ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 9, Issue, 7(E), pp. 28019-28022, July, 2018

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

EFFICACY OF AMYGDALIN ON ORAL CANCER CELL LINES-AN INVITRO STUDY

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DOI: http://dx.doi.org/10.24327/ijrsr.2018.0907.2377

ARTICLE INFO

Article History:

Received 4th April, 2018
Received in revised form 25th May, 2018
Accepted 23rd June, 2018
Published online 28th July, 2018

Key Words:

Amygdalin, Oral Cancer, Anticancer

ABSTRACT

Introduction: Malignant tumors are the major cause of concern to human health, and have been listed as an important factor which have threatened human health by World Health Organization (WHO). In recent years the development of antitumor drugs has gradually moved from cytotoxic drugs to improving the selectivity of drugs along with overcoming multidrug resistance, development of new targeted drugs with low toxicity and high specificity drugs. Many natural occurring products have been tried in the treatment of cancer. Amygdalin is one such product.

Vitamin B-17, commonly known as "Amygdalin", or "Laetrile", is a natural substance that can be found in a variety of species in the Vegetable kingdom. The greatest concentration is found in the seeds of the rosaceous fruits, such as apricot kernels and other bitter nuts. Amygdalin is a cyanogenic compound and belongs to the aromatic cyanogenic glycoside group. Moreover it has numerous pharmacological effects on the various system of the body.

It has anticancer function by decomposing carcinogenic substances in the body, killing cancer cells, blocking nutrient source of tumor cells, inhibiting cancer cell growth. This study is designed to investigate the effect of Vitamin B17 (amygdalin) on oral squamous cell carcinoma cell lines.

Materials and methods: Apricot oil obtained from dried apricot seeds are a rich source of amygdalin. The apricot oil was freshly dissolved in cell culture medium (1–10 mg/ml) and applied to tumor cells in culture flasks for 24 h. In all experiments, treated tumor cell cultures were compared to non-treated cultures. Oral Squamous cell line (KB mouth cell line) were obtained and cytotoxicity of the cells was observed based on the morphological changes.

Results: Apricot oil caused a significant decrease in cell viability of the cell line.

Conclusion: Based on the literature review and the present study, Amygdalin, present in the apricot seed oil had a noteworthy antineoplastic activity on the oral cancer cells and may be further used for animal experiments and later subjected to clinical trials.

INTRODUCTION

Oral cancer represents a common entity comprising a third of all head and neck malignant tumors of which 90% are squamous cell carcinomas approximately.[1] Despite advances in the field of surgery, radiotherapy, and chemotherapy, the survival of patients with oral squamous cell carcinoma has not significantly improved over the past several decades. Treatment options for recurrent or refractory oral cancers are limited.[2] Alternative therapy for oral cancer is currently under investigation in clinical trials with the goal to introduce new material without toxicity to the normal tissues. Amygdalin is one such product.[2]

Amygdalin (D-mandelonitrile-fl-D-glucosido-6-fl-Dglucoside) is a cyanogenic glucoside.This is found in a variety of plant species, mainly in the seeds of apricots and bitter almonds.[3] Amygdalin is derived from the fruit kernels of the Rosaceae family, which includes *Prunus persica* (peach), *Prunus armeniaca* (apricot) and *Prunus amygdalus* var. amara (bitter almond). Amygdalin, in the United States, has been administered to cancer patients since the 1920s. In the 1950s, an intravenous, chemically different form of amygdalin was synthesized and patented as laetrile. Although laetrile differs from amygdalin, the terms are often used interchangeably, making data interpretation difficult.[4]

Amygdalin was isolated by two French chemists, Robiquet and Boutron, in 1837, and was named 'emulsin' by Liebig and Wöhler.[5] Amygdalin hydrolyzes and generates prunasin and mandelonitrile under the glucosidase action, such as amygdalase and prunase, and finally decomposed into benzaldehyde and hydrocyanic acid (HCN). Amygdalin itself is non-toxic, but its product HCN, decomposed by some enzymes

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can act as a poisonous substance. Numerous studies have been documented that amygdalin has antitussive and antiasthmatic effects, along with the effects on the digestive system. Moreover, the pharmacological effects also include antiatherogenic, inhibition of renal interstitial fibrosis, prevention of pulmonary fibrosis, resistance to hyperoxia induced lung injury, immune suppression, immune regulation, antitumor, antiinflammatory and antiulcer.[3]

