Azal hamoody jumaa et al /J. Pharm. Sci. & Res. Vol. 10(9), 2018, 2236-2241

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

# Esomeprazole and Amygdalin combination cytotoxic effect on human cervical cancer cell line (Hela cancer cell line)

**Azal hamoody jumaa[1] Wissam Sajid Hashim Al Uboody[2] Ahmed mhdy hady[1]**

*1- Pharmacologist, Al-Yarmouk University College/Iraq.*
*2- Physiologist, College of Medicine, Al Muthanna University, Iraq.*

**Abstract**

Cancer cells exert a resistance against the chemotherapeutic agents which make the treatment of cancer facing a real challenge. To overcome the resistance of cancer cells, the researchers have developed discrepant strategies comprising the use of drugs combination or the use of drugs which are not classified as anticancer but their properties as anticancer considered promising. In this study, we have used a combination of amygdalin and esomeprazole to identify the possibility of synergism between them as anticancer.

For this purpose, a mixture of (esomeprazole and amygdalin) was used to see their activity on human cervical cancer cells (Hela cancer cell line) in vitro at concentrations ranged between 1- 10000 µg/ml at three incubation periods and compared with the cytotoxicity of (esomeprazole alone and amygdalin alone), with identifying the style of combination between the esomeprazole and amygdalin.

The results of the study demonstrate that the cytotoxic effect of (esomeprazole and amygdalin) combination was significantly more than the cytotoxicity of each one alone on Hela cancer cell line (human cervical cancer cell line) specially at concentration equal to (1000 and 10000) µg/ml at the three incubation periods. The result shows also the cytotoxicity of (esomeprazole and amygdalin) combination increases with the increase in the concentration which ranged between (1-10000) µg/ml, at incubation periods equal to (24, and 72) hrs.

The results also reveal that the style of esomeprazole cytotoxicity on Hela cancer cell line was increase with increase in their concentration except the smallest concentration, where the results shows the cytotoxicity of (1 µg/ml) was significantly more than the cytotoxicity of (10 ,100 and 1000) µg/ml at(24 and 48 hr) incubation periods. Besides, was also seen that the cytotoxicity of esomeprazole was significantly more than the cytotoxicity of amygdalin especially at (1, 1000 and 10000) µg/ml at (42 and 72) hr. incubation periods.

## INTRODUCTION

Cervical cancer treatment is relied majorly on chemotherapy. Surgery or radiation combined with chemotherapy are used in earliest stages of cervical cancer. In later stages, radiation combined with chemotherapy is usually the main treatment (1). Drugs that are used as chemotherapy kills the cancer cells but they do also kill the normal proliferating cells which results in real side effects that depends on duration and dose of the drug used (13). Such side effects includes for instance neurotoxicity and nephrotoxicity which are caused by vincristine and methotrexate respectively (11).

To get rid of or to belittle the chemotherapy side effects, different strategies were adopted like the use of medicaments that are not anticancer but might exert anticancer activity such as aspirin which has a chemopreventative activity against colorectal cancer by a mechanism linked to its antiplatets effect (7). The sensitivity of a pre-B acute lymphoblastic leukemia cell line (ALL) to vinblastine has been found to be increased by omeprazole by modification of cellular pH gradients of tumor B cell which leads to inhibition of the proliferation of these cells (3), and it is also found to modulate the lysosomes transport and autophagy leading to programmed cell death and hence it modulates the tumor chemoresistance (2). It was mentioned that melanoma cell proliferation in vitro is inhibited by esomeprazole and cellular death of tumor cells might occur by activation of caspases and acidification inside the cells. Besides, it was noticed that surviving rate of the animals that bear melanoma of human was elevated when esomeprazole was offered and there were no toxic effects (4). As an inhibitor of proton pump, esomeprazole is famous which acts on H/K-ATPase causing its inhibition and then lead to reduce the acid secretion from gastric cells. It is of a great anti cancer activity especially against melanoma and human cancer cells B by the mechanisms mentioned above (3,4). A lot of compounds were being isolated from plants and they were of great functions in biological fields (17, 27). These plant sources compounds are without side effects as compared with chemotherapies and a good example about the is amygdalin. Amygdalin is a type of a glycosides which is derived or being isolated from the plant the biter almond or in more certain word the seed of the bitter almond or *Prunus dulcis* (24). Amygdalin and its modified forms the laetrile or what is called vitamin B17 were found to be effective or can kill the cancer cells by selective mechanism and without exerting side effects (18).

## AIM OF STUDY

The aim of study was to determine the cytotoxicity of esomeprazole -amygdalin combination on Hela cancer cell line in vitro.

## MATERIAL AND METHODS:

**(amygdalin)**

Amygdalin was purchased from Santa Cruz (Santa Cruz, CA, USA) used at different concentration which ranged between (1-10000) µg/ml, these concentration achieved by dilution of amygdalin with free serum media.

