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THE HARMONIOUS INHIBITORY EFFECT OF NATURAL DRUG AMYGDALIN (VITAMIN B17) AND CHEMOTHERAPY (CISPLATIN) ON ORAL SQUAMOUS CELL CARCINOMA CELL LINE

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ABSTRACT

BACKGROUND: Oral squamous cell carcinoma (OSCC) represents the majority of all cancers of the head and neck. In recent times, the development of antitumor drugs has been transformed from cytotoxic drugs to ameliorate the selectivity of the drug, overcoming multidrug-resistant using natural alternatives. Amygdalin is one of the most common non-conventional anti-cancer drugs. Cisplatin has been used for the treatment of numerous human cancers. Recently combination therapy is the treatment of choice for cancer patients for the reason that it may enhance the efficiency of the combined agents and decreases their toxicities by decreasing the dose required for therapeutic benefits.

AIM: This study was organized to investigate the cytotoxic effect of both amygdalin and cisplatin on OSCC, compare between two effects on OSCC, and investigate the synergistic effect on cytotoxicity of both drugs on OSCC.

METHODS: The cytotoxic effect was determined using methyl tetrazolium assay, microscopic examination, and statistical analysis (ANOVA).

RESULTS: Our findings showed that the cytotoxic effect of both drugs on HNO97 cells was dose-dependent. Microscopic examination showed that apoptotic criteria became more apparent in cisplatin/amygdalin mix-treated HNO97 group. Our statistical analysis results revealed significant differences in statistical analysis among mean values of NAF of 50 cells in each group, with lower mean values of NAF of mix 48 group ($p < 0.0001$).

CONCLUSION: Both Amygdalin and cisplatin have anti- cancer effect on HN097 separately and their combination induced harmonious inhibitory effect on HNO97 cells.

KEYWORDS: OSCC, amygdalin, cisplatin, apoptosis, ic50.

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INTRODUCTION

Cancer is an important public health problem in many parts of the world, and oral cancer is among the 10 most common cancer worldwide. According to the International Agency for Research on Cancer of the World Health Organization (IARC-WHO), cancer rates were expected to increase from 10 million new cases in 2000 to 15 million in 2020 around the world. [1]

Oral squamous cell carcinoma (OSCC) represents 95% of all cancers of the head and neck, and over the last decade, its incidence had increased by 50%.[2]Tobacco and alcohol use are traditionally the greatest risk factors for oral cancer in the western world. [3, 4]

Recently the development of antitumor drugs has been transformed from cytotoxic drugs to ameliorate the selectivity of the drug, overcoming multidrug-resistant development of a new drug with low toxicity and high specificity. So, oncologists and oncology researchers shifted toward natural methods to reduce the toxic effects of the drugs that were used to treat such type of cancer. [5]

Recent studies have shown that amygdalin (vitamin B17) is a natural plant extract that has an anti-carcinogenic effect on several types of cancers. Amygdalin is found in considerable quantities in members of the Rosacea family including apricot, peaches, and almond. [6]

Amygdalin is called bitter apricot, laetrile, and almond. It induces apoptotic cell death in cells by down regulating the excretion of P53 (tumor suppressor gene) so could be used as an anticancer drug[7-9]. It is composed of two molecules of glucose, one molecule of Benz aldehyde, and one molecule of hydro cyanide. Studies have shown that the Benz aldehyde in amygdalin can induce an analgesic effect and the hydro cyanide in amygdalin can induce an anti-cancerous effect. Moreover amygdalin has the ability to decompose carcinogenic substances in the human body, killing cancer cells, blocking the nutrient source of tumor cells, inhibiting cancer cell growth,[10,11] and can also reduce the incidence of prostate cancer.[12] Cervical cancer,[13] liver cancer,[14] bladder cancer, [15] and SCC in the buccal pouch of hamsters,[16] by promoting apoptosis.

Cisplatin, cisplatinum, or cis-diamminedichloroplatinum (II), is a world-famous chemotherapeutic drug. It has been used for the treatment of numerous human cancers including bladder, head and neck, lung, and testicular cancers [17] Its mode of action is cross-linkage with the purine bases on the DNA to form DNA adducts [18], interfering with DNA repair mechanisms through induction of P53 signaling and cell cycle arrest, down regulation of proto-oncogenes and anti-apoptotic proteins, and activation of both intrinsic and extrinsic pathways of apoptosis. [19-21]

Other platinum-containing anti-cancer drugs such as carboplatin, oxaliplatin are preferably used because of drug resistance and undesirable side effects such as severe kidney problems, allergic reactions, decrease immunity to infections, gastrointestinal disorders, bone marrow problems, hemorrhage, and hearing loss, especially in younger patients. [17]

Combination therapy of cisplatin with other natural anti-cancer extracts such as Amygdalin drugs has been utilized as incoming therapeutic strategies for many human cancers to overcome drug resistance and reduce the toxicity of chemotherapy drugs. [22]

Apoptosis is a genetic program of cell death, being initiated by various physiologic and pathologic stimuli that function primarily to eliminate altered cells that are useless or harmful in a living organism. [23]Oral carcinogenesis correlates with the progressive accumulation of genetic mutations in molecules that regulate apoptosis.[24,25] The morphological characteristics of apoptosis include cell shrinkage, cytoplasmic vacuolation, and

condensation of nuclear chromatin, which is called **pyknosis.** [26]Apoptosis depends on cysteine proteases called **caspases.** Caspase-3 is a member of the cysteine protease family and plays an important role in the regulation of programmed cell death (apoptosis) suggesting its use as a prognostic marker for cancers. [27, 28]

The research aimed to investigate the cytotoxic effect of amygdalin and cisplatin on oral Squamous cell carcinoma, Compare between two effects on oral Squamous cell carcinoma, and investigate the synergistic effects on cytotoxicity of both drugs on oral Squamous cell carcinoma.

