of 72 and 24 hours. The aforementioned observation is substantiated by the table of combination index (5,7,9) and figure (5,7,9), which present a comparative analysis of the growth inhibition effects of the mixture, laetrile, and vinblastine across three-time intervals: 24, 48, and 72 hours. The aforementioned results are additionally demonstrated in (Table 4, 6, and 8) The growth inhibitory effects of the combination of vinblastine and laetrile were significantly

Table 3. Impact of Concentration and Time on the Rate of Growth suppression for a Combination of Laetrile and Vinblastine on the Hela neoplastic Cell Line

| Concentration (µg/ml) | Growth suppression | | | LSD value |
|---|---|---|---|---|
| | 24 hr. | 48 hr. | 72 hr. | |
| 1 | C 25 b | C 20 c | C 45 a | 4.62 |
| 10 | BC 35 b | BC 25 c | C 50 a | 3.26 |
| 100 | BC 36 b | BC 33 b | BC 55 a | 10.02 |
| 10,00 | AB 45 b | B 36 c | B 65 a | 3.26 |
| 10,000 | A 55 b | A 60 b | A 80 a | 7.04 |
| LSD value | 16.94 | 14.16 | 14 | - |

* (P<0.05); NS, Non-significant; Different capital letters indicate significant differences (P< 0.05) between the means of the respective columns. Significant differences (P< 0.05) between the means of each row are denoted by different lowercase letters.

Table 4. Hela Growth Suppression Effect of Vinblastine, Laetrile, and the Combination of Vinblastine and Laetrile after 24 hours Line

| Concentration (µg/ml) | Growth suppression | | | LSD value |
|---|---|---|---|---|
| | combination | Laetrile | Vinblastine | |
| 1 | C 25 a | D 5 c | C 15 b | 4.62* |
| 10 | BC 35 a | C 15 b | BC 20 b | 9.78* |
| 100 | BC 36 a | BC 20 b | BC 25 ab | 10.32* |
| 1,000 | AB 45 a | AB 27 b | AB 30 b | 8.2* |
| 10,000 | A 55 a | A 35 b | A 46 ab | 11.19* |
| LSD value | 16.94* | 9.86* | 16.94 * | - |

* (P<0.05); NS, Non-significant; Different capital letters indicate significant differences (P< 0.05) between the means of the respective columns. Significant differences (P< 0.05) between the means of each row are denoted by different lowercase letters.

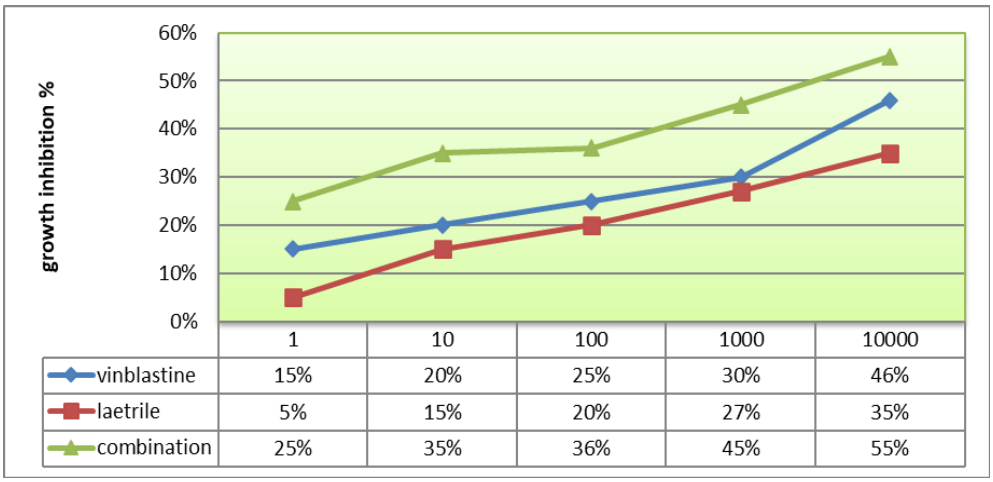


Figure 4. Growth Inhibition Effects of Vinblastine, Laetrile, and the Combination of Vinblastine and Laetrile over 24 hours.