Amygdalin can be decomposed to hydrocyanic acid, which is an antitumor compound, and benzaldehyde, which can induce an analgesic action. Hence, it can be used for the treatment of cancer and relief of pain. Therefore the anti-tumor effect of amygdalin can be considered for research purpose. It has anticancer function by decomposing carcinogenic substances in the body, killing cancer cells, blocking nutrient source of tumor cells, inhibiting cancer cell growth, and can also reduce the incidence of prostate cancer, lung cancer, colon cancer and rectal cancer.[3]

Although amygdalin is used by many cancer patients as an antitumor agent, there is a lack of information on the efficacy and toxicity of this natural compound. In the present study, the inhibitory effect of amygdalin on the growth of oral squamous cell carcinoma (OSCC) cell lines was examined based on the morphological changes.

MATERIALS AND METHOD

Oral Squamous cell carcinoma cell lines, (KB Mouth) were cultured in Minimum Essential Medium (MEM) supplemented with 1% (v/v) Penicillin-Streptomycin and 10% (v/v) Fetal Bovine Serum (FBS) obtained from Sigma-Aldrich. The cells were maintained at 37°C in a 5% CO2 incubator. Cells at exponential stage were used for experimentation and medium was changed every 2-3 days. The cytotoxic activity was measured using MTT (3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay $1x10^5$ cells/ml were seeded in 96-well microplates and incubated at 37^oC in a CO_2 incubator for 24 hrs. The plate was observed under inverted microscope after 24hrs. Cells were treated with various concentrations (2–250 µg/mL) of organic compressed apricot oil for 48 hours. At the end of incubation period, the medium was removed. 100 µl fresh MEM medium along with 10µlof 5mg/ml MTT reagent were added in each well followed by an incubation period for a further 4 hours at 37°C. Later, 200 µl of acidic isopropanol was added to each well for solubilisation of the formazan products. Absorbance was taken at 492 nm using a 96 well Plate reader.

The percent cell cytoxicity was calculated by means of the formula:

$$\% \text{ Cytotoxicity} = \frac{\text{O.D of Control sample- O.D of Test sample}}{\text{O.D of Control Sample}}$$

After 48 hours, cells were visualized to assess the changes in cell morphology and photographed under a inverted microscope. The results of cell lines are shown in Figure 1, 2, 3 and 4.

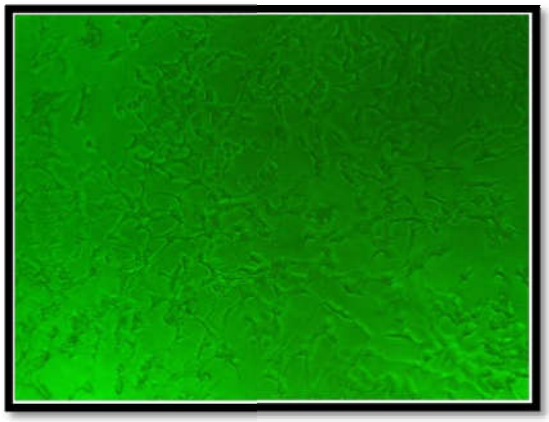

Fig 1 Oral Squamous Cell Cancer Cell Line (Control)

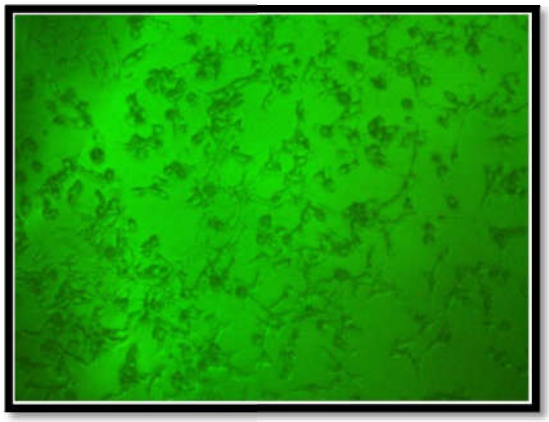

Fig 2 OSCC cells were inhibited after incubating with apricot oil (5 µg/ml)