MATERIALS AND METHODS

Cell culture

Human oral squamous cell carcinoma (HNO97 cell line) was obtained from Cell Culture Department-VACSERA-EGYPT in the form of a frozen vial from cell lines services (California, USA)

Cell culture protocol

HNO97 (tongue SCC) were established from surgical tissue samples of SCCHN patients, then were grown as a single layer culture on a flask containing Dulbecco' modified Eagle' medium **(DMEM)** supplemented with 15% (V/v)* fetal bovine serum **(FBS)**, (100IU/mL) of penicillin, (100IU/mL) of streptomycin and finally, cells were preserved in a humidified incubator which contained 5% CO_2 at 37%°C [Jouan – France]. HNO97 cells were divided into four groups: a control group of untreated HNO97 cells, a group of Amygdalin treated HNO97 cells, a group of cisplatin-treated HNO97 cells, and a group of a mix of Amygdalin/cisplatin-treated HNO97 cells. These groups were cultured under high-efficiency sterilization conditions. The last three groups were treated according to standard protocols.

Reagents

Cisplatin is a well-known chemotherapeutic drug, is slightly soluble in water, and is soluble in dimethylprimanide and N, N-dimethylformamide, and has chemical formula [Pt. $(NH_3)_2cl_2$].

Cisplatin was obtained from Sigma Aldrich Products (St, Louis, MO, USA), was dissolved in 0.5% dimethyl sulfoxide **DMSO** and enoxaparin sodium in phosphate-buffered saline **PBS**, and all manipulations with cisplatin and enoxaparin sodium were performed under subdued lighting.

Amygdalin is a natural drug extracted from apricot kernels ≥ 99%, Cat# A6005, Sigma- Aldrich, Empirical Formula [$C_{20}H_{27}NO_{11}$], was dissolved in 0.5% dimethyl sulfoxide DMSO.

Methyl Thiazol Tetrazolium [MTT] Assay Protocol

Cell viability and cytotoxic activity of cancer drugs was measured using MTT assay (3-(4,5 dimethylthiazol diphenyltetrazolium bromide) assay which is a sensitive, quantitative and reliable colorimetric method, it's based on the ability of mitochondrial lactate dehydrogenase enzyme (LDH) in living cells to convert into the water-soluble substrate.

Briefly, cells were seeded in 96-well plates and treated with 2 folds serially diluted test materials starting from 100μg. At 37c. After 24 hrs. Cells were administered the treatments diluted with **DMSO**, and incubated for 24 -48 hrs.

To measure cell viability at 24hrs and 48hrs, 50μl MTT solution was added directly to each well, and the plates were incubated for 4 h. Solubilization buffer (50 μl) **DMSO** was then added to each well without removing the medium to dissolve formazan crystals and stop this reaction.The plates were then incubated overnight at 37c.

For the reading, we used microplate reader (scanning multi-well spectrophotometer) ELISA plate reader (BioTek- EL800-USA) was used at a

wavelength of 570nm. The result obtained indicates the optical density, since the darker the color obtained, the greater the MTT metabolism of the cells under study. Consequently, a higher optical density results in less toxicity of the extract tested. The data obtained were analyzed using the Master plex 2010 Fit program.

Viability percentage = (Mean Optical Density of treated cells/ Mean Optical Density of untreated cells) ×100

The data generated were used to plot a dose-response curve that determines the extract concentration capable of killing 50% of the cell population tested, indicating **IC50 (inhibitory concentration).**

Briefly in this research, we work on four groups of HNO97 cells: control group, amygdalin -treated HNO97 group, cisplatin -treated HNO97 group, and amygdalin /cisplatin mix-treated HNO97 group. The cytotoxicity effect of Amygdalin, Cisplatin, and Amygdalin /Cisplatin mix on HNO97 cell line in vitro was evaluated for 24 &48 hrs. incubation.

TABLE (1): Concentrations of Amygdalin-treated cells, Amygdalin / Cisplatin mix– treated cells, and Cisplatin –treated cells after 24 hrs.

conc.μg/ml		conc.μg/ml		conc.μg/ml	
Amygdalin		Cisplatin		Mix	
10000	20.98765	1000	21.7284	5500	24.93827
5000	21.97531	500	25.18519	2750	26.79012
2500	23.33333	250	34.19753	1375	29.38272
1250	25.18519	125	35.06173	687.5	29.75309
625	46.41975	62.5	41.23457	343.75	32.96296
312.5	58.2716	31.25	42.09877	171.875	60.74074
156.25	60.12346	15.625	45.92593	85.9375	64.07407
78.125	79.38272	7.8125	60.37037	42.96875	81.85185
39.0625	80.8642	3.90625	62.09877	21.48438	86.17284
19.53125	92.09877	1.953125	71.97531	10.74219	88.76543
9.765625	92.22222	0.976563	92.09877	5.371094	92.09877
4.882813	95.4321	0.488281	101.4815	2.685547	104.0741

TABLE (2): Concentrations of Amygdalin-treated cells, Amygdalin / Cisplatin mix– treated cells, and Cisplatin –treated cells after 48 hrs.

