Anticancer Effects of Triterpenoid Extracted from Apple Peel on Human B cell Lymphoma

Mehrdad Hashemi¹, and Mahmoud Yousefzadeh²

Abstract—Apple is a rich source of nutrition as well as other components such as fiber, minerals and vitamins. Apple peel contains considerable amount of lipophilic triterpenoids that are concentrated in cuticular wax layer and have potential antioxidant activity. Ursolic acid and Olea-nolic acid are the most predominant triterpenoids apple peels.

The purpose of this study is to determine anticancer activity of Olea-nolic acid on human B cell Lymphoma. In this study and human leukemia pre-B-cells (Nalm-6) were cultured in RPMI 1640[Sigma], supplemented with 10% fetal bovine serum (FBS), penecilin-streptomycin and L-glutamine. The cultures were incubated at 37°C, 5% CO2 and then inhibitory effect of Olea-nolic acid on their proliferation was measured by MTT assay.

MTT assay showed that Olea-nolic acid inhibited proliferation of Nalm-6 cell line in dose-dependent manner significantly (P<0.01). The IC50 values for Olea-nolic acid was 60μmol/L. This study demonstrates the anticancer effect of Olea-nolic acid therefore, we can say that apple peel has a powerful anticancer activity.

Keywords— Apple peel, Anticancer, Olea-nolic acid, MTT, Nalm-6 cell line

I. INTRODUCTION

In recent years, the morbidity and mortality of cancer still reaches a high plateau and is a major public health problem worldwide [1,2]. Cancer is the major cause of human’s death because of high incidence and mortality. The identification of new cytotoxic drug with low side effects on immune system has developed as important area in new studies of immunopharmacology [3].

Many studies report that a high diet in fruits and vegetables lowers the incidence of cancer [4,5]. Some of fruits and vegetables are considered as the main anticancer foods, because of their abundant antioxidants such as phenols, vitamin C, vitamin E, beta-carotene and lycopene [6]. It has been reported that various fruit and vegetable extracts are capable of inhibiting the proteasome activity and this inhibition is associated with tumor cell apoptosis [7].

Table: Material and Methods

A. Drugs and reagents

Olea-nolic acid was gifts from Iran medical sciences university and was extracted from apple peel with purity 98%. The drugs were dissolved in 100% ethanol and then diluted 10 times with RPMI-1640 as the working solution, the final concentration of ethanol being less than 2%. This material was purchased from the Sigma Company too. (USA).

B. Cell culture

Nalm-6 cells were cultured in RPMI 1640 with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in 5% CO2 incubator. Upon reaching appropriate confluence, the cells were passaged and incubated with IC50 with 0.5, 10, 50, 75 and 100 μmol/L. Concentrations of triterpenoid extracted from apple peel for a defined time. The cells were treated with ethanol at the same dose of that used in the maximum dose (100 μmol/L) group too.

C. MTT staining

In this technique, color effect of MTT (dimethylthiazol diphenyl tetrazolium bromide) on cells has been used in which alive cells, contained purple crystals as a result of color reduction by mitochondrial dehydrogenase of alive cells, would be countered and alive cells percentage would be determined by the following formula:

\[
\text{Viability} = \left( \frac{\text{alive cells number}}{\text{whole cells cultured}} \right) \times 100
\]

After 18 hours in order to full adherence of cells to the plate, different concentrations of the ethanolic extract have been added to cells and plates were incubated for 48 hours at 37°C and 5% CO2.

MTT staining is on the basis of MTT reduction into an insoluble blue-purple product (Formazan) by mitochondrial reductase in alive cells. Nalm-6 cells were seeded into 96-well plate (5-7×10³ cells per well). After 24 h incubation ethanolic extract in different concentrations were added to each well and incubated for 48 hours, followed by incubation with 5
mg/ml MTT for 4h. The supernatant was removed after centrifugation, finally 100 μL of DMSO was added to each well. 48 wells are used for the MTT assay. The absorbance of cells was measured at 570 nm with Eliza reader. Toxicity level was calculated by the following formula:

\[
\text{Cytotoxicity} \% = 1 - \frac{\text{Mean absorbance of toxicant}}{\text{Mean absorbance of negative control}} \times 100
\]

\[
\text{Viability} \% = 100 - \text{Cytotoxicity} \%
\]

To diminish test error level, MTT strain was added to some wells without cells and along with other wells, absorbance level was read and ultimately subtracted from whole the absorbance. The data were analyzed with Tukey Test measured by one way ANOVA.