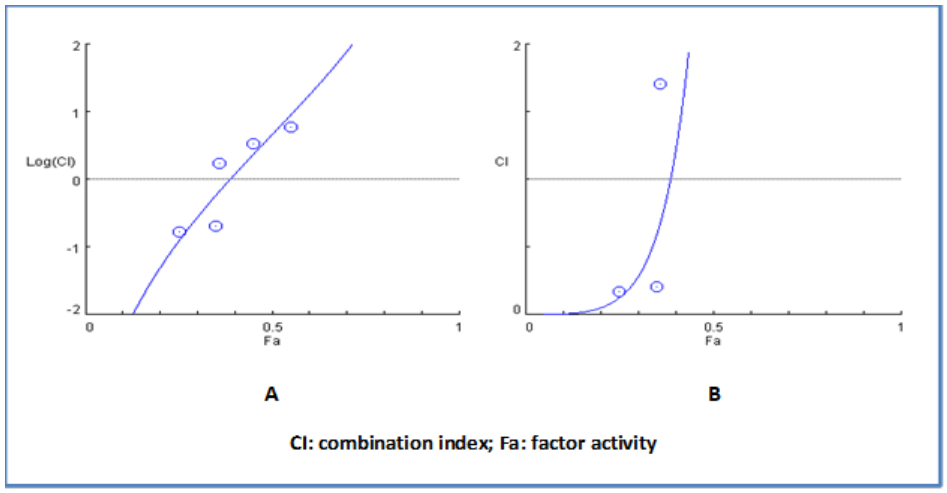


Figure 5. (A) combination index plot and, (B) logarithmic combination index plot for a mixture at a 24-hour incubation period. The interaction between the mixture's components is determined by the combination index (CI). A CI less than one implies synergism, a CI of one indicates an additive impact, and a CI larger than one indicates antagonism (Chou et al.,2019)

greater at a concentration of 10000 µg/ml compared to the separate effects of vinblastine and laetrile at the same concentration. This evaluation was conducted at three distinct time intervals (24, 48, and 72 hours).

## Discussion

Our study result is compatible with the outcome of another study suggested, the sensitivity of women uterine cervical cancer cell lines to various concentrations of vinblastine is evident since the cytotoxic effects of vinblastine appear to be influenced by both the concentration of the medication and the duration of exposure to it, the mechanism of action of vinblastine

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

| cumulative dosage of the mixture | The value of the combination index | Pattern of combination |
|---|---|---|
| 1 µg/ml | 0.1684 | strong synergism |
| 10 µg/ml | 0.2062 | strong synergism |
| 100 µg/ml | 1.7065 | Nearly additive |
| 1,000 µg/ml | 3.2878 | antagonism |
| 10,000 µg/ml | 6.0509* | Strong antagonism |

Table 6. Growth Inhibition Effects of Vinblastine, Laetrile, and Combined Vinblastine and Laetrile at 48 Hours.

| Concentration (µg/ml) | Growth suppression | | | LSD value |
|---|---|---|---|---|
| | Vinblastine | laetrile | combination | |
| 1 | B 10 | B 20 | C 20 | N.S |
| 10 | B 15 b | AB 30 a | BC 25 ab | 14.82 |
| 100 | B 21 b | AB 34 a | BC 33 a | 5.1 |
| 1,000 | AB 25 b | A 36 a | B 36 a | 9.6 |
| 10,000 | A 37 b | A 39 b | A 60 a | 13.84 |
| LSD value | 15.64 * | 14.88* | 14.16* | - |

* (P<0.05); NS, Non-significant; Different capital letters indicate significant differences (P< 0.05) between the means of the respective columns. Significant differences (P< 0.05) between the means of each row are denoted by different lowercase letters.