conc.μg/ml		**conc.μg/ml**		**conc.μg/ml**	
Amygdalin		**Cisplatin**		**Mix**	
10000	19.40476	1000	20.71429	5500	20
5000	18.69048	500	21.54762	2750	20.83333
2500	18.57143	250	22.02381	1375	22.85714
1250	18.45238	125	22.61905	687.5	23.45238
625	19.40476	62.5	22.7381	343.75	34.16667
312.5	71.90476	31.25	25.47619	171.875	55.2381
156.25	84.88095	15.625	27.7381	85.9375	61.66667
78.125	98.92857	7.8125	78.45238	42.96875	68.21429
39.0625	105.9524	3.90625	86.19048	21.48438	73.45238
19.53125	112.0238	1.953125	112.381	10.74219	78.69048
9.765625	116.1905	0.976563	113.4524	5.371094	95.47619
4.882813	120.7143	0.488281	114.2857	2.685547	101.4286

Microscopic examination

Slides Preparation

The same steps of cells maintenance and subculture protocol were repeated but the cells were dispensed in 25 ml total volume to have a larger quantity of cells sufficient for cytological examination. Pelleted cells were re-suspended in PBS and a part (50µL) was dispended on the clean ethanol washed glass slide, air-dried, and fixed using methanol as a preparatory step for cytological examination.

Hematoxylin and Eosin Staining

- The fixed slides were rehydrated in descending concentrations of alcohol (100%, 90%, 75%, then 50%), then washed in distilled water for 5 min.
- The slides were immersed in filtered hematoxylin stain for 3 min then washed with distilled water twice.
- The slides were immersed in filtered eosin stain for 5 seconds then washed with distilled water.
- Dried slides were immersed in xylene, mounted with Canada balsam then coverslips were placed and left to dry.

Photomicrography and Cytological Evaluation

Slides were photomicrographed at the power of x1000 oil. The photomicrographed fields analyzed using image analysis software (Image J, 1.27z, NIH, USA). Nuclear area factor (NAF) was calculated using the formula:

NAF = Circularity × Object area.

Statistical analysis:

Results were expressed as mean ± standard deviation SD. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 22.0 window software using One-way Analysis of Variance (ANOVA) and Bonferroni Post Hoc multiple comparisons test to determine the significance of differences between groups. With $P < 0.05$ deemed as statistically significant.

RESULTS

[MTT] cytotoxicity Assay:

Data obtained showed that the cytotoxicity was time-dependent. The mean viability percentage of treated cells is inversely proportional to drug concentration.

In 24hrs incubation, the mean viability percentage of treated cells decreased as the drug concentrations increased. The half-maximal inhibitory concentration (IC_{50}). Values were in order 402.87 μg/ml, 14 μg/ml, 196.50μg/ml for Amygdalin-treated cells, Cisplatin –treated cells, and Amygdalin / Cisplatin mix–treated cells as shown.

In 48hrs incubation, the mean viability percentage of treated cells decreased as the drug concentrations increased. The half-maximal inhibitory concentration (IC_{50}). The half-maximal inhibitory concentration (IC_{50}) values were in order 381.60 μg/ml, 11,19 μg/ml, 189 μg/ml for Amygdalin-treated cells, Cisplatin –treated cells, and Amygdalin / Cisplatin mix–treated cells as shown in figure (1).

Microscopic examination

Control cells of HNO97 line

Microscopic examination showed that control HNO97 cells were almost rounded with minimum folding in the cellular and nuclear membranes. Cells showed criteria of malignancy such as hyperchromatism, nuclear pleomorphism, poikailokarynosis, and increased mitotic activity with abnormal mitotic figures, increased nuclear-cytoplasmic ratio, anisocytosis, and anisonucleosis.

Only few cells among control cells showed criteria of apoptosis, mainly confined to nuclear shrinkage and peripheral chromatin condensation.

TABLE (3): pre IC50, IC50, and post IC50 concentrations of Amygdalin-treated cells, Cisplatin –treated cells, and Amygdalin / Cisplatin mix– treated cells after 24 &48 hrs.

	MTT					
	24h			48h		
	Pre IC_{50}	IC_{50}	Post IC_{50}	Pre IC_{50}	IC_{50}	Post IC_{50}
Amygdalin	312.5	402.87	625	312.5	381.6	625
Cisplatin	7.813	14	15.625	7.813	11.19	15.625
mix	171.88	196.5	343.75	171.88	189	343.75

