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Original Article

The Role of *Olea Europaea* L. Fruit on A2780, A172 and HFFF2 Proliferation

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Abstract

Olea europaea L. commonly known as olive has been traditionally used for the prevention and treatment of many diseases since ancient times. Olive has been reported to possess a broad spectrum of pharmacological properties. In the present study, we investigated the activity of aqueous extract of *Olea europaea* L. fruit at various concentrations on A2780, A172, and HFFF2 cell lines proliferation by MTT assay. Aqueous extract of olive significantly increased cell proliferation in a dose dependent manner in the cell lines. It has been previously reported that olive has chemoproventive and anti-tumor effects. These disagreements can be explained by differences in cell line properties, type of olive and different solvents in the extracts. However, further investigation is needed to clarify the exact role of olive in cell proliferation and cancer. In this study fruit extract of *Olea europaea* L. showed more activatory effects on A2780 cell line in comparison with A172 and HFFF2. These differences in the activatory effects may be related to the activation of different signaling pathways in different cell lines.

Keywords: Activatory effects, A2780, A172, Cell Proliferation, HFFF2, Olea europaea L, Olive.

1. Introduction

Olea europaea L. (Oleaceae) known as olive is native to the Mediterranean and widely cultivated in the worldwide especially in southeastern Europe, northern Africa, and northern Iran at the south end of the Caspian Sea [1]. Mediterranean region is the major production area of olive and there is a strong association with the high level of olive consumption and its associated products in this

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region and reduced incidence of cardiovascular diseases and cancers of the breast, skin, and colon [2, 3]. It has been also reported the low incidence of chronic degenerative disease in Mediterranean countries [4]. Olive is traditionally used as an herbal remedy for the treatment of aphrodisiacs, emollients, laxatives, nutritive, sedatives, tonics, colic, alopecia, paralysis, rheumatic pain, sciatica, and hypertension [5]. In the context of religious importance, olive is narrated over several times in the Bible as well as is praised as a blessed fruit in Chapter 24 Al-Nur Quran [6]. The fruit and compression extracted oil, olive oil, possess various nutritional and medicinal properties since ancient times. Olive fruit contains valuable nutrients and bioactive compounds such as proteins, flavonoids, oleic acid, and phenolic compounds [l, 7]. Oleuropein is one of the most abundant phenolic constituents of olive with a considerable antioxidant activity [8]. Several studies have demonstrated that olive possesses a broad spectrum of pharmacological and therapeutic effects such as anti-inflammatory [9] and anticancer [10]. hepatoprotective activity [11], cardiotonic [12] , antiatherogenic [13], anticarcinogenic [14], antimicrobial [15], antihypertensive [16], and antidyslipidemic [17]. In this investigation it was aimed to explore the role of aqueous extract of *Olea europaea* L. fruit on A2780, A172 and HFFF2 cell proliferation by MTT assay. A2780 and A172 are human ovarian cancer cell line and human glioblastoma cell line, respectively; while HFFF2 is a normal human embryonic cell line. In this way we can evaluate the effects of olive on cancerous and normal cells proliferation in a new point of view.

2. Material and Methods

2.1. Materials

Fetal bovine serum (FBS), phosphate-buffered saline (PBS), trypsin, penicillin, streptomycin, DMSO, 3-4, 5-dimethylthiazol-2-yl-2, 5-diphenyl tetrazolium bromide (MTT), and the RPMI-1640 medium supplemented with 10% heat inactivated FBS were purchased from Autocell. The cell lines were provided by Pasteur Institute of Iran.