Proton pump inhibitor:

**Esomeprazole (nexium)** ® vial 40 mg (astra Zeneca), used at different concentration which ranged between (1-10000) µg/ml, these concentration achieved by dilution with free serum media.

**Cell culture**

Human cervical cancer Hela cell line was purchased from tissue culture unit/ Iraqi Centre for Cancer and Medical Genetics Research (ICCMGR). The cells were cultured in 75 $cm^2$ tissue culture flasks under humidified 5% CO2 atmosphere at 37°C in RPMI-1640 medium (Sigma chemicals, England) with 10% fetal bovine serum (FBS) provided by ICCMGR, Iraq, and penicillin-streptomycin 1% (100 U/ mL penicillin and 100 µg/mL streptomycin) (Lilly, Italy) During the course of the experiment (9).

**Cytotoxicity Assay**

Hela cancer Cells that's culture in microtiter plate (96wells) were exposed to range of concentration from (esomeprazole, amygdalin, (amygdalin with vincristine)), the concentration of cancer cells in each well will be increase during the log phase of growth and the cytotoxic effect of tested agents will be determined after several incubation periods. (9) , every well contained $7x10^3$cells, Serum calf medium (10%) was used for cancer cells seeding, after seeding the plates incubated for 24hrs at 37 °C to achieve cancer cells attachment, then By using maintenance medium, fivefold serial dilution were prepared starting from (1-10000 µg/ml) for each amygdalin , esomeprazole and a mixture of (amygdalin with esomeprazole) , After

incubation for 24 hrs, cells were exposed (Six replicate at 200µl for each tested concentration), 200 µl of maintenance medium added to each well of control group, the times of exposure were 24, 48 and 72 hrs. The plates were sealed with self-adhesive film then returned to incubator, cells where staining with MTT stain, The optical density of each well was read by using a micro-ELISA reader at a transmitting wavelength 550 nm (17; 9).

The inhibitor rate measuring by using of the following equation (10):

$$\text{Inhibitor rate \%} = \frac{\text{The optical density of control} - \text{The optical density of test}}{\text{The optical density of control}} \times 100$$

**Statistical Analysis:**

The Statistical Analysis System (SAS) (21) was used to identify the effect of different factors in study parameters. Least significant difference –LSD test was used to compare between means in this study significantly

## RESULTS AND DISCUSSION:

**The cytotoxic effect of amygdalin:**

The result revealed the maximum cytotoxic effect of amygdalin on Hela cancer cell line occur at (10000) µg/ ml for each three incubation period with a significance variation comparing with control at level ( $p$ <0.05), without a significance variation between (10000,100 and 10) µg/ ml comparing with other concentration for each (48 and 72) hr. with without a significant variation for (10000) µg/ ml between (24,48 and 72) hr table (1) figure (1).

**Table (1): effect of concentration and time in growth inhibition rate for amygdalin on Hela cancer cell line**

| Conc. | 24hr. | 48hr. | 72hr. | LSD |
|---|---|---|---|---|
| 10000 µg/ml | A 25 a | A 26 a | A 34 a | N.S |
| 1000 µg/ml | AB 17 b | A 24 ab | AB 30 a | 9.875 |
| 100 µg/ml | BC 10 b | A 20 a | AB 25 a | 9.325 |
| 10 µg/ml | BC 7 a | B 12 a | B 22 a | N.S |
| 1 µg/ml | C 0 a | C 0 a | C 0 a | N.S |
| - | 10.143 | 6.9989 | 10.545 | - |

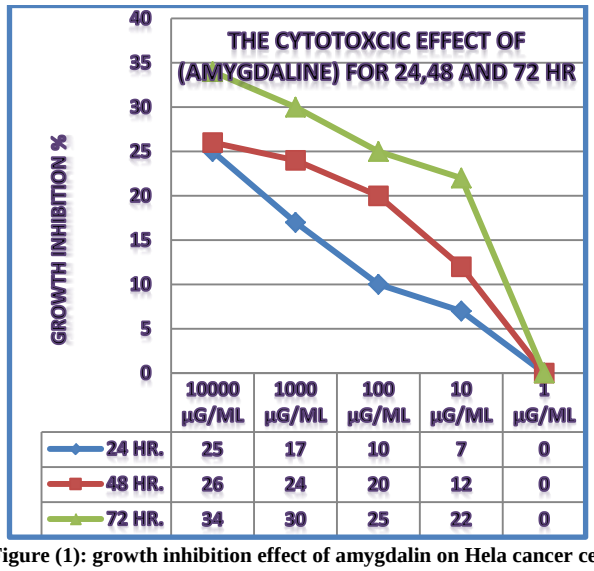



**Figure (1): growth inhibition effect of amygdalin on Hela cancer cell**

This result showed, there is an increase in the cytotoxicity occur with increasing of the (concentration and time of incubation) i.e. its (dose depend and time depend) , Amygdalin show an increase in their cytotoxicity toward Hela cancer cell line with increase in the amygdalin concentration and duration of exposure (concentration and time depends) , this cytotoxicity mainly related to the effect of hydrocyanic acid and benzaldehyde that liberated inside the cancer calls under the analytical effect of glucosidase enzyme (18) .

