

## Inhibition of oral cancer cell line KB by hydroxytyrosol through induction of apoptosis

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### ABSTRACT

**Introduction:** Cancer is a disorder with a high mortality rate that leads to many psychological and economic conflicts. Herbal compounds that induce apoptosis are one of the methods for the treatment of cancer. The aim of the present study was to evaluate the anticancer effects of hydroxytyrosol on oral cancer cell line KB and the regulation of *BAX* and *BCL2* genes expression.

**Materials and Methods:** Anti-proliferation effects of hydroxytyrosol against oral cancer cell line KB were investigated by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay. Moreover, mRNA expression of *BAX* and *BCL2* genes were investigated by quantitative Real-Time PCR method.

**Results:** We observed a significant decrease in proliferation of the oral cancer cell line treated with hydroxytyrosol. In addition, expression of *BAX* and *BCL2* genes was significantly increased (3.3 fold) and decreased (2.2 fold) in the oral cancer cell line treated with Hydroxytyrosol, respectively ( $P < 0.05$ ).

**Conclusion:** The study indicated a high antiproliferation effect of hydroxytyrosol against oral cancer cell line KB through regulating the expression of some apoptotic genes.

**Keywords:** Oral cancer, Hydroxytyrosol, Apoptosis, Anticancer

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## Introduction

Cancer is the most common human genetic disease characterized by abnormal cell growth and regarded as the second-leading cause of death globally (1). Cancer cells often have the ability to invade adjoining parts or spread throughout the body (2). Oral cancer is the sixth most common malignancy in the world (3, 4). The global incidence of this cancer has been reported as about 354,864 new cases and 177,384

deaths in 2018 (5, 6). Conventional oral squamous cell carcinoma (OSCC) accounts for more than 90% of cancer cases in the head and neck region, and it may affect any region of the oral cavity and oropharynx (7, 8). Pathological studies have demonstrated that environmental factors such as tobacco and alcohol are associated with an increased risk of oral cancer (8, 9).

Current clinical treatments for oral cancer include surgery, chemotherapy, and radiation therapy. According to recent

studies, these treatments are not only completely free of side effects, but also it may reduce proper organ function and life quality (10, 11). Previous investigations have reported that various herbal medicines are important sources of several therapeutic compounds.

Due to fewer side effects compared to current chemotherapeutic drugs, natural compounds have attracted the interest of scientists (12, 13).

Olive is one of the most important medicinal plants with anti-cancer, anti-inflammatory, antioxidant, and antibacterial activity. Hydroxytyrosol is one of the major compounds in olive oil with an anti-cancer effect through several signaling pathways, such as induction of apoptosis (14, 15). Previous studies have reported that hydroxytyrosol has an appropriate anti-proliferative effect against several cancer cells (16, 17). However, very limited studies have been conducted on the anti-cancer effects of hydroxytyrosol on oral cancer cells and underlying molecular mechanisms.

The present study aimed to evaluate the potential anti-proliferative effect of hydroxytyrosol against oral cancer cell line KB.

## Materials and Methods

### Cell Culture

KB cell line was purchased from the Pasteur Institute of Iran. The cancer cells were cultured in RPMI-1640 medium supplemented with 10% FBS, 1% penicillin-streptomycin (5,000 units/mL-5,000 mg/mL), and incubated at 37°C and 5% CO<sub>2</sub> (18).

### Cell Viability Assay

The cancer cells were seeded in a 96-well cell culture plate ( $1.5 \times 10^4$  cells/well) and incubated for 24 hours. Then, treatment of the cancer cells was performed by hydroxytyrosol (10, 32, 100, 320  $\mu$ M) for 24, 48, and 72 hours. After this, the old

medium was substituted with fresh culture medium (200  $\mu$ L) contains 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) reagent (50  $\mu$ L, 5 mg/mL reagent in culture medium), and incubated for 4 hours. Next, the old culture medium was discarded, and 50  $\mu$ L dimethyl sulfoxide (DMSO) was added and then incubated for 30 minutes. The optical densities (OD) of all wells were measured at 570 nm, and cancer cells viability was calculated (19).

### Gene Expression Assay

The cancer cells were seeded in a 6-well cell culture plate ( $1.5 \times 10^5$  cells/well) and incubated for 24 hours. Then, treatment of the cancer cells was performed by hydroxytyrosol (20  $\mu$ M) for 72 hours. The treated cancer cells were trypsinized and resuspended in TRIzol reagent in order to extract total RNA. Next, the obtained total RNA was reversely transcribed into cDNA using oligo-dT primers. The quantitative Real-Time PCR (qRT-PCR) by specific primers was used to investigate *BAX* and *BCL2* genes mRNA expression. The primers sequences were as follows: *BAX* forward primer: 5'-AACTGGACAGTAACATGGAG-3' and reverse primer: 5'-TTGCTGGCAAAGTAGAAAAG-3'; *BCL2* forward primer: 5'-CCTTTGGAATGGAAGCTTAG-3' and reverse primer: 5'-GAGGGAATGTTTTCTCCTTG-3'. The expression of the *ACTB* gene ( $\beta$ -actin) was investigated as endogenous control. The expression of mentioned genes was analyzed by a comparative  $2^{-\Delta\Delta C_t}$  threshold cycle (20).