2.2. Plant Materials and the Preparation of the Extracts

Olea europaea L. fruit were collected from Tarom, Zanjan province of Iran. The collected fruits were scientifically approved by the Department of Botany, Shahid Beheshti University (Voucher number: 1115, deposited in: Shahid Beheshti University Herbarium). Fresh fruits were cleaned and then dried in the shade at room temperature. For the preparation of aqueous extract the fruits were decocted in water for 30 min. Then, the extract was filtered

and concentrated to the desired level (honey-like viscosity), and stored at -20°C. The moisture level of the extract was determined as follows: 2g of final extract was placed in an oven at 60–65°C for 72 h and then weighed. Weight loss was used as a moisture indicator. The final extract contained 24% water. These extracts were dissolved in distilled water at the desired concentrations just before use [18, 19].

2.3. *Cell Culture and Cell Proliferation Assay By MTT*

Activity effects of aqueous fruit extract of *Olea europaea* L. were assessed on human cancer cell lines (A2780 and A172) and human embryonic cell line (HFFF2) by using MTT (3-4, 5-dimethylthiazol-2-yl -2, 5 diphenyl tetrazolium bromide) assay as previously described [20, 21]. The cells were cultured in RPMI 1640 medium (A2780) or DMEM (A172 and HFF-2) enriched with 10% FBS (Fetal Bovine Serum) and incubated at 37o C with 5% CO2 and 96% humidity. After several subcultures, cells were distributed in 96-well plates at 1,000 cells in 100 µL of culture medium and incubated for 24 h at same condition to allow attachment of cells to the bottom of wells. Then culture medium removed and 100 µL of ten-fold serially diluted concentrations of plant extracts (0.0001-100 mg/ml) added to each well in pentaplicate. Microtiter plates further incubated for 5 days in same condition. Culture medium without extract was used as negative control. After the incubation time, the extract containing medium discharged and for evaluation of cell survival, 25 µL of MTT solution (4 mg/ml in PBS) added to each well and plates incubated for 3 h (in same condition). Then 100 µL of DMSO added to each well and plates were gently shaken to dissolve the formed formazan crystals. The absorbance of each well measured at 540 nm using an ELISA plate reader (Infinite M200, Tecan). The GI% (Growth Inhibition percent) was calculated using the formula %Growth Inhibition = 100 – (ODtest - ODcontrol) × 100, where ODtest is the mean absorbance of treated cells and ODcontrol is the mean absorbance of a negative control. The cell survival of control assumed 100% and cell growth values generated from dose-response curves for each cell line.

2.4. *Statistical Analysis*

All experiments were done in pentaplicate and the results were calculated as a mean ± standard deviation (SD). The experimental data were processed using the paired sample t-test and one way ANOVA analysis of the SPSS version 16.0 software for Windows.

3. Results and Discussion

In this study, human ovarian cancer cells (A2780) and human glioblastoma (A172) and also human embryonic cell line (HFFF2) were treated with different concentrations (0.0001-100 mg/ml) of aqueous extract of *Olea europaea* L. fruit. The cell lines were observed by an inverted Microscope and it was observed that

Figure 1. Determination of cell viability by an inverted microscope. The cell lines viability was detected by aninverted microscope (Axiovert 25 Inverted Microscope) just before MTT assay. A: A2780, B: A172, C: HFFF2.

they were viable during the study and before MTT assay (Figure 1). Cell viability was determined5 days after the treatment. As indicated in figure 2, *Olea europaea* L. fruit extract increased A2780 proliferation at different concentration in comparison with control one (without extract). Also, the extract of *Olea europaea* L. induced the proliferation of A172cell line at different concentration in comparison to control after the exposure (Figure 3). Again, as shown in figure 4, olive aqueous extract exhibited activatory effects on normal cell line HFFF2 proliferation at different concentration. Interestingly, *Olea europaea* L. extract had more activatory effect on A2780 in comparison with other two cell lines (Figure 5).