The antineoplastic effect of hydrocyanic acid occurs through its ability to inhibition of cytochrome C oxidase in the respiratory electron transport chain of the mitochondria, impairing both oxidative metabolism and the associated process of oxidative phosphorylation, eventually causing energy deprivation (19), while benzaldehyde cytotoxicity occurs through its ability to induce apoptosis by caspase 3, 8 and 9 activation (25). Amygdalin by depending on dose and time of exposure have ability to induce apoptosis by caspase-3 activation through downregulation of antiapoptotic Bcl-2 protein and up regulator of proapoptotic Bax protein in prostate cancer cells (8).

**The cytotoxic effect of esomeprazole:**

The result of esomeprazole growth inhibition of Hela cancer cells demonstrate increase in the growth inhibition occurs with increasing in the esomeprazole concentration which ranged between (10-10000) µg/ml , for each three incubation periods, except (1 µg/ml) , where the growth inhibition was significantly more than 10 µg/ml for all incubation periods, without significant variation between the growth inhibition of (1 µg/ml) with (10000 µg/ml) for 48hr. and (1 µg/ml) with (100 µg/ml) for 72hr. and 72hr. incubation periods table (2) figure (2).

**Table (2): effect of concentrations and time in growth inhibition rate for esomeprazole on Hela cancer cell line**

| Conc. | 24hr. | 48hr. | 72hr. | LSD |
|---|---|---|---|---|
| 10000 µg/ml | A 83 a | AB 27 b | A 82 a | 12.9 |
| 1000 µg/ml | C 30 b | B 23 b | A 80 a | 11.18 |
| 100 µg/ml | D 16 b | C 2 c | B 28 a | 7.48 |
| 10 µg/ml | D 10 a | C 7 a | C 3 a | NS |
| 1 µg/ml | B 44 a | A 33 ab | B 23 b | 12.74 |
| LSD | 10.072 | 9.9449 | 9.0233 | _ |

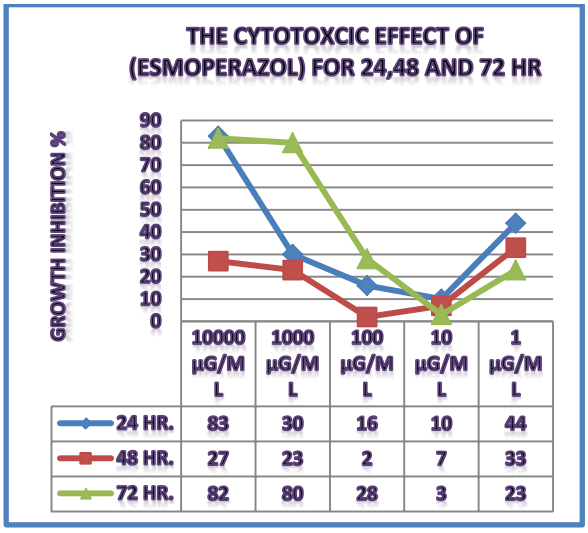



**Figure (2): growth inhibition effect of esomeprazole on Hela cancer cell line**

The versatile inhibitory specialty of esomeprazole on inhibition can be explained depending upon its hormotic effect where hormosis is well known in field of toxicology as a phenomenon featured by low concentration reverse action comparing with the higher concentrations (15). The precise mechanism for the hormosis is still vague but it is postulated that the toxin of low doses might induce bodily repairing mechanism. The damage of low level that might accumulate beyond the mechanism of repair is also comprised in the process of repair in addition to the repair of the toxin caused damage (5). The obstinate diseases like Parkinson and Alzheimer`s might be treated with exploiting this

phenomenon as it depends upon the use of low dose pollutants or toxins as these compounds have the capacity to devastate the cancer cells without demolishing the normal cells and some those factors or compounds can ameliorate the activity of anticancer agents and some of the has the ability to alleviate the genotoxicity of some agents (7). Of the mechanisms involved in the esomeprazole action that what is related to its lysosomal inducing effect to cause them permeable through cell membranes hence to cause passage of lysosomal contents into cytoplasm which will cause lysis of cell components and finally cellular death. Besides, enzymes of lysosomes might provide acidic umbworld that can devastate the cells of tumor (6, 12, and 14). Furthermore, esomeprazole acts by offering acidification of inside the cells and triggering the enzymes caspases which will kill melanoma cells in vitro (4). Also for esomeprazole, it can generate reactive oxygen species in the B cells of human which will trigger: 1) the lysosomal pH will be alkalinized, 2) permeability of lysosomal membrane, and 3) acidification of lysosomes via the destabilizing of the vesicular acidic constructors (3).