**D. IC\(_{50}\) determination**

The 50% inhibition concentration (IC\(_{50}\)) values of extract on Nalm6 cells at 48h were determined. IC\(_{50}\) was determined by probit analysis using the Pharm PCS (Pharmacologic Calculation System) statistical package (Springer-Verlag, USA).

### III. Results

The results of MTT test on cancerous cells under various concentrations of triterpenoid extracted from apple peel has been shown in figure 1. There was a significant difference between extract effect on growth depression of cancerous cells \((P = .01)\). The IC\(_{50}\) after 48h was 60.1±0.45 μmol/L calculated \((P = .01)\).

![Fig1: Results of MTT test on cancerous cells under various concentrations of OA(Treatment) and ethanol (Control)](image)

**IV. Discussion**

Since usual methods on cancer treatment (surgery, chemical treatment, radiotherapy) have an effect on natural dividing cells, in addition to tumor cell, and kill or arrest their cell division[9]. In recent years, herbs found widespread use in prevention and treatment of cancer which in this procedure, tumor cells are controlled while natural cells remain intact[10]. The effect of diverse antioxidant foods on cancer and cardiovascular disease has been proved and it has been revealed that these materials cause to enhance long life by 60% [11]. During laboratory researches on poly metoxilated flavonoides including tungertin, it has been revealed that these materials have antioxidant and anticancer effects and preservative effect on neurons[12].

Oxidative stress by free radicals is an important event in the cell that can cause aging and human degenerative diseases including cancer, heart diseases, multiple sclerosis, Parkinson’s disease, autoimmune disease and senile dementia. Stresses, physical damage, viral infection, cytotoxic or carcinogenic compounds as a consequence of chemical or biological aggression may cause peroxidation of polyunsaturated fatty acids of cell membranes and liberation of toxic substances such as free radicals. Studies concerning the relationship between the morbidity due to cancer and heart diseases and the consumption of fruits and vegetables indicated that polyphenols present in large amount in fruits and vegetables have a significant impact on the morbidity decrease from these diseases [13-15]. Recently, attention has been focused on antioxidant products of natural sources isolated of plant products. Polyphenolic compounds are found mainly in fruits and vegetables as secondary plant metabolites. Many polyphenols such as kaempferol, quercetin, luteolin, myricetin and catechin express strong antioxidative, antiinflamatory, antiallergic and antineoplasic properties [16]. The high antioxidative activity of plant phenolic compounds attractive to the food industry, prompting their use as replacements for synthetic antioxidants and also as nutraceuticals, playing a role in preventing many diseases. Reactive oxygen species such as hydroxyl, superoxide and peroxyl radicals are formed in human tissue cells result in extensive oxidative damage that leads to age-related degenerative conditions, cancer and wide range of other human diseases [15-17]. Antioxidants from natural sources increase the shelf-life of foods[18]. Therefore, consumption of antioxidant and addition of antioxidant in food materials protect the body as well as foods against these events. Antioxidative properties of the essential oils and various extracts from many plants are of great interest in both academia and the food industry, since their possible use as natural additives emerged from a growing tendency to replace synthetic antioxidants by natural ones.

OA has antifungal, insecticidal, anti-HIV, diuretic, complement inhibitory, blood sugar depression and gastrointestinal transit modulating activities. OA also possess liver-protection and anti-inflamatory effects. In recent years, it was found that they had marked anti-tumor effects and exhibited cytotoxic activity toward many cancer cell line in culture[19].

**V. Conclusion**

This study demonstrates the anticancer effect of OA therefore, we can say that apple peel has a powerful anticancer activity.

**REFERENCES**