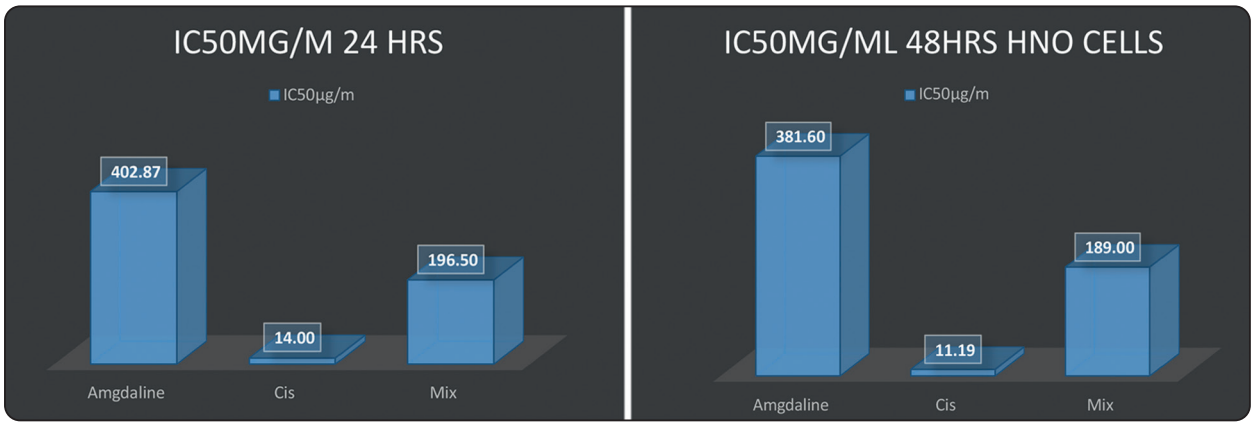

Fig (1): Column chart showing IC50 concentrations of Amygdalin-treated cells, Amygdalin / Cisplatin mix–treated cells, and Cisplatin –treated cells after 24 & 48 hrs.

Treated HNO97 cell line:

HNO97 treated cells showed nuclear morphological modifications that correspond to the morphological parameters of apoptosis. These criteria were obvious in cisplatin-treated HNO97 cells and amygdalin -treated HNO97cells, but they became more apparent in cisplatin/amygdalin mix-treated HNO97 group.

These criteria included peripheral condensation of chromatin against the nuclear membranes, irregularities in the nuclear and the cellular membranes, membrane blebbing, nuclear shrinkage (Pyknosis), cellular shrinkage, and nuclear fragmentation (Karyorrhexis).

In addition to apoptotic criteria, some cells revealed nuclear alterations that resembled the morphological hallmarks of necrosis such as nuclear and cellular swelling, increased eosinophilia of the cytoplasm, and cell membrane rupture leads to loss of membrane integrity with amygdalin-treated HNO97 cells, cisplatin/amygdalin mix-treated HNO97 cells and increased with cisplatin-treated HNO97cells.

In amygdalin-treated HNO97 cells, cisplatin -treated HNO97cells, and cisplatin/amygdalin mix-treated HNO97cells, the presence of secondary necrotic cells with both apoptotic and necrotic characteristics such as nuclear fragmentation and cytoplasmic swelling were detected.

Statistical analysis:

ANOVA and Bonferroni Post Hoc multiple comparison tests revealed significant differences in statistical analysis among mean values of NAF

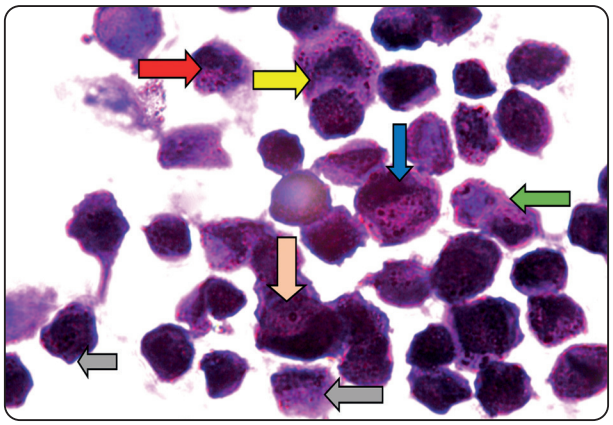

Fig. (2) : A photomicrograph of control HNO97 cell line showing: increased mitotic activity (green arrow), increased nuclear/cytoplasmic ratio (yellow arrows), peripheral chromatin condensation (blue arrows), nuclear fragmentation (grey arrows), anisocytosis & anisonucleosis (pink arrows), and abnormal mitotic figure (red arrows) (H & E 1000x oil).

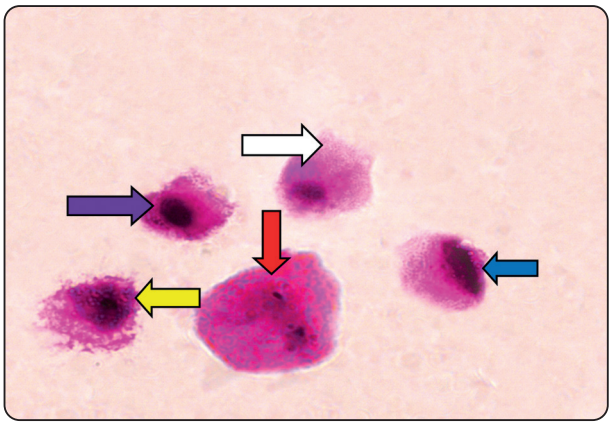

Fig. (3): A photomicrograph of HNO97 cell line 24 hours treated with amygdalin showing: peripheral chromatin condensation (blue arrow), membrane blebbing (yellow arrow), secondary necrotic cell (white arrow), nuclear shrinkage (Pyknosis) (purple arrow), and nuclear fragmentation (red arrows)

(H & E 1000x oil immersion).

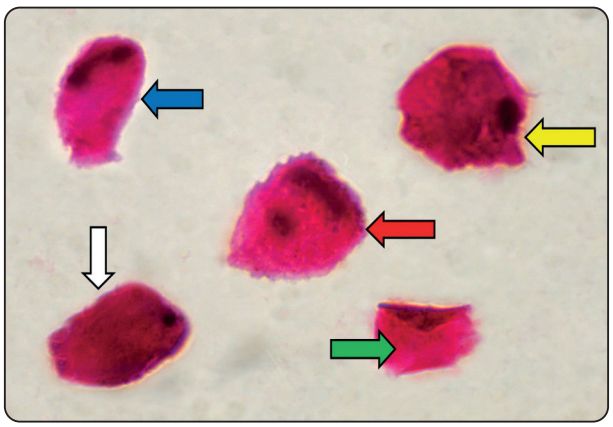

Fig. (4): A photomicrograph of HNO97 cell line 48 hours treated with amygdalin showing: peripheral chromatin condensation (blue arrow), membrane blebbing (yellow arrow), necrotic tissue (green arrow) and nuclear fragmentation (red arrows), secondary necrotic cell (white arrow)

(H & E 1000x oil immersion).