**The cytotoxic effect of the mixture:**

The result of (esomeprazole and amygdalin) combination growth inhibition of Hela cancer cells demonstrate increase in the growth inhibition occurs with increasing in the mixture concentration which ranged between (1-10000) µg/ml, for (24 and 72) hr. incubation periods, the result indicated also there is no significant variation between (24 and 72) hr. incubation periods for (10000, 100, 10 and 1) µg/ml, table (3), figure (3).

**Table (3): effect of concentration and time in growth inhibition rate for a mixture of (esomeprazole and amygdalin) on Hela cancer cell line**

| Conc. | 24hr. | 48hr. | 72hr. | LSD |
|---|---|---|---|---|
| **10000 µg/ml** | **A 90 a** | **A 36 b** | **A 92 a** | **12.9** |
| **1000 µg/ml** | **B 37 b** | **A 28 b** | **A 91 a** | **11.18** |
| **100 µg/ml** | **C 23 a** | **BC 5 b** | **B 28 a** | **8.71** |
| **10 µg/ml** | **C 22 a** | **C 2 b** | **BC 17 a** | **6.32** |
| **1 µg/ml** | **C 20 a** | **B 14 a** | **C 12 a** | **N.S** |
| | **9.0233** | **9.6952** | **10.39** | - |

The style of mixture component combination between (vincristine and amygdalin) was synergetic at concentration 1µg of mixture in 24hr. , at (1µg, 10000 µg) in 48hr. and (1,10)µg/ml at 72hr. incubation period as shown in table (4,5,6) and figure (4,5,6,7,8,9), the pattern of combination can be concluded after comparing the combination index value of each concentration at three incubation period with the guideline combination index value table (7).

The growth inhibition is linked to amygdalin and esomeprazole cytotoxicity feature beside the ability of potentiation participating between them which comprises the enhancing ability of esomeprazole to elevate amygdalin cytotoxicity besides the amygdalin feature to delineate the Hella cancer cells resistance towards the cytotoxic repercussions of esomeprazole. Depending upon the levels of cyanide inside cytoplasm, the amygdalin activity as anticancer emerges. By the action of glucosidase enzyme, the decomposition of amygdalin will upshots the cyanide and benzaldehyde. For that, the enzyme glucosidase is considered to be a key role for the cytotoxicity of amygdalin. Hereby, the agents which can elevate the fluency of glucosidase inside the cells which will lead to elevate cytotoxicity of amygdalin. Esomeprazole also assists in elevating the cytotoxicity of amygdalin by offering a lot of the enzyme glucosidase and this is accomplished by the permeablizing of membrane lysosomes cause by vincristine which will upshot plenty of glucosidase in the cytoplasm (6, 12, 14). The cyanide which is liberated from the decomposition of amygdalin inside cancer cells has the ability to decrease the resistance of the cancer cell against cytotoxicity of esomeprazole and this is done via the capacity of cyanide to deprive energy (19). This mechanism of cyanide also decrease development of resistance of cancer cells against the permeabilization of lysosomes of membrane which is done by esomeprazole and this is done via decreasing the Hsp70 chaperones protein family from being overexpressed inside the cancer cells (16). The later action is done via declination of ATP which is paramount for the overexpression of these protein family. When this expression is being reduced, the resistance should be reduced too towards the acidic milieu (20) which will yield elevation in the lysosomal membrane permeability and finally leakage of the contents of lysosomes into the cancer cell cytoplasm. Apoptosis induced by mixture could be induced by two variant pathways: the first is through the benzaldehyde which is liberated from decomposed amygdalin where it can induce apoptosis by its activating action on the caspases 3, 8, and 9 (22). The second pathway relates to the ability of esomeprazole to trigger activation of many caspases and to yield ROS which can produce: lysosomal PH alkinazation, permeability of membrane lysosomes and acidification of cytosol via earlier destability of the vesicular acidic constituent (3).