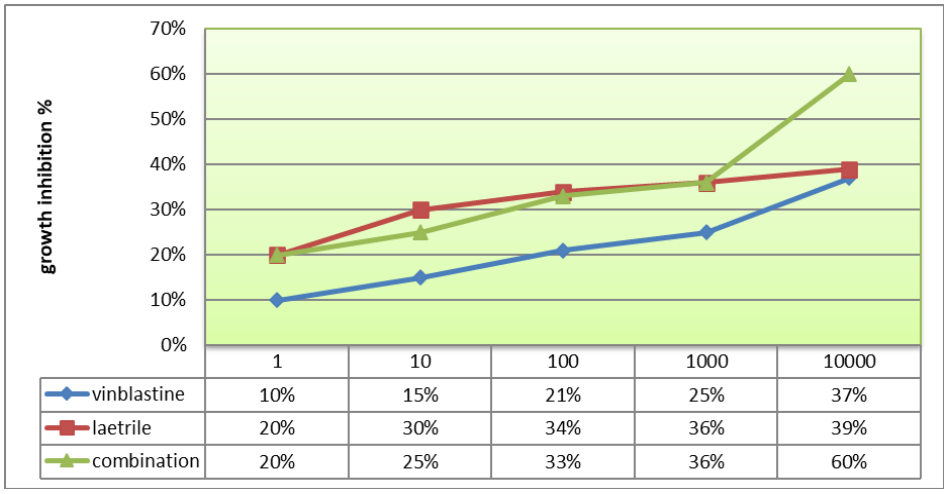


Figure 6. Growth Inhibition Effects of Vinblastine, Laetrile, and the Combination of Vinblastine and Laetrile after 48 hours.

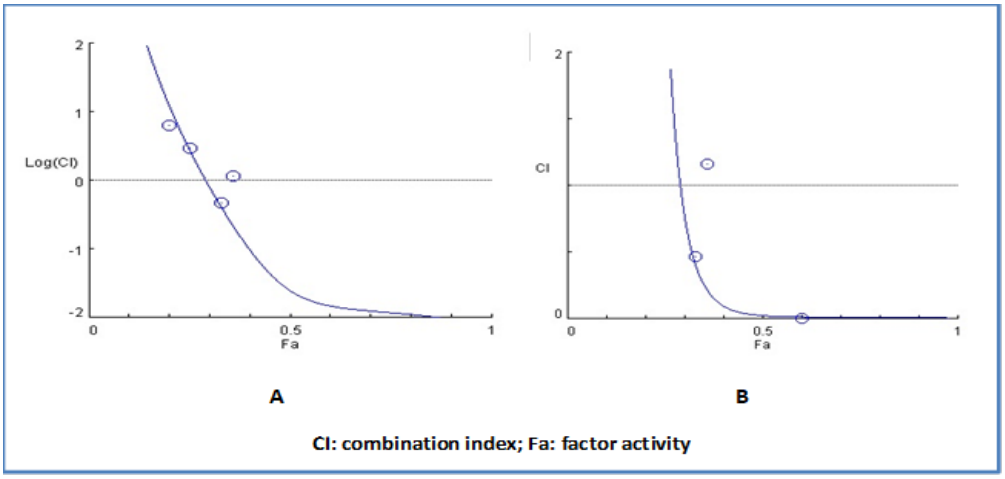


Figure 7. (A) combination index plot and, (B) logarithmic combination index plot for a mixture at a 48-hour incubation period, The interaction between the mixture's components is determined by the combination index (CI). A CI less than one imply synergism, a CI of one indicates an additive impact, and a CI larger than one indicates antagonism (Chou et al.,2019)

is characterized by its cytotoxic effects observed after a 72-hour incubation period. These effects are attributed to the prolonged presence of vinblastine within cancer cells, leading to a heightened cytotoxicity. The observed effect can be ascribed to the capacity of vinblastine to stimulate the development of microtubule crystals in 28 types of lymphoid malignancies. The time of exposure to vinblastine greatly influences both the percentage of cells exhibiting microtubule changes and the level of progression.

Table 7. Combination Index Value for the Mixture after a 48-hour Incubation Period.

| Total mixture Dose | Combination index Value | Pattern of combination |
|---|---|---|
| 1 µg/ml | 320.56 | Very strong antagonism |
| 10 µg/ml | 147.92 | Very strong antagonism |
| 100 µg/ml | 23.47 | Very strong antagonism |
| 1,000 µg/ml | 58.9 | Very strong antagonism |
| 10,000 µg/ml | 0.1761 | strong synergism |

A further study provided analogous findings, demonstrating a negative correlation between the concentration of vinblastine and the survival rate of murine leukemia, as well as the inhibition of human lymphoblastoid leukemia cells (Della Corte et al., 2020;

Table 8. Growth Inhibition Effect of Vinblastine, Laetrile, and the Combination of Vinblastine and Laetrile after a 72-hour Period

| Concentration (µg/ml) | Group suppression | | | LSD value |
|---|---|---|---|---|
| | Vinblastine | Laetrile | combination | |
| 1 | B 15 c | B 30 b | C 45 a | 11.14 |
| 10 | A 35 b | AB 33 b | C 50 a | 11.14 |
| 100 | A 41 b | AB 37 b | BC 55 a | 11.14 |
| 1,000 | A 47 b | AB 39 c | B 65 a | 6.24 |
| 10,000 | A 48 b | A 44 b | A 80 a | 10.14 |
| LSD value | 13.8 * | 13.32* | 14 | |

* (P<0.05); NS, Non-significant; Different capital letters indicate significant differences (P< 0.05) between the means of the respective columns. Significant differences (P< 0.05) between the means of each row are denoted by different lowercase letters.

