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Preventive Role of Amygdalin in Squamous Cell Carcinoma Induced in Hamsters

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Abstract:

Background and Aim of the study:

Oral squamous cell carcinoma (OSCC) represents 95% of all forms of head and neck cancer, In recent years the development of antitumor drugs has been gradually transformed from cytotoxic drugs to develop new targeted drugs with low toxicity and high specificity. Amygdalin is extracted from bitter apricot kernels and has been used for the treatment of many cancers. Amygdalin is considered as a natural product that owns antitumor activity, less side effects and it is widely sourced. We investigate in this study the preventive role of amygdalin in squamous cell carcinoma induced in the buccal pouch of hamsters, by detecting its correlation to the the main proteins in cell cycle and apoptosis(P53 and BCL2, respectively).

Materials and Methods:

30 hamsters were incubated and have been treated by amygdalin orally, interchangeably with the carcinogenic agent (DMBA) for 3.5 months. Then hamsters have been sacrificed and we prepared the buccal pouch for traditional and immunohistochemical stains, using P53 and BCL2.

Results:

There was a delayed in initiation of the carcinoma. Only a variant of dysplastic changes were observed during the 3.5 months. In addition, a decrease in the expression of the markers of cell cycle and apoptosis (P53 and BCL2) in the samples treated by amygdalin was detected.

Conclusion:

Amygdalin has a preventive role as to the oral squamous cell carcinoma induced in hamsters. It has this role by controlling the main proteins associated with the cell cycle and apoptosis (P53 and BCL2, respectively)

Keywords: Amygdalin, DMBA, Squamous cell carcinoma, P53, BCL2, Hamsters.



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Introduction:

Malignant tumors are the major disease that cause serious damage to human health, and have been listed as a seriously threatened human health by World Health Organization . (1).

So it is a challenge to treat this carcinoma and other types of cancers as well, in such a traditional management or a complementary and alternative approaches that are spreading wildly.(2) (3) (4) (5).

Laetril (amygdalin) is also an important example of alternative therapy for cancer and it is related to cyanogenic glycosides received from kernels of various fruits (almonds, apricots, peaches, ...etc). It is a natural product that owns antitumor activity and less side effects. It is a promising antitumor drugs, if combined with conditional surgical therapy and chemotherapy drugs (6, 7). Mounting evidence has supported the anti-cancer effects of amygdalin and its selective killing effect on cancerous cells (8) (9) (10) (11).

Amygdalin can be used for the treatment of cancer and relieving pain as well(8, 12, 13) (14) (15).

Materials and methods:

Study design:

This study composed of 30 Golden Syrian Hamster. We induced S.C.C in the buccal pouch three days by week interchangeable with amygdalin orally (200 mg). This study lasted for 3.5 months, the time needed for inducing squamous cell carcinoma in the buccal pouch. All of the hamsters were sacrificed after 3.5 months. We prepared the traditional and the immunohistochemical stains for the histopathologic study.

The experimental study was achieved at the animal incubators at faculty of pharmacy, Damascus university. While preparation of specimens and the staining techniques were done at the oral pathology laboratory, faculty of dentistry, Damascus university.

Sample size calculation:

A minimum sample size of 30 hamsters was proposed to the current study requirements of demonstrating a 2.5 fold. This calculation set the power of the test at 80% and the level of significant at 5%.

Materials:

Amygdalin: amygdalin from Terezia company was used orally at concentration reached 200 mg/kg.

Carcinogenic agent: we used DMBA, a polycyclic aromatic hydrocarbon carcinogen, we applied it in the buccal pouch mucosa of hamsrers using a painting brush for three months and a half.



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Staining: We used the traditional stains (hematoxylin and eosin). In addition to the immunohistochemical stains; P53 and BCL2, from the American company Bio-SB .

Methods:

DMBA was applied at the left buccal pouch of the hamster using a painting brush for three months and a half. It is the proposed period of time to induce squamous cell carcinoma within this technique.

Amygdalin was prepared to be used orally at the dose of 200 mg/kg.

Hamsters were sacrificed all together after 3.5 months.

Specimens were prepared for the traditional and the immunohistochemistry stains.