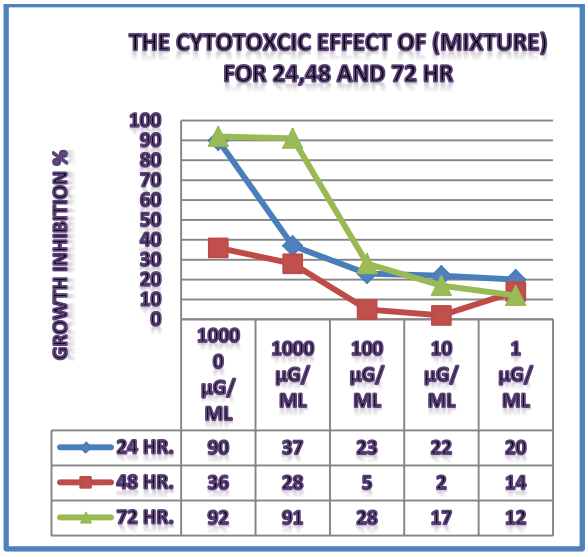


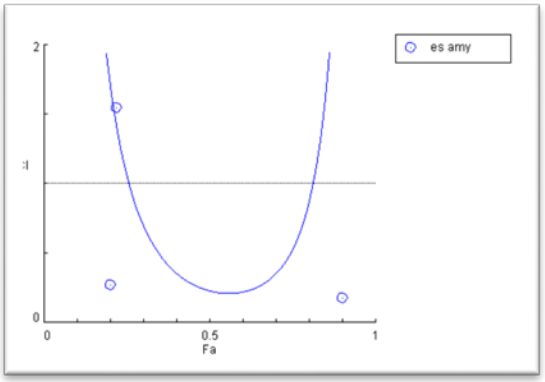

**Figure (3): growth inhibition effect of (esomeprazole and amygdalin) mixture on Hela cancer cell line**

**Table (4): growth inhibition effect and combination index value of esomeprazole, amygdalin and the mixture of them on Hela cancer cell line at 24 hr.**

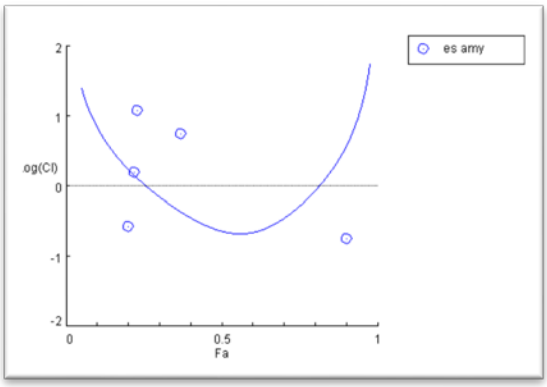
| Conc. | esomeprazole | amygdalin | mix | LSD | CI Value | Pattern of combination |
|---|---|---|---|---|---|---|
| **10000** | A 83 a | A 25 b | A 90 a | **12.896** | 1.20291 | Moderate antagonism |
| **1000** | C 30 a | AB 17 b | B 37 a | **11.267** | 2.09964 | antagonism |
| **100** | D 16 ab | BC 10 b | C 23 a | **8.7086** | 2.95372 | antagonism |
| **10** | D 10 b | BC 7 b | C 22 a | **7.4084** | 1.90646 | antagonism |
| **1** | B 44 a | C 0 c | C 20 b | **12.262** | 0.25843 | Strong synergism |
| **LSD** | **10.072** | **10.143** | **9.0233** | - | - | - |

**Table (5): growth inhibition effect and combination index value of esomeprazole, amygdalin and the mixture of them on Hela cancer cell line at 48 hr.**

| Conc. | esomeprazole | amygdalin | mix | LSD | CI Value | Pattern of combination |
|---|---|---|---|---|---|---|
| **10000** | AB 27 a | A 26 a | A 36 a | **12.896 NS.** | 0.85837 | Slight Synergism |
| **1000** | B 23 a | A 24 a | A 28 a | **11.267 NS.** | 1.78564 | antagonism |
| **100** | C 2 b | A 20 a | BC 5 b | **7.4754** | 1.75017 | antagonism |
| **10** | C 7 ab | B 12 a | C 2 b | **6.3179** | 109.826 | Very Strong antagonism |
| **1** | A 33 a | C 0 c | B 14 b | **9.8554** | 735.000 | Very Strong antagonism |
| **LSD** | **9.9449** | **6.9989** | **9.6952** | - | - | - |




A




B

**CI: combination index; Fa: factor activity (growth inhibition)**
**Figure (4): A combination index plot; B logarithmic combination index plot for mixture at 24hr. incubation period (CI < 1 indicates Synergism, CI = 1 indicates Additive Effect, CI > 1 indicates Antagonism), (23).**