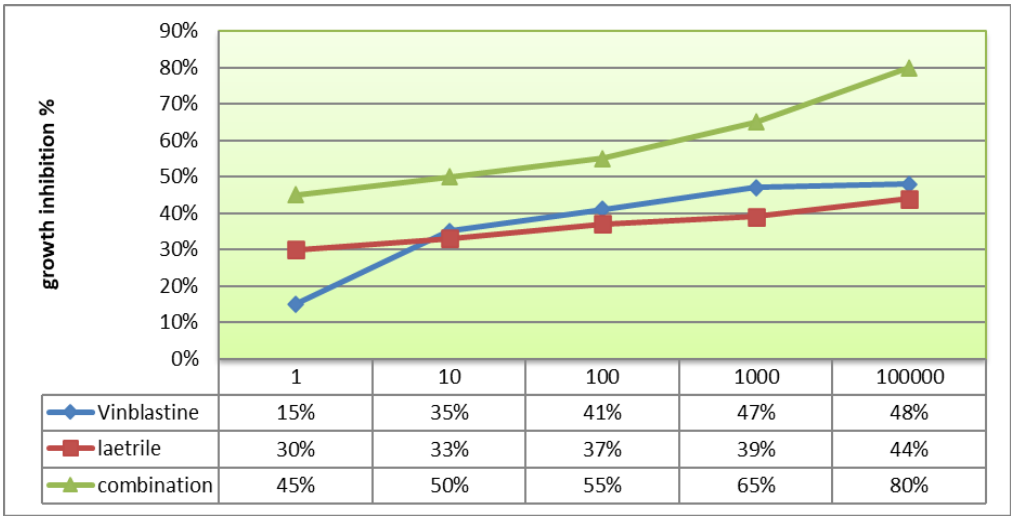


Figure 8. Growth Inhibition Effects of Vinblastine, Laetrile, and the Combination of Vinblastine and Laetrile over a 72-hour Period.

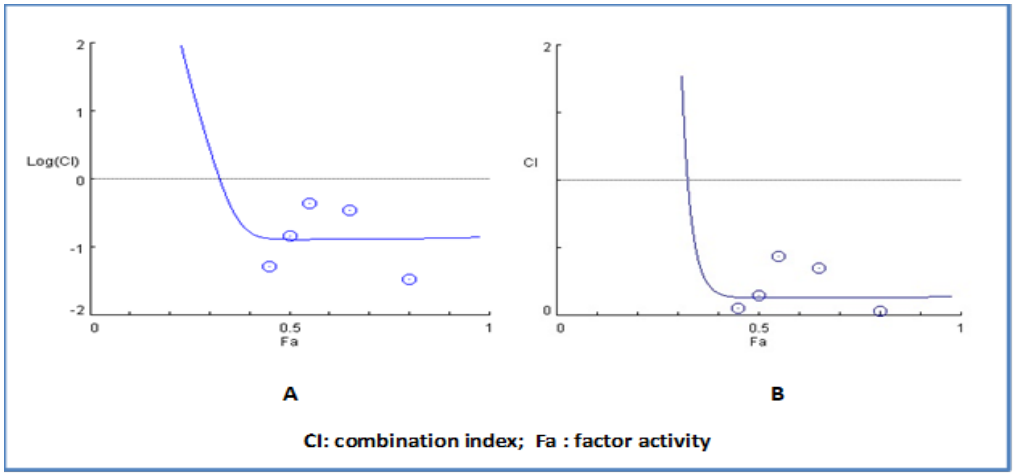


Figure 9. (A) combination index plot and, (B) logarithmic combination index plot for a mixture at a 72-hour incubation period. The interaction between the mixture's components is determined by the combination index (CI). A CI less than one implies synergism, a CI of one indicates an additive impact, and a CI larger than one indicates antagonism (Chou et al.,2019).