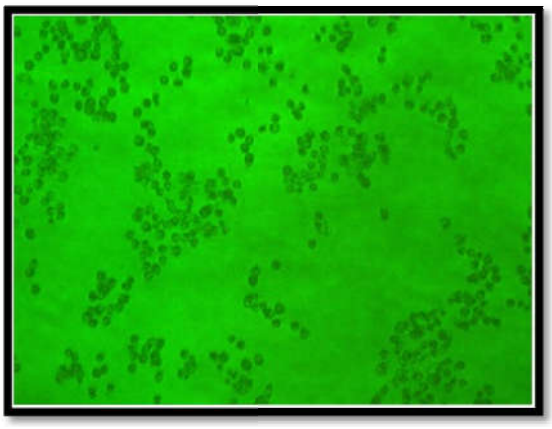

Fig 3 OSCC cells were inhibited after incubating with apricot oil (100 µg/ml)

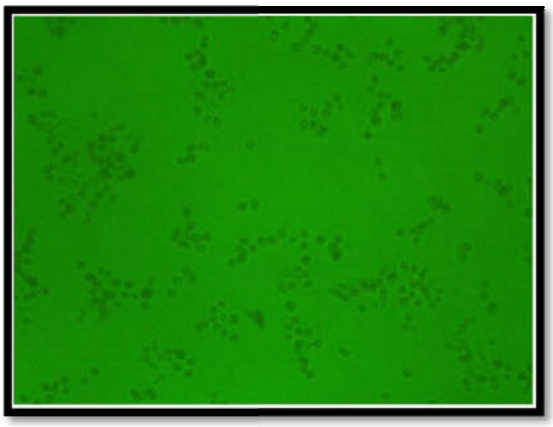

Fig 4 OSCC cells were inhibited after µg/ml) incubating with apricot oil (25 µg/ml)

RESULTS

The viability of the HeLa cells treated with amygdalin decreased in a dose-dependent manner. The cell viability decreased as the concentration of apricot oil increased in a ascending manner. This signifies the cytotoxic effect of apricot oil on cancer cell lines. Standard deviation was calculated at different concentrations and are represented in Table 1.

Table 1 MTT Cell Viability Data of Apricot Oil

	Optical Density (O.D)	%Viability	Standard Deviation
Control	0.107	100%	0.008
2ul	0.106	99.06%	0.013
5ul	0.067	61.68%	0.015
25ul	0.020	17.76%	0.004
50ul	0.016	14.95%	0.007
100ul	0.011	10.28%	0.002

DISCUSSION

Oral cancers constitute about one-third of head and neck malignant tumors and face diverse challenges for optimal management to minimize the morbidity and increase the chance for cure. The great majority being squamous cell carcinomas. During the past 3 decades, there has been an outburst of knowledge of different aspects of oral cancer. But still, the 5-year overall survival of this disease has not shown much improvement, though the quality of life of patients has undoubtedly improved.[6]

Scientific studies have recently shifted their focus from conventional therapy to alternative and complementary medicine as a wide spread method in treating oral cancer.[7] Dissatisfaction with conventional therapy and the desire to reduce side-effects have led many patients to accept complementary and alternative medicine.[8]

Many Natural compounds have been in trail over a period of years for the treatment of cancer due to minimum or no side effects. But the information about the efficacy of natural compounds is sparse, and few of these compounds, such as the cyanogenic diglycoside amygdalin (D-mandelonitrile-β-gentiobioside), remain controversial. Amygdalin and other cyanogenic sugar, are also considered to be a potential alternative antitumor drug.

Amygdalin is derived from the fruit kernels of the Rosaceae family, which includes *Prunus persica* (peach), *Prunus armeniaca* (apricot) and *Prunus amygdalus* var. amara (bitter almond). As early as in 1803, Schrader found this substance in the study of ingredients of bitter almond ingredients. In the present study we have used the oil of apricot seeds which is considered to be a rich source of amygdalin. Around 1830, Robiquet separated amygdalin from the bitter almond, which has always been used as auxiliary medicine of cough expectorant agent and cancer therapy. [9]. Amygdalin, mainly in the United States, has been administered to cancer patients since the 1920s. In the 1950s, an intravenous, chemically different form of amygdalin was synthesized and patented as laetrile.[8] Although laetrile and amygdalin can both represent amygdalin, they are different substances. Natural amygdalin exists as a right-handed structure (R-amygdalin), which is the active form. Laetrile is the acronym of laevorotatory and mandelonitrile.