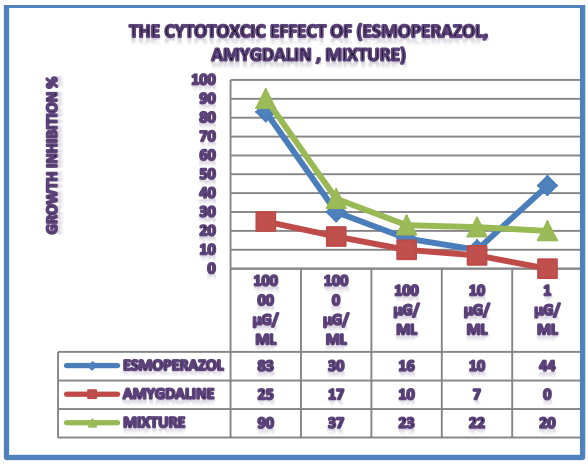
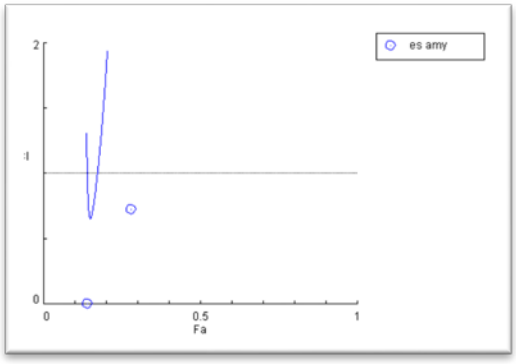
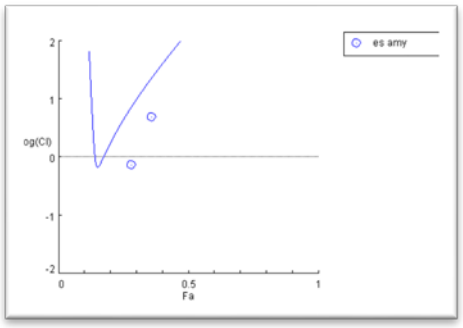



**Figure (5): growth inhibition effect of esomeprazole, amygdalin and the mixture of them on Hela cancer cell lone at 24 hr.**




A




B

**CI: combination index; Fa: factor activity (growth inhibition)**
**Figure (6): A combination index plot; B logarithmic combination index plot for mixture at 48hr. incubation period (CI < 1 indicates Synergism, CI = 1 indicates Additive Effect, CI > 1 indicates Antagonism), (23).**

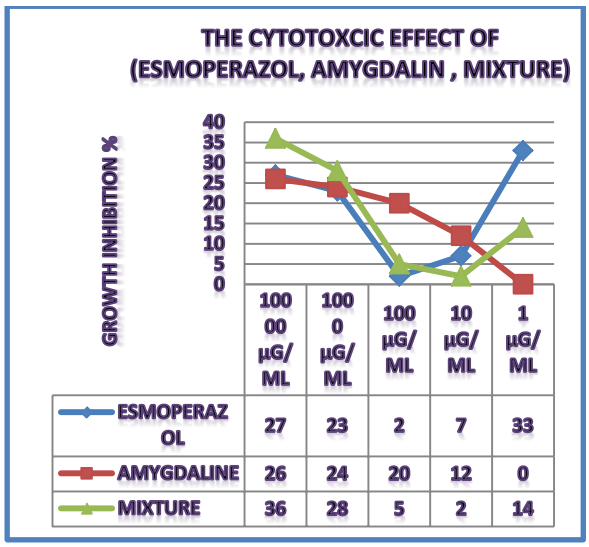
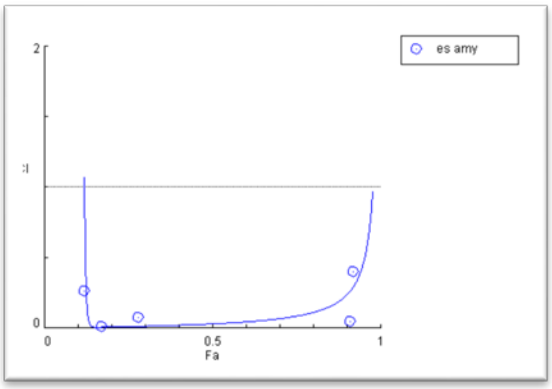


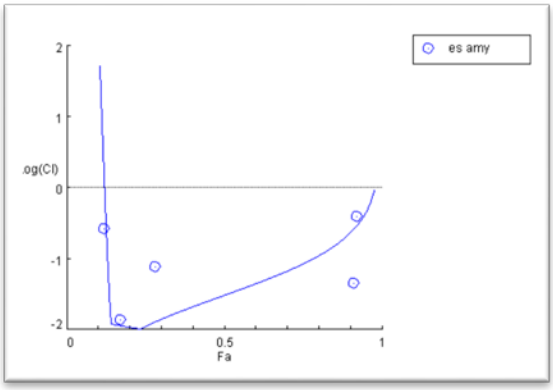

**Figure (7): growth inhibition effect of esomeprazole, amygdalin and the mixture of them on Hela cancer cell lone at 48 hr.**