Kadhim and Mohamad, 2023; Škubník et al., 2021). On other side laetrile cytotoxic effect is primarily attributed to hydrocyanic acid and benzaldehyde, which are released within the cancer cells due to the catalytic activity of the glucosidase enzyme (Sekino and Teishima, 2020).

The antineoplastic action of hydrocyanic acid is believed to be associated with its ability to inhibit the activity of cytochrome C oxidase, An essential component of the electron transport chain in mitochondrial respiration. The inhibition indicated above impedes both the process of oxidative metabolism and the subsequent mechanism of oxidative phosphorylation, resulting in a depletion of energy stores, The harmful effects of benzaldehyde are attributed to its capacity to initiate activations of caspase 3,

Table 9. Combination Index Value for the Mixture Reported at a 72-hour Incubation Period.

| Total mixture Dose | Combination index Value | Pattern of combination |
|---|---|---|
| 1 µg/ml | 0.0518 | Very strong synergism |
| 10 µg/ml | 0.149 | strong synergism |
| 100 µg/ml | 0.4408 | synergism |
| 1,000 µg/ml | 0.3509 | synergism |
| 10,000 µg/ml | 0.0339 | Very strong synergism |

Table 10. Guidelines for the Determination of Antagonism and Synergism Style Using Combination Index analysis (CIA) (18) (Chou et al.,2019)

| Pattern of combination | Combination index |
|---|---|
| Very Strong Synergism | < 0.1 |
| Strong Synergism | 0.1–0.3 |
| Synergism | 0.3–0.7 |
| Moderate Synergism | 0.7–0.85 |
| Slight Synergism | 0.85–0.90 |
| Nearly Additive | 0.90–1.10 |
| Slight Antagonism | 1.10–1.20 |
| Moderate Antagonism | 1.20–1.45 |
| Antagonism | 1.45–3.3 |
| Strong Antagonism | 3.3–10 |
| Very Strong Antagonism | > 10 |

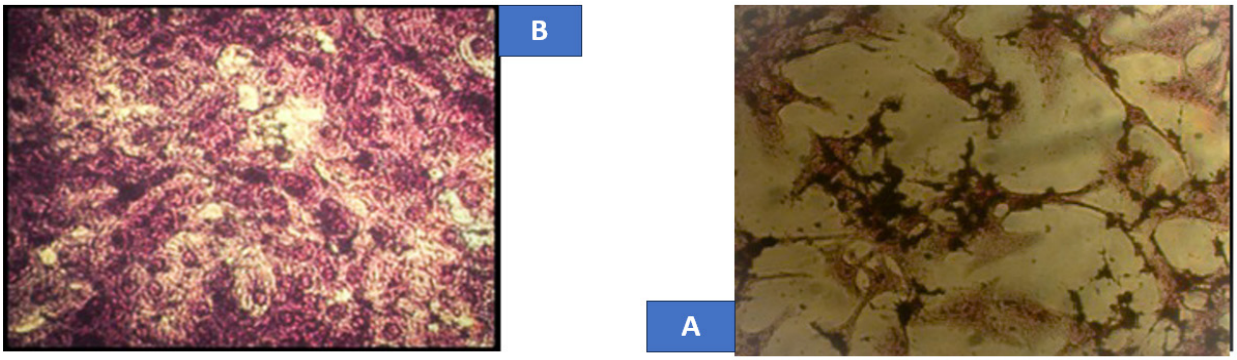


Figure 10. Shown the Morphology of HeLa Cells that have been Stained with MTT., (A): The HeLa cells were subjected to a concentration of 10000 (µg/ml) of Vinblastine and Laetrile for 72 hours. As a result, the cells developed feathering (shown by an arrowhead) and loss of polarity, and the nuclei displayed crowded hyperchromasia (indicated by an arrow),100x (B): The control groups' HeLa cells, the specimens that underwent no treatments displayed a collection of neoplastic cells characterized by a tightly packed arrangement and little cytoplasm, The cells also exhibited coarsely granular nuclear chromatin (shown by an arrow), and the presence of nucleoli was not seen,100x

8, and 9, hence inducing apoptosis, the potential influence of laetrile on prostate cancer cells is in its capacity to initiate apoptosis through the activation of caspase-3,The extent of this phenomenon is dependent on variables such as the amount and length of time of the exposure. As mentioned earlier, the methodology entails the inhibition of the antiapoptotic Bcl-2 protein and the augmentation of the proapoptotic Bax protein (Makarević et al., 2016).