The olive tree, Olea europaea L., is a small evergreen tree that cultivated in different part of the world especially in Mediterranean [22, 23]. Olives and their constituents show important roles in diseases prevention through different mechanisms such as: anti-oxidant, anti-inflammatory, hepatoprotective activity, cardiotonic activity. It is believed that olive exerts its biological effects mainly via constituent antioxidants, including oleic acid, phenolics, and squalene [24, 25]. It has been also reported that hydroxytyrosol is one of the main phenolic components of olive oil that is responsible for chemoproventive effects of olive and olive oil [14, 26]. In addition, this active compound showed a preventive role in cardiovascular diseases and acquired immunodeficiency syndrome (AIDS). It has been proven that these preventive effects are mediated by its potent antioxidant and anti-inflammatory properties [27].

As shown in figure 2-4, aqueous extract of *Olea europaea* L. increased cell proliferation inhuman ovarian cancer cells (A2780), human glioblastoma (A172) and human fetus derivedcells (HFFF2) in a dose dependent manner. However, the previous reports have been shown that*Olea europaea* L. fruit has antimutagenic effects [10]. These differences can be explained by different properties of cell lines, different types of date fruits and constitutes that fractionated in different solvents.

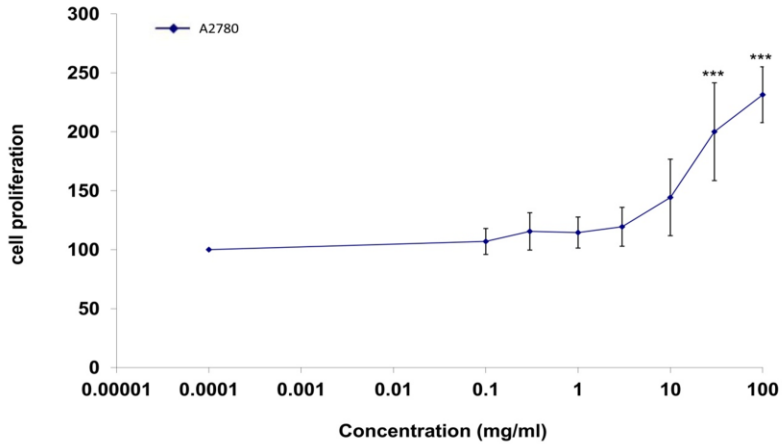

Figure 2. Effects of aqueous extract of *Olea europaea* L. fruit on cell proliferation of A2780. MMT assay was used for cell viability detection. Values are presented as mean±SD. ****P* < 0.001 compared with control.

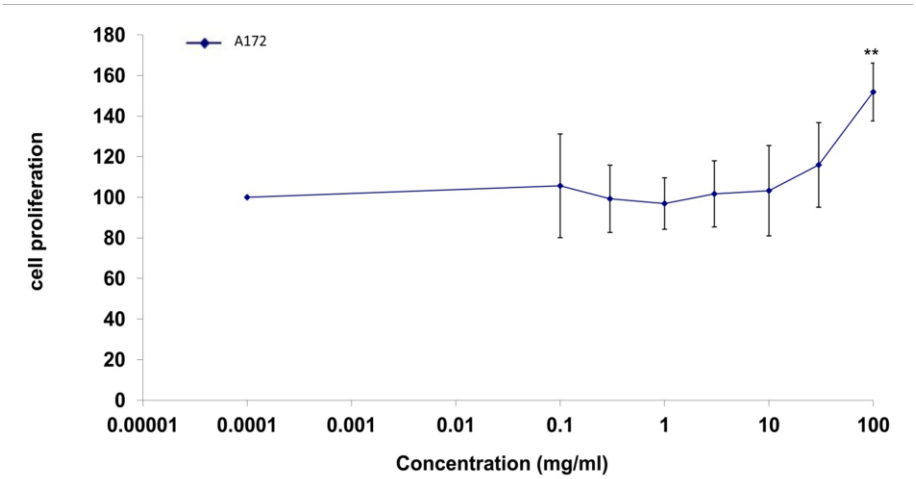

Figure 3. Effects of aqueous extract of *Olea europaea* L. fruit on cell proliferation of A172.MMT assay was used for cell viability detection. Values are presented as mean±SD. ** *P* < 0.01 compared with control.