**Table (6): growth inhibition effect and combination index value of esomeprazole, amygdalin and the mixture of them on Hela cancer cell line at 72 hr.**

| Conc. | esomeprazole | amygdalin | mix | LSD | CI Value | Pattern of combination |
|---|---|---|---|---|---|---|
| **10000** | A 82 | A 34 | A 92 | **12.896** | 1.18101 | Slight Antagonism |
| **1000** | A 80 | AB 30 | A 91 | **11.267** | 1.96960 | Antagonism |
| **100** | B 28 | AB 25 | B 28 | **7.4754** | 2.21888 | Strong Antagonism |
| **10** | C 3 | B 22 | BC 17 | **7.4084** | 0.69168 | Synergism |
| **1** | B 23 | C 0 | C 12 | **12.741** | 0.81640 | Moderate Synergism |
| **LSD** | **9.0233** | **10.545** | **10.39** | - | - | - |




**A**




**B**

**CI: combination index; Fa: factor activity (growth inhibition)**
**Figure (8): A combination index plot; B logarithmic combination index plot for mixture at 72hr. incubation period (CI < 1 indicates Synergism, CI = 1 indicates Additive Effect, CI > 1 indicates Antagonism), (23).**

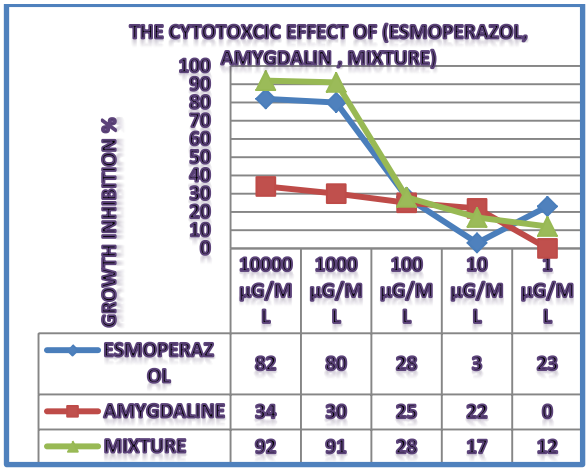



**Figure (9): growth inhibition effect of esomeprazole, amygdalin and the mixture of them on Hela cancer cell lone at 72 hr.**

**Table (7); guideline for determine pattern of Synergism and Antagonism by using Combination index analysis[23].**

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

## CONCLUSION:

The result of this study demonstrate that the combination of (amygdalin , esomeprazole) has ability to inhibit the growth of human cervical cancer cells in vitro by a suggested mechanism including a potentiation ability of esomeprazole to the cytotoxicity of amygdalin on Hela cells , and the ability of amygdalin to minimized the development of Hela cancer cells resistance to esomeprazole activity .

## REFERENCES:

1. American Cancer Society.(2016): Cancer Facts and Figures 2016. Atlanta, Ga: American Cancer Society.
2. Andrej Udelnow;Andreas Kreyes; Stefan Ellinger; Katharina Landfester; Paul Walther; Thomas Klapperstueck; Johannes Wohlrab, et al., (2014): Omeprazole Inhibits Proliferation and Modulates Autophagy in Pancreatic Cancer Cells , LoS ONE | www.plosone.org , Volume 6 | Issue 5 | e20143.
3. Angelo De Milito; Elisabetta Iessi; Mariantonia Logozzi; Francesco Lozupone; Massimo Spada; Maria Lucia Marino; Cristina Federici; et al., (2007): Proton Pump Inhibitors Induce Apoptosis of Human B-Cell Tumors through a Caspase-Independent Mechanism Involving Reactive Oxygen Species, Cancer Res;67:5408-5417 .
4. Angelo De Milito; Rossella Canese; Maria Lucia Marino; Martina Borghi; Manuela Iero; Antonello Villa; Giulietta Venturi. et al.,(2010) : pH-dependent antitumor activity of proton pump inhibitors against human melanoma is mediated by inhibition of tumor acidity, International Journal of Cancer: 127, 207–219 .
5. Axelrod, Deborah; Burns, Kathy; Davis, Devra and von Larebeke, Nicolas (2004):"'Hormesis'—An Inappropriate Extrapolation from the Specific to the Universal".International Journal of Occupational and Environmental Health 10 (3): 335– 9.
6. Boya, P. and Kroemer, G.(2008): Lysosomal membrane permeabilization in cell death. Oncogene 27: 6434–6451,.(Z).
7. Calabrese, E.J and Baldwin, L.A.(2003): Hormesis: the dose-response revolution Annu.Rev.pharmacol.Toxico.143:175-197.
8. Chang, h.k. ; shin, m.s. ; yang, h.y. ; lee, j.w. ; kim, y.s. ; lee, m.h ; kim, j. ; et al., (2006): Amygdalin induces apoptosis through regulation of bax and bcl-2 expressions in human du145 and lncap prostate cancer cells. In *biological and pharmaceutical bulletin,* vol. 29, no. 8, p. 1597-1602.
9. Freshney,R.I.(1994):Culture of Animal Cells.( 3rd. Ed.).Wiley-Liss,U.s.A.,pp:267-308.(7).