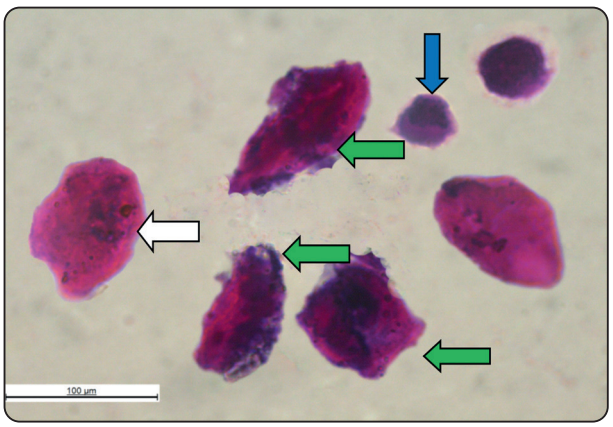

Fig. (5): A photomicrograph of HNO97 cell line 24 hours treated with cisplatin showing: peripheral chromatin condensation (blue arrows), secondary necrotic cell (white arrow) and necrotic cell (green arrow).

(H & E 1000x oil immersion).

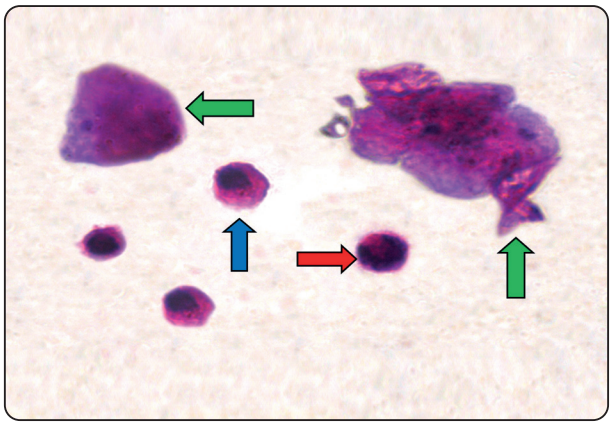

Fig. (6): A photomicrograph of HNO97 cell line 48 hours treated with cisplatin showing: peripheral chromatin condensation (blue arrows), necrotic cell (green arrow) and nuclear fragmentation (red arrows)

(H & E 1000x oil immersion).

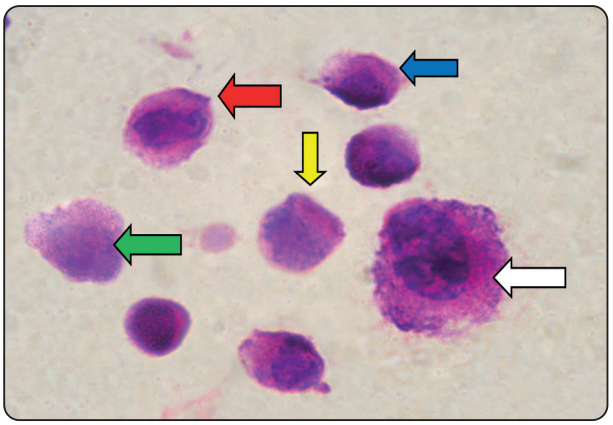

Fig. (7): A photomicrograph of HNO97 cell line 24 hours treated with amygdalin /cisplatin mix. Showing: peripheral chromatin condensation (blue arrows), necrotic cell (green arrow), membrane blebbing (yellow arrow), secondary necrotic cell (white arrow) and nuclear fragmentation (red arrows)

(H & E 1000x oil immersion).

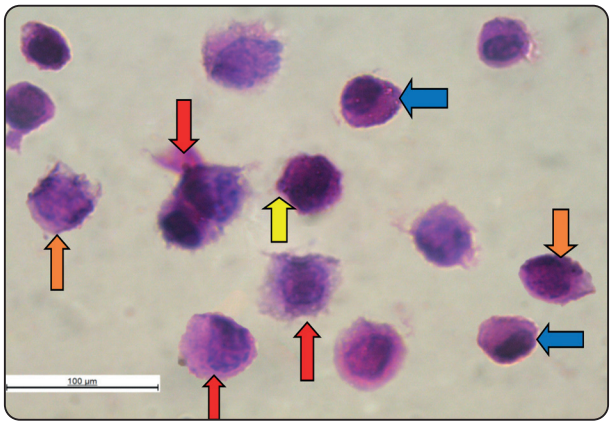

Fig. (8): A photomicrograph of HNO97 cell line 48 hours treated with amygdalin /cisplatin mix. Showing: peripheral chromatin condensation (blue arrows), irregularities in the nuclear and the cellular membranes (orange arrow), membrane blebbing (yellow arrow) and nuclear fragmentation (red arrows)

(H & E 1000x oil immersion).

of 50 cells in each group (control HNO97 cells, Amygdalin-treated cells, Cisplatin –treated cells, and Amygdalin / Cisplatin mix–treated cells after 24 & 48 hrs) and the difference is statistically significant (p value<0.0001) as shown in table (1).