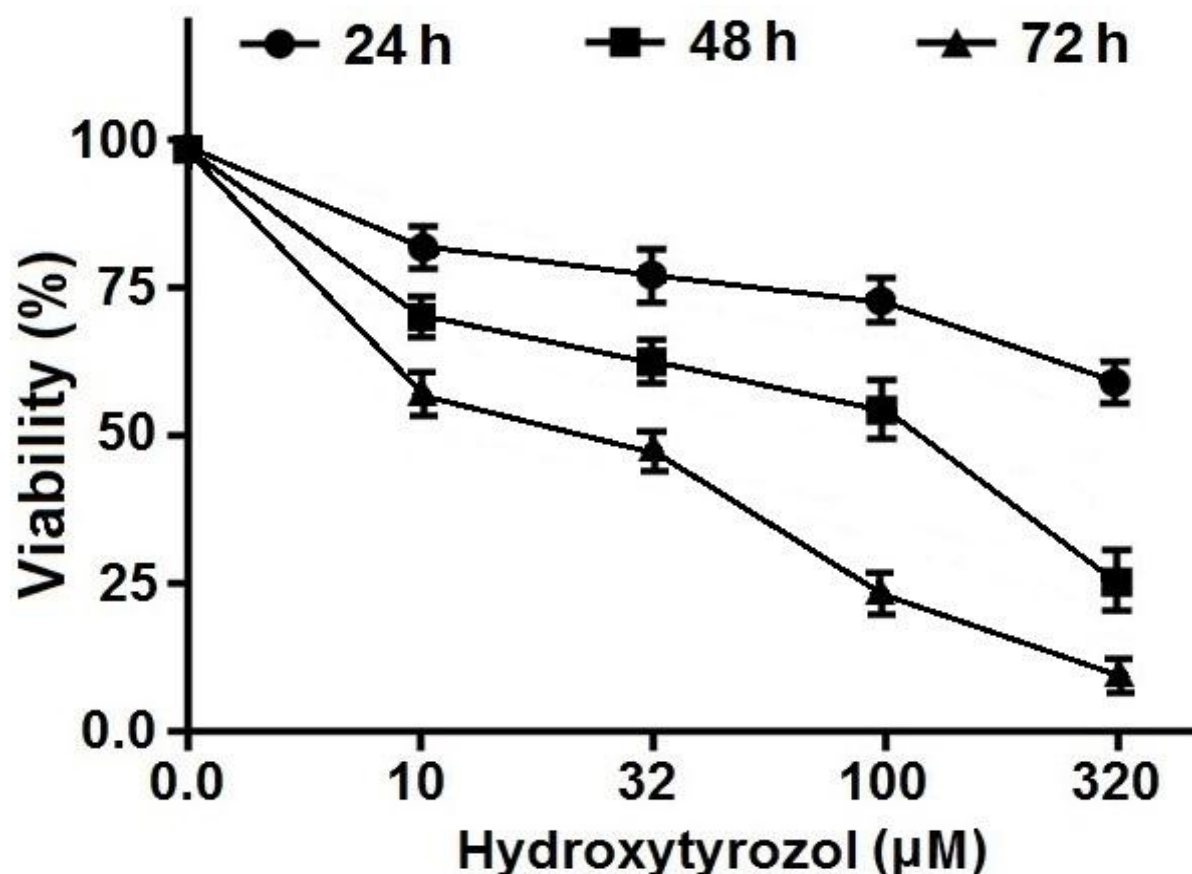
The statistical package for the social sciences (SPSS) software (version 20, SPSS Inc., Chicago, IL) was used for the statistical analysis of the obtained data. The obtained data were analyzed by one-way analysis of variance (ANOVA). Tukey (post-hoc) test was used to compare the treatment groups (21).

## Results

### Cancer Cell Viability

We have found that hydroxytyrosol has an appropriate anti-proliferation effect against

oral cancer cell line KB. We observed that the anticancer effects of hydroxytyrosol were in a time- and dose-dependent manner. In this study, the half-maximal inhibitory concentration (IC<sub>50</sub>) of hydroxytyrosol was 28  $\mu$ M (Figure 1).



**Figure 1.** Inhibitory effects of hydroxytyrosol on the viability of oral cancer cell line KB. Anticancer effects of hydroxytyrosol were in a time- and dose-dependent manner, and IC<sub>50</sub> was 28  $\mu$ M.