The combination of vinblastine and laetrile exhibited a synergistic effect after 72 hours of incubation across all mixture concentrations. A synergistic effect was also observed at a 10000 µg/ml dose after 48 hours. And 1 and 10 µg/ml concentrations at 24 hours, the results of the incubation periods, as presented in Tables (5, 7, and 9), and Figures (5, 7, and 9), were compared to one another to find the common thread, The objective was met by comparing the combination index reading for each concentration throughout all three incubation times to the appropriate value in the table (Chou et al., 2019; Ueda et al., 2022).

The present study investigates the relationship between mixture growth inhibition and the cytotoxicity of vinblastine and laetrile. Additionally, it explores the potential synergistic effects between these compounds, specifically Moreover, this study examines the possibility of laetrile as a means to alleviate the resistance of Hela neoplastic cells to the cytotoxic effects of vinblastine. The anticancer activity of laetrile primarily relies on the concentration of cyanide and benzaldehyde released by the degradation of laetrile through the catalytic action of the glucosidase enzyme inside the cytoplasm. Hence, glucosidase is regarded as a pivotal factor in the cytotoxicity of laetrile, certain medications has been observed to increase the glucosidase enzyme's intracellular concentration, potentially intensifying the cytotoxic properties of laetrile. Specifically, studies have shown that Vinblastine can enhance laetrile's cytotoxicity by raising the glucosidase enzyme's activity. The observed phenomenon is accomplished by the capacity of Vinblastine to cause the permeabilization of lysosomal membranes, leading to an augmented presence of the glucosidase enzyme within the cytoplasm. The glucosidase enzyme belongs to the lysosomal hydrolytic enzymes located within lysosomes (Domagala et al., 2018; Jumaa et al., 2018; Jumaa et al., 2020; Oliveira et al., 2020; Serrano-Puebla and Boya, 2016).

The release of cyanide, which is a byproduct of laetrile breakdown inside malignant cells, is another proposed mechanism of synergism in the combination. It is believed that this procedure will help cancer cells become less resistant to vinblastine's toxic effects. The P-glycoprotein (P-gp) transport mechanism is dependent on adequate quantities of adenosine triphosphate (ATP), which cyanide may deplete. Cancer cells are successfully cleared of vinblastine via the ATP-dependent transport pathway (Falfushynska et al., 2019).

Cancer cells' capacity to develop resistance to vinblastine-induced lysosomal membrane permeabilization is diminished due to the depletion of energy resources caused by cyanide use. By lowering ATP levels, the quantity of overproduced Hsp70 family chaperone proteins in cancer cells may be reduced. The Hsp70 family of chaperone proteins is significantly impacted by this decline (Tegeder and Kögel, 2021).

Reduced production of the Hsp-70 chaperone protein could impede the rate of resistance evolution. To achieve this decrease, the lysosomal membrane's resistance to acidic conditions inside the lysosome is diminished (Lie and Nixon, 2019). It results in an increase in the lysosomal membrane's permeability, which in turn permits lysosomal contents to migrate into the cancer cell's cytoplasm.

The induction of apoptosis by combinations occurs via two separate pathways. Firstly, benzaldehyde, released during the disintegration of laetrile, triggers caspase 3.8 and 9, leading to apoptosis (Sánchez-Pérez, 2023). Secondly, vinblastine triggers the translocation of lysosomal contents, including proteases such as cathepsin B, CD, and cathepsin L, to the cytoplasm, resulting in cell extinction. These proteases create a series of sequential reactions conducting to the activation of apoptotic effectors, such as mitochondria and caspases (Serrano-Puebla and Boya, 2016).

In conclusion, this investigation demonstrated that laetrile and vinblastine inhibit the growth and division of human cervical cancer cells within an experimental laboratory setting. The observed phenomenon is thought to be elucidated by the synergistic interplay between vinblastine and laetrile, wherein vinblastine boosts the cytotoxic effects of laetrile on Hela cells. Moreover, laetrile has been found to potentially ameliorate the development of resistance to the effects of vinblastine in Hela cancer cells.

## Author Contribution Statement

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

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*Conflicts of interest*

There are no conflicts of interest.

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