TABLE (3): ANOVA test for the mean values of NAF ± stranded deviation of different groups

Group	N	NAF		P-value
		Mean±SD	**Standard error**	
Control	50	0.170± 0.067	0.006	
Amygdalin 24	50	0.068± 0.088	0.012	
Amygdalin 48	50	0.064± 0.107	0.015	
Cisplatin 24	50	0.095± 0.076	0.011	<0.0001
Cisplatin 48	50	0.051± 0.039	0.005	
Mix 24	50	0.061± 0.062	0.009	
Mix 48	50	0.049± 0.057	0.008	

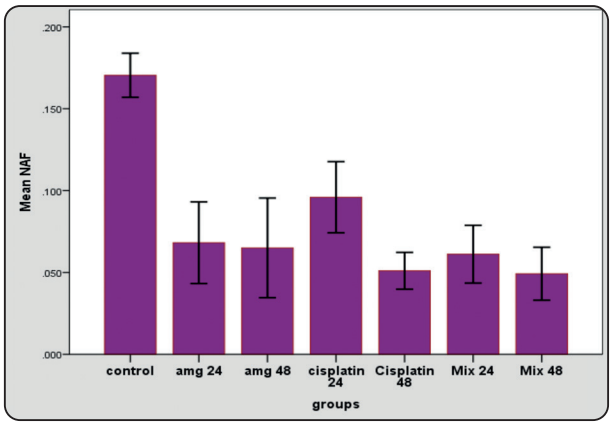

Fig. (9): Bar diagram of mean ± standard error of control and treated HNO97 cells for 24 & 48 hrs. incubation.

DISCUSSION

Cancer is the second leading cause of death worldwide, and exposure to risk factors plays an important role in the biology and burden of many cancer types.[29]

OSCC, which affects the epithelial layer of the oral cavity, is a common malignant tumor of the head and neck with low survival rates and high risks of recurrence.[30] Clinically, OSCC includes lip cancer, which accounts for the majority of OSCC, and intra-oral cancer, which mainly affects the tongue.[31]

In the Middle East, population witnessed a surprisingly accelerated incidence of some cancers, which are often secondary to adverse lifestyle choices and westernization. Despite this, the incidence is far lower that reported figures from Western countries. Several clinical trials are warranted to study the effect of caloric restriction, fasting, and type of food in the Middle East on cancer incidence.[32] Another recent study showed that Egypt had the lowest mortality crude rate of oral cancer 0.4%.[33]

Combination therapy of **cisplatin** with other natural anti-cancer extracts such as **Amygdalin** drugs has been utilized as incoming therapeutic strategies for many human cancers to overcome drug resistance and reduce the toxicity of chemo-therapy drugs.[34]

In our research, the cytotoxicity effect of Amygdalin, Cisplatin, and Amygdalin /Cisplatin mix on HNO97 cell line in vitro was evaluated for 24 & 48 hrs.

Cell viability and cytotoxic activity of cancer drugs at different concentrations were measured using an MTT assay (3-(4, 5 dimethylthiazol diphenyltetrazolium bromide) assay which is a sensitive, quantitative and reliable colorimetric method, it's based on the ability of mitochondrial lactate dehydrogenase enzyme (LDH) in living cells to convert into the water-soluble substrate.[35]

Our obtained data showed that the cytotoxicity was dose-dependent. The mean viability percentage of treated cells is inversely proportional to drug concentration. The (IC50) value of Cisplatin was less than those of Amygdalin and Amygdalin / Cisplatin mix, so Cisplatin had a higher cytotoxic effect on malignant cells with lower concentrations.

Those findings were consistent with **Said A. Barakat & et al. 2021**, study was undertaken to investigate antitumor and antioxidant effects of vitamin B17 beside platinum-based drugs in Ehrlich ascites carcinoma (EAC) -bearing female rats. Results showed that administration of vitamin B17 alone or in combination with cisplatin or oxaliplatin led to a decrease of tumor markers together with enhancement of antioxidant indicators compared with EAC bearing rats.[36]

And agreed with D Sireesha, Basireddy Siva Reddy & et al.2019 who proved that both almond and apricot extracts showed antitumor activity on KB cell line (Oral cancer cell line). The viability decreased in a dose-dependent manner. The cell viability decreased as the concentration of the extracts increased. Almond exhibited maximum efficacy at 50 µg/mL by killing 78% of cells, whereas apricot required a concentration of 100 µg/mL to attain 82% of activity. Based on the results of this study, amygdalin extracted from the seeds of almonds and apricots showed cytotoxic effect on human oral cancer cell lines.[37]

At the microscopic and cellular levels, HNO97 treated cells demonstrated a considerable increase in apoptotic cells after 24 & 48 hrs. of amygdalin , cisplatin, and amygdalin /cisplatin mix therapy. These criteria were obvious in cisplatin-treated HNO97 cells and amygdalin -treated HNO97cells, but they became more apparent in cisplatin/ amygdalin mix-treated HNO97 group. In addition to apoptotic criteria, some cells revealed necrotic and secondary necrotic characteristics. Secondary necrosis is beneficial in cancer therapy because it indicates the death of malignant cells.