In addition, the amounts of anticancer compounds that are responsible for antitumor effects of olive may be low in olives of our study. We believe that further investigation is needed to clarify the exact role of *Olea europaea* L. in cell proliferation and cancer prevention. In this study the extract of *Olea europaea* L. showed more activatory effects on A2780 cell line in comparison with A172 and HFFF2. These differences in the activatory

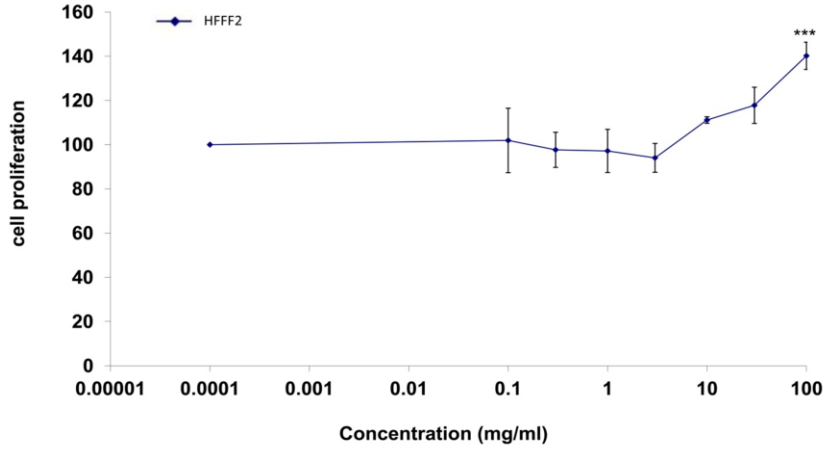

Figure 4. Effects of aqueous extract of *Olea europaea* L. fruit on cell proliferation of HFFF2.MMT assay was used for cell viability detection. Values are presented as mean±SD. *** $P < 0.001$ compared with control.

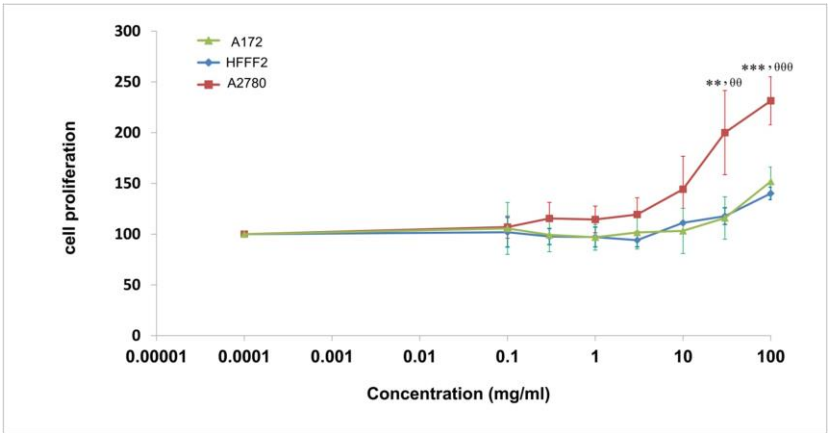

Figure 5. Effects of *Olea europaea* L. aqueous extract on cell proliferation of A2780, A172, and HFFF2. MMT assay was used for cell viability detection. Values are presented as mean±SD. ** $P < 0.01$,*** $P < 0.001$ compared with HFFF2; **00** $P < 0.01$; **000** $P < 0.001$ compared with A172.

effects may be related to the activation of different signaling pathways in different cell lines.

4. Conclusion

In this study, the activatory effects of aqueous extractof *Olea europaea* L. were investigated in different cancerous (A2780 and A172) and fetus derived cell line (HFFF2). The aqueous extract increased cell proliferation when exposed to these cell lines. On the basis of observed results further studies are necessary to confirm our findings. In the next steps the active constitutes of aqueous extract should be isolated and tested for the confirmation and validation of our findings.

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