There has been a lot of work carried out in the analysis of amygdalin and a large number of studies have shown that amygdalin plays a supporting role in the treatment of cancer, diabetes, atherosclerosis, immune suppression, leprosy and other diseases.[3] The earlier study on amygdalin dates from late1970's. A static and regressive effect was observed by Ardenne and Reitnauer on carcinosarcoma bearing rats.[10]The authors observed a reduction in PH and toxic process indicating β –glucosidase driven amygdalin metabolization and cyanide synthesis.But similar results were not observed by others,though the life span was found to be increased in animal groups treated with amygdalin.[11]

In an investigation done by Stock *et al*, animals treated with amygdalin were found to be in better health than untreated animals and an antitumour effect of amygdalin was observed.[12] Application of amygdalin (1000mg/kg/day) in a mouse strain reduced tumour development from 82% to 72% and lung metatstasis from 81%to 17%. But the subsequent trials did not show positive results. Although direct antitumour effects of amygadlain were not assessed , many studies have described the cyanide effect on the tumour cells. Both cyanate and thiocyanate exerted inhibitory effects on transplanted hepatomas and colon cancer.[3] The antitumour effect of amygdalin has also been reported by studies done on cell lines.The present study also showed a cell viability of only 10% at a concentration of 250µl/ml. This is supported by various experiment results (Table 2). In the present study we have shown the cytotoxic efficacy of Amygdalin in the form of apricot oil on cancer cell lines and non toxic effect on Normal cell lines.

Table 2n Review of Effect of Amygdalin in various cancers

S.No	Author	Effect of Amygdalin
1.	Kwon *et al* [13]	Induced apoptosis in human promyelocytic leukemia (HL-60) cells;
2.	Park *et al* [14]	Amygdalin inhibited the proliferation of human colon cancer SNU-C4 cell, and the mechanism is the inhibition of expression of cell cycle related genes
3.	Chang *et al* [15]	Induced apoptosis in prostate cancer DU145 and LNCaP cells by regulating the expression of Bax and of Bcl-2
4.	Chen Y*et al*. [16]	Inhibit the survival rate of HeLa cells, in a concentration dependent manner. Induced apoptosis of HeLa cells mediated by endogenous mitochondrial pathway. JNK/c-Jun pathway is involved in the process of amygdalin induced apoptosis in HeLa cells.
5.	Makarevic J *et al* [17]	Amygdalin dose-dependently reduced growth and proliferation in all three bladder cancer cell lines which, reflected in a significant delay in cell cycle progression and G0/G1 arrest.
6.	Mirmiranpour *et al* [18]	Showed Antiangiogenic effect of amygdalin

Amygdalin is usually used alongside conventional cancer treatments or in combination with other unconventional methods such as metabolic therapy, urine therapy, diet therapy, fruit seeds intake (apricot, bitter almond, peach), oral supplements, and injections of betaglucuronidase . An advanced new research on anti-tumor effect of amygdalin

showed that amygdalin is a natural product that owns antitumor activity, less side effects, widely sourced and relatively low priced. All these features make the amygdalin a promising antitumor drug, if combined with conditional chemotherapy drugs, which can produce synergistic effect, providing new insights for the development of new anticancer drug.[7] Nevertheless, the antitumor mechanism of amygdalin is not completely clear. Clinical trials and large retrospective studies have shown that bitter almond had unstable antitumor effect. Most important aspect is association of some adverse reactions after large dose application, such as gastrointestinal tract reaction and headache. But in view of the limited quantity and quality of clinical data, so far clinical studies have no paired and reliable design, so it is liable to conduct more carefully designed controlled clinical trials for bitter almond, and prove its effect *in vivo*.

CONCLUSION

Based on the results of our study and Literature review, Amygdalin showed cytotoxic effect on cancer cells. But Controlled clinical trials are required to further substantiate the anticancer property of amygdalin on oral cancer.

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How to cite this article:

Bindiya Narang *et al.* 2018, Efficacy of Amygdalin on Oral Cancer Cell Lines-an Invitro Study. *Int J Recent Sci Res.* 9(7), pp. 28019-28022. DOI: http://dx.doi.org/10.24327/ijrsr.2018.0907.2377

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