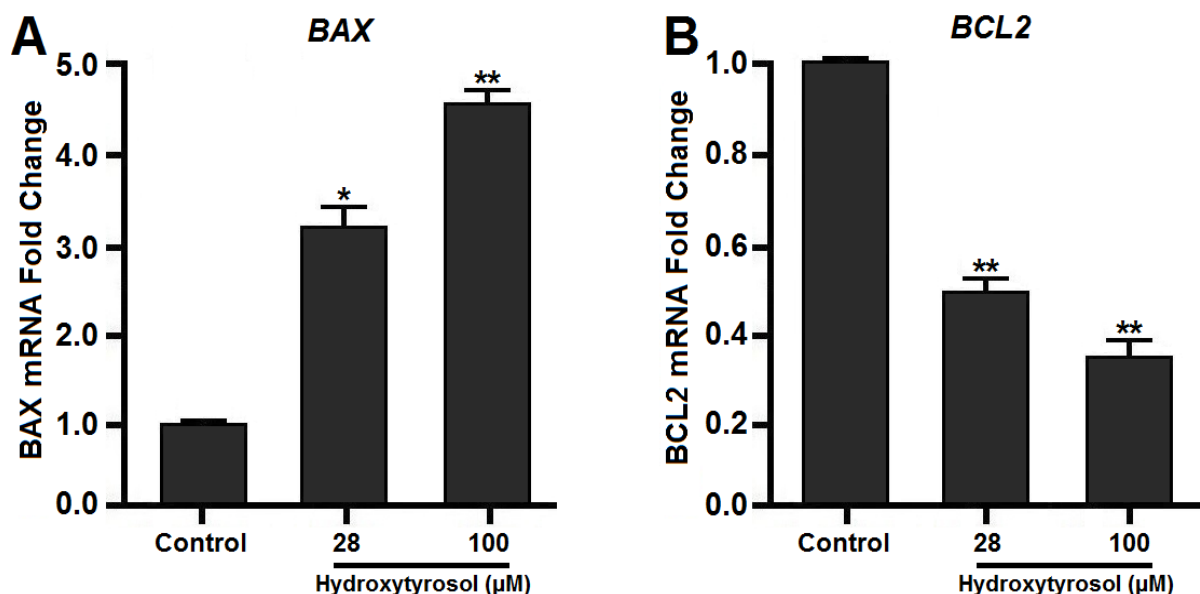
### Expression of Apoptosis-related Genes

Expression of *BAX* gene was upregulated (3.3 fold) in oral cancer cell line KB after treatment by hydroxytyrosol (28  $\mu$ M). In addition, expression of the *BCL2* gene was downregulated (2.2 fold) in treated cancer cells with hydroxytyrosol (28  $\mu$ M). A higher concentration of hydroxytyrosol (100  $\mu$ M) indicated more regulatory effects in mRNA expression of apoptosis-related genes (Figure 2).

### Discussion

For a large percentage of the world's population, especially in developing

countries, herbal medicines are used to treat various disorders. This is because they believe that herbal medicines, in addition to being cheap and available, have no side effects (22, 23). The world today is facing a high prevalence of cancer, which is the second cause of death after heart disease. Identifying important mechanisms involved in cancer progression is important for the improvement of the cancer therapy approach (24, 25). Different mutations can make cells more resistant to apoptosis, so the use of chemical compounds that induce apoptosis is one of the main approaches to cancer therapy (26, 27).



**Figure 2.** Effects of hydroxytyrosol on mRNA expression of apoptosis-related *BAX* and *BCL2* genes. (A) Expression of *BAX* gene was significantly upregulated 3.3 fold, and 4.6 fold in oral cancer cell line KB treated with 28  $\mu$ M and 100  $\mu$ M hydroxytyrosol, respectively. (B) Expression of the *BCL2* gene was significantly downregulated 2.2 fold, and 3.1 fold in oral cancer cell line KB treated with 28  $\mu$ M and 100  $\mu$ M hydroxytyrosol, respectively. (\*  $P < 0.01$  and \*\*  $P < 0.001$ ).

In the present study, we investigated the anti-proliferative effect of hydroxytyrosol against oral cancer cell line KB, as well as the expression of apoptosis-related genes. Our results demonstrated that hydroxytyrosol could inhibit the proliferation of oral cancer cells in a time- and dose-dependent manner. In our study, IC<sub>50</sub> of hydroxytyrosol was 28  $\mu$ M. In addition, mRNA expression of the *BAX* gene was significantly upregulated in oral cancer cells after treatment with hydroxytyrosol; whereas mRNA expression of the *BCL2* gene was significantly downregulated in treated cancer cells with hydroxytyrosol. Numerous anticancer agents are derived from herbal medicine and are used to treatment of various metastatic and non-metastatic cancers (28). Various studies have demonstrated that herbal medicine has a significant effect on the prevention and treatment of cancer. These compounds work by different mechanisms, but apoptosis induction is a common point of many of these compounds. Olive oil is one of the common natural compounds, and hydroxytyrosol is the main

component of olive oil. Evidence suggests that hydroxytyrosol decreases the growth and proliferation of numerous cancer cells through several molecular mechanisms, includes cell cycle arrest and apoptosis induction (29, 30). Both *BAX* (pro-apoptotic) and *BCL2* (anti-apoptotic) are the most important genes involved in the apoptosis pathway. We observed that hydroxytyrosol significantly downregulates *BCL2* and upregulates *BAX*.

## Conclusion

Generally, our study demonstrated that hydroxytyrosol has a significant anti-proliferative effect against oral cancer cell line KB. We suggested that the anti-proliferative effect of hydroxytyrosol may be due to the regulation of apoptosis-related genes expression. However, more studies are required to obtain more accurate results.

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