10. Gao, S; Yu, B; Li, Y; Dong, W. and Luo, H. (2003): Antiproliferative effect of Octreotide on gastric cells mediated by Inhibition of Akt/PKB and telomerase. World J. Gastroenterol, 9: 2362-5.(8).
11. Grewal, J; Dellinger, CA and Yung, WK.(2007): Fatal reactivation of hepatitis B with temozolomide. N. Engl. J. Med., 356:1591–1592.
12. Groth-Pedersen L and Jaattela M.(2010): Combating apoptosis and multidrug resistant cancers by targeting lysosomes. Cancer Lett [Epub ahead of print]; DOI: 10.1016/j.canlet. 05.021.(Y).
13. Gullatte, MM and Gaddis, J.(2004): Chemotherapy. In: Varrichio CG, ed. A Cancer Source Book for Nurses. 8th ed. Sudbury, Mass: Jones and Bartlett; :103-130.
14. Johansson, AC; Appelqvist, H; Nilsson, C; Kagedal, K; Roberg, K and Ollinger, K.(2010): Regulation of apoptosis-associated lysosomal membrane permeabilization. Apoptosis 15: 527– 540, (U).
15. Kaiser, Jocelyn. (2003): "Sipping from a Poisoned Chalice". Science 302 (5644): 376– 9.
16. Kirkegaard, T; Roth, AG; Petersen, NH; Mahalka, AK; Olsen, OD; Moilanen, I; Zylicz, A; et al., (2010): Hsp70 stabilizes lysosomes and reverts Niemann-Pick disease-associated lysosomal pathology. Nature 463: 549–553.
17. Al-Uboody, W. S.; and Al-Diwan, M. A. (2015). Study the protective role of quercetin against o-anisidine toxicity on some hematological parameters of laboratory male rats (Rattus norvegicus). Bas.J.Vet.Res.Vol.14, No.1.
18. Marek, Halenár; Marína, Medveďová; Nora, Maruniaková; Adriana, Kolesárová. (2013): AMYGDALIN AND ITS EFFECTS ON ANIMAL CELLS. 1Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic. Journal of Microbiology Biotechnology and Food Sciences (Special issue 1) 1414-1423
19. Nelson, David. L and Cox, Michael. M. (2000): *Lehniger Principles of Biochemistry* (3rd ed.). New York: Worth Publishers. Pp. 668,670–71,676
20. Saftig, P and Klumperman, J.(2009): Lysosome biogenesis and lysosomal membrane proteins: trafficking meets function. Nat Rev Mol Cell Biol 10: 623–635.
21. SAS. (2012): Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.(19).
22. Swain, E; Poulton, J.E. (1994): "Utilization of Amygdalin during Seedling Development of Prunus serotina". Plant physiology 106 (2): 437–445.
23. Ting-Chao Chou; and Nick Martin. (2004): A Computer Program for Quantitation of Synergism and Antagonism in Drug Combinations, and the Determination of IC50 and ED50 Values. ComboSyn, Inc. 599 Mill Run, Paramus, NJ, 07653, USA
24. *Ueda K; Clark DP; Chen CJ; Roninson IB; Gottesman MM; Pastan I . (1987):* "The human multidrug resistance (mdr1) gene. cDNA cloning and transcription initiation". *The Journal of Biological Chemistry 262 (2): 505–8.*
25. Yu, Liu; Hiroshi, Sakagami; Ken, Hashimoto; Hirotaka, Kikuchi; Osamu, Amano; Mariko, Ishihara; Yumiko, Kanda; et al., (2008): Tumor-specific Cytotoxicity and Type of Cell Death Induced by Cyclodextrin Benzaldehyde Inclusion Compound Anticancer Research *28*: 229-236
26. ZHOU, C.; QIAN, L.; MA, H. ; YU, X. ; ZHANG, Y. ; QU, W. ; ZHANG, X. ;XIA, W. (2012): Enhancement of amygdalin activated with β-D-glucosidase on hepg2 cells proliferation and apoptosis. In Carbohydrat*e Polymers*, vol. 90, , p. 516-523. (28).
27. Al-Uboody, W. S.; and Al-Diwan, M. A. (2015). Ameliorating and protective role of quercetin against o-anisidine toxicity on some reproductive aspects of laboratory male rats (Rattus norvegicus). Bas.J.Vet.Res.Vol.14, No.1.