The classification of the epithelium injury was divided into 7 categories, normal epithelium (value 0), hyperplasia (value 1), hyperplasia and dysplasia (value 2), mild dysplasia (value 3), moderate dysplasia (value 4) and sever dysplasia (value 5)

Statistics:

We used T-student test for two separated groups, to compare the expression of P53 and BCL2 between the different periods of time of using the drug, and chi- square test to compare the difference between the frequencies of positivity expression of the immunohistochemistry stains and between the frequencies of normal lung specimens and inflamed ones.

Results:

Some samples of epithelium of the buccal pouch revealed a hyperplastic and dysplastic changes (24 cases), while it revealed normality in other samples (6 cases).

Impact of amygdalin on the frequencies of changes within the epithelium:

We used chi-square test to study the significance of differences between the frequencies of normal epithelium and dysplastic one (table 1).

As it has been shown in table 1, there were significance changes between the frequencies of normal epithelium and dysplastic one ($P= 0.005$).

Results of the expression of the immunohistochemistry stains: (table 2)

Impact of amygdalin in frequencies of the immunohistochemistry stains:

We used chi-square test to study the significance of the differences between the frequencies of the expression of the immunohistochemistry stains: (table 3)



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No significance between the frequencies of negative samples and positive samples for BCL2 ($P=0.796$), nor for P53 ($P=0.439$).

We used T student test to study the significance of differences between (o) and values of expression of BCL2 and P53. (table 4)

This table shows the significance differences that were demonstrated between the standard value (o) and the mean values of the positive expressions regarding the two investigated proteins (BCL2 and P53) at the confidence level of 95 percent (P value < 0.05). The positive sign of the difference indicates that the values of the proteins expression were greater than the standard value (o) in the amygdalin treated group.

Discussion:

In this study we detected the role of amygdalin extracted from apricot kernel as a preventive drug of squamous cell carcinoma induced in the buccal pouch of the Hamsters. Amygdalin consists of two molecules of glucose units, one unit of benzaldehyde and hydrocyanic acid (HCN). Benzaldehyde and hydrocyanic acid both has anti- neoplastic properties. Amygdalin has been used to treat various type of cancers.(9-11, 16-20)

In this study, the amygdalin that was used at dose 200 mg/kg orally for 3.5 months significantly suppressed the initiation of squamous cell carcinoma development in hamsters.(15, 21, 22).

While reviewing the literature reviews, we found no previous studies about the preventive role of amygdalin in squamous cell carcinoma or another cancers.

The new researches have confirmed the role of this drug in treatment of different types of human cancers. Its role was related to its down regulated effect of proteins such as BCL2 (an apoptic protein) and up expression of BAX (an anti apoptic protein). (6, 9, 11, 18, 20, 22-25).

Conclusion:

In concern of malignant tumor and its management, researches today are going for targeted therapy rather than the traditional therapies. More researches and studies should take into consideration this view of point. Furthermore, it is more important to have the ability to prevent the incidence of this initiates among individuals who have tendency to develop such malignancies. To be concluded, Amygdalin merits further evaluation for its preventive role.

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Table 1 : Results of qui-square test showing differences between the frequencies of normal and dyplastic epithelium

	Chi-square value	Degree of freedom	P value	Significance
Changes within epithelium	8.067	1	0.005	Sig.

Table 2: Results of the expression of the BCL2 and P53

IHC	Number of hamsters			Percentage		
	Negative	Positive	Total	Negative	Positive	Total
BCL2	16	14	30	53.3	46.7	100
P53	18	12	30	60.0	40.0	100

Table 3: Results of chi-square test to study the significance of differences between the frequencies of the expression of BCL2 and P53

IHC	Chi-square	Degree of freedom	P value	Significance
BCL2	0.067	1	0.796	No sig
P53	0.600	1	0.439	No sig



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Table 4: The significance of differences between (o) and the values of expression of BCL2 and P53.

	IHC	T value	Degree of freedom	Differences between the standard value (o) and mean value	P value	Significance
Value of the positivity of IHC	BCL2	3.371	14	11.33	0.005	Sig
	P53	2.921	14	9.33	0.011	Sig