Our results agreed with **El-Desouky A. Mohamed, Fahmi A & et al., 2020**, who examined the effect of amygdalin with and without zinc on hepatocellular carcinoma (HepG2) cell line. They proved that amygdalin promotes apoptosis via the intrinsic cell death pathway (the mitochondria-initiated pathway) and cell cycle arrest at G/M. (38)

And agreed with **Gómez-Ruiz, Santiago & et al., 2012, who** summarized mode of action of cisplatin on the tumor cells and found that the net effect of intracellular interaction of cisplatin with DNA and non-DNA targets is the cell cycle arrest and subsequent death in sensitive clones. There are two type of death signals resulting from cellular intoxication by this drug .Fundamentally, the drug concentration presents the critical point for cell decision to undergo apoptotic or necrotic cell death. Primary cultures of proximal tubular cells isolated from mouse died by necrosis if they were exposed to high doses of cisplatin just for a few hours while apoptotic cell death is often triggered by long-term exposure to significantly lower concentrations. (39)

Finally our statistical analysis results revealed significant differences in statistical analysis among mean values of NAF of 50 cells in each group ,with lower mean values of NAF of mix 48 group that proved the synergistic cytotoxic effect of amygdalin and cisplatin on HNO97cells.(P value < 0.0001).

CONCLUSION

Both Amygdalin and cisplatin have anti- cancer effect on HN097 separately, and their combination induced harmonious inhibitory effect on HNO97 cells.

REFERENCES

1. Mignogna MD, Fedele S, Lo Russo. The World Cancer Report and the burden of oral cancer.Eur J Cancer Prev 2004; 13(2):139-42.
2. Rivera C Venegas B. Histological and molecular aspects of oral squamous cell carcinoma (Review). Oncology Letters. 2014; 8(1):7-11.Epub 2014/06/25.
3. Chole RH, Patil RN, Basak A, Palandurkar K, Bhowate R. Estimation of serum malondialdehyde in oral cancer and precancer and its association with healthy individuals, gender, alcohol, and tobacco abuse. J Cancer Res Ther 2010; 6: 487-91.
4. Zygogianni AG, Kyrgias G, Karakitsos P, et al. Oral squamous cell cancer: early detection and the role of alcohol and smoking. Head Neck Oncol 2011; 3: 2.
5. Rayan A, Raiyn J, Falah M. Nature is the best source of anticancer drugs: Indexing natural products for their anticancer bioactivity. PLoSOne.2017; 12(11):e0187925. .
6. Ioannis, P.; Anastasis, S.; Andreas, Y. Tripterygium wilfordii extract (Triptolide) and amygdalin promotes cell death in cancer cells: true or a myth. Am. J. Can. Prevent., 2015, 3(4), 77-83.
7. Holzbecher MD, Moss MA, Ellenberger HA. The cyanide content of laetrile preparations, apricot, peach, and apple seeds. Journal of toxicology Clinical toxicology. 1984; 22(4):341-7. Epub 1984/01/01.
8. Song Z, Xu X. Advanced research on anti-tumor effects of amygdalin. Journal of cancer research and therapeutics. 2014; 10 Suppl 1:3-7. Epub 2014/09/11.
9. Amira Nour DDS, MSc Basel Azar DDS, MSc Anas Rabata DDS, MSc Prof. Ahmad Manadili DDS, MSc, PhD. Department of oral pathology- Damascus university. The effect of Amygdalin in the treatment of squamous cell carcinoma.IOSR Journal of Dental and Medical Sciences (IOSR-JDMS). IX (Feb. 2016), PP 75-79.
10. Ellison et al., 1978; Sim et al., 2000; Fukuda et al., 2003.

11. Shin, M.C., Jang, M.H., Chang, H.K., Kim, Y.J., Kim, E.H., Kim, C.J. Modulation of cyclooxygenase-2 on glycine- and glutamate-induced ion currents in rat periaqueductal gray neurons. Brain Res Bull 2003, 59, 251–256.

12. Chang HK, Shin MS, Yang HY, Lee JW, Kim YS, LeeMH, et al.Amygdalin induces apoptosis through regulation of Bax and Bcl2expressions in human DU145 and LNCaP prostate cancer cells. BiolPharm Bull 2006; 29:1597602.

13. Chen Y, Ma J, Wang F, Hu J, Cui A, Wei C, et al. Amygdalin induces apoptosis in human cervical cancer cell line HeLa cells.Immunopharmacol Immunotoxicol 2013; 35:4351.

14. Yang C, Li X, Rong J. Amygdalin isolated from Semen Persicae (Tao Ren) extracts induces the expression of follistatin in HepG2 and C2C12 cell lines. Chin Med 2014; 9:23.

15. Makarević J, Rutz J, Juengel E, Kaulfuss S, Reiter M, Tsaur I, et al.Amygdalin blocks bladder cancer cell growth in vitro by diminishing cyclin A and Cdk2. PLoS One 2014;9:e105590.

16. 16-Nour A, Azar B, Rabata A, Manadili A. The effect of amygdalin in the treatment of squamous cell carcinoma induced in the buccal pouch of the golden Syrian hamster. IOSR J Dent Med Sci 2016; 15:759.

17. Shaloam Dasari, Paul Bernard Tchounwou. Cisplatin in cancer therapy: molecular mechanisms of action. Eur J Pharmacol. 2014 October 05; 740: 364–378. doi:10.1016/j.ejphar.2014.07.025.

18. Rossi A, Maione P, Gridelli C. Safety profile of platinum-based chemotherapy in the treatment of advanced non-small-cell lung cancer in elderly patients. Expert Opin Drug Saf 2005; 4:1051-67.

19. Galluzzi L, Senovilla L, Vitale I, Michels J, Martins I, Kepp O, et al. Molecular mechanisms of cisplatin resistance. Oncogene 2012; 31:1869-83.

20. Jiang T, Zhou C, Gu J, Liu Y, Zhao L, Li W, et al. Enhanced therapeutic effect of cisplatin on prostate cancer in tumor-bearing mice by transfecting the attenuated Salmonella carrying a plasmid co-expressing p53 gene and mdm2 siRNA. Cancer Lett 2013; 337:133-42.

21. Mantoni TS, Reid G, Garrett MD. Androgen receptor activity is inhibited in response to genotoxic agents in a p53-independent manner. Oncogene 2006; 25:3139-49.

22. Aydın, Davut.The Combination of Amygdalin with Some Anticancer, Antiparasitic, and Antigout Drugs Against MG63, Saos2, SW1353, and FL Cells In Vitro. J Med Food. 2021 Nov; 24(11):1230-1234. doi: 10.1089/jmf.2020.0143. Epub 2021 Mar 17.

23. Nikitakis NG, Sauk JJ, Papanicolaou SI. The role of apoptosis in oral disease: mechanisms; aberrations in neoplastic, autoimmune, infectious, hematologic, and developmental diseases; and therapeutic opportunities. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004; 97:476–490.

24. Schoelch ML, Le QT, Silverman S Jr, et al. Apoptosis-associated proteins and the development of oral squamous cell carcinoma.Oral Oncol 1999;35:77–85.

25. Polverini PJ, No¨r JE. Apoptosis and predisposition to oral cancer. Crit Rev Oral Biol Med 1999; 10:139–152.

26. Pistritto G, Trisciuoglio D, Ceci C, Garufi A, D'Orazi G. Apoptosis as an anticancer mechanism: Function and dysfunction of its modulators and targeted therapeutic strategies. Aging (Albany NY), 2016; 8(4):603.

27. Budihardjo I, Oliver H, Lutter M, Luo X, Wang X (1999) Biochemical pathways of caspase activation during apoptosis. Annu Rev Cell Dev Biol 15: 269-290.

28. Fiandalo MV, Kyprianou N (2012) Caspase control: protagonists of cancer cell apoptosis. Exp Oncol 34: 165-175.

29. Kocarnik JM., Compton K., Dean FE &et al. Cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life years for 29 cancer groups from 2010 to 2019: a systematic analysis for the Global Burden of Disease Study 2019.JAMA Oncol. 2022; 8: 420-444.

30. Adnan, Yumna et al. "High CD44 Immunoexpression Correlates with Poor Overall Survival: Assessing the Role of Cancer Stem Cell Markers in Oral Squamous Cell Carcinoma Patients from the High-Risk Population of Pakistan." International journal of surgical oncology vol. 2022 9990489. 7 Mar. 2022.

31. Scully, Crispian, and Jose Bagan. "Oral cancer." Oral Complications of Cancer and its Management. Oxford, UK: Oxford University Press. , Oxford Medicine Online. Date Accessed 29 Jun. 2022.

32. Arafa, M.A., Farhat, K.H. Why cancer incidence in the Arab counties is much lower than other parts of the world?. J Egypt Natl Canc Inst 34, 41 (2022).

33. Sung WW, Hsu YC, Dong C, Chen YC, Chao YC, Chen CJ. Favorable Lip and Oral Cancer Mortality-to-Incidence Ratios in Countries with High Human Development Index

and Expenditures on Health. Int J Environ Res Public Health. 2021; 18(11):6012. Published 2021.

34. Davut Aydın, Korhan Özkan, and Ali Aydın. The Combination of Amygdalin with Some Anticancer, Antiparasitic, and Antigout Drugs Against MG63, Saos2, SW1353, and FL Cells In Vitro .Journal of Medicinal Food.Nov 2021.1230-1234 .

35. Ghasemi, M.; Turnbull, T.; Sebastian, S.; Kempson, I. The MTT Assay: Utility, Limitations, Pitfalls, and Interpretation in Bulk and Single-Cell Analysis. Int. J. Mol. Sci. 2021, 22, 12827

36. Ashraf Barakat Said; Saleh Yousef; Ibrahim Ibrahim; Yasmina Mahmoud; Marwa El-Beltagy. "Biochemical Evaluation of Antitumor Activity of Vitamin B17 Alone or in Combination with Platinum Based Drugs Against Ehrlich Ascites Carcinoma in Female Rats". Suez Canal Veterinary Medical Journal. SCVMJ, 26, 1, 2021, 189-218. doi: 10.21608/scvmj.2021.184983.

37. Sireesha, D et al. "Effect of amygdalin on oral cancer cell line: An in vitro study." Journal of oral and maxillofacial pathology: JOMFP vol. 23, 1 (2019): 104-107.

38. El-Desouky A. Mohamed, Fahmi A. Abdelgawad, Abdelkader Y. Ibrahim and Nasraldin M. Karima, Anticancer Effect of Amygdalin (Vitamin B-17) on Hepatocellular Carcinoma Cell Line (HepG2) in the Presence and Absence of Zinc, Anti-Cancer Agents in Medicinal Chemistry 2020; 20(4) .

39. Gómez-Ruiz, Santiago & Maksimovic-Ivanic, Danijela & Mijatovic, Sanja & Kaluđerović, Goran. (2012). On the Discovery, Biological Effects, and Use of Cisplatin and Metallocenes in Anticancer Chemotherapy. Bioinorganic chemistry and applications. 2012. 140284. 10.1155/2012/140284.