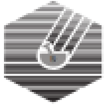

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Original Article

Phoenix dactylifera L. Fruit Induces Cell Proliferation of A2780, A172 and HFFF2 Cell Lines

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Abstract

Dates fruit has been used as staple food in the Middle East for thousands of years and various types of dates are found worldwide. Dates and their constituents show various roles in diseases prevention and treatment through anti-oxidant, anti-inflammatory, and anti-bacterial activity. In the present study we investigated the activities of aqueous and n-hexane extracts of *Phoenix dactylifera* L. fruit at various concentrations on A2780, A172 and HFFF2 cell lines proliferation by means of MTT (3-4, 5-dimethylthiazol-2-yl-2, 5 diphenyl tetrazolium bromide) assay. Aqueous and n-hexane extracts of date showed activatory effects on the cell lines and increased cell proliferation in a dose dependent manner. It has been previously reported that dates fruit possesses anticancer and antimutagenic effects. These disagreements can be explained by differences in cell line properties, type of date fruits, and different solvents in the extracts. However, further investigations are needed to clarify the exact role of date in cell proliferation and cancer. In addition, n-hexane extract acted more powerful than the aqueous extract in increasing the cell lines proliferation. Therefore, it can be concluded that the active components that are responsible for the activatory effects are present in the n-hexane extract.

***Keywords*:** Activatory effects, A2780, A172, Cell Proliferation, Date, HFFF2, *Phoenix dactylifera L.*

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1. Introduction

The date palm *Phoenix dactylifera* L., a tropical and subtropicaltree, belonging to the family Palmae (Arecaceae) is one of mankinds oldest cultured plants, and in the Arabian Peninsula it has played a fundamental role in the day-to-day life of the people for the last 7000 years [1]. The fruit of the date palm (*Pheonix dactylifera*) is a significant commercial crop in Middle Eastern countries. Date fruits are still considered by many people in this part of the world as a main food [1-2]. Date fruits presume great significance in human nutrition because of their rich content of vital nutrients which comprise carbohydrates, salts and minerals, dietary fibre, vitamins, fatty acids, amino acids and protein. They have vast scope and potential for use as food for generations to come due to their notable nutritional, health and economic worth [3-5]. The nutritional value of dates is owing to their high potassium (2.5 times more than bananas), calcium, magnesium, sugar content (around 50–60%), and iron as well as vitamins (B1, B2) and niacin [6]. In the folklore, date fruits have been attributed to have many therapeutic properties when consumed either alone or in combination with other herbs or fruits. In recent years dates have drawn larger attention because of their various health benefits and accordingly many *in vitro* and animal studies besides the identification and quantification of different classes of phytochemicals are being pursued worldwide [7]. A comprehensive analysis of the phytochemistry and validated pharmacological properties of date fruits (*P. dactylifera* L.) and the seeds were extensively reviewed recently [8]. Preclinical studies have shown that the date fruits possess free radical scavenging, antioxidant, antimutagenic, antimicrobial, anti-inflammatory, gastro protective, hepatoprotective, nephroprotective, anticancer, and immunostimulant activities [7]. These reviews indicated that in addition to its dietary use the dates have medicinal value and are extensively used to treat a variety of ailments in

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the various traditional systems of medicine. The aim of this study was to investigate the effects of aqueous and n-hexane extracts of *P. dactylifera* L. fruit on A2780, A172 and HFFF2 cell proliferation by means of MTT assay.

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

P. dactylifera L. fruits were collected from Jam, Busher province of Iran. The collected fruits were scientifically approved by the Department of Botany, Shahid Beheshti University (Voucher number: 8071, 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 [9]. For the preparation of the n-hexane extract, 10 g of the obtained paste of *P. dactylifera* L. extract was mixed with 100 mL of water and transferred to a decanter funnel. The n-hexane solvent was added to the funnel, and the n-hexane fraction was then extracted [10].

2.3. Cell Culture and Cell Proliferation Assay by MTT

Activity of n-hexane and water extract of *P. dactylifera* were assessed on human cancer cell lines (A2780 and A172) and human embryonic cell line (HFFF2) using MTT (3-4, 5-dimethylthiazol-2-yl -2, 5 diphenyl tetrazolium

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bromide) assay as previously described with some modifications [11-13]. 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 37° C with 5% CO_2 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 the same condition to allow attachment of cells to the bottom of wells. Then culture medium was removed and 100 μL of ten-fold serially diluted concentrations of plant extracts (0.0001-100 mg/ml) were 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 – (OD_{test} - $OD_{control}$) × 100, where OD_{test} is the mean absorbance of treated cells and $OD_{control}$ is the mean absorbance of a negative control. The cell survival of control was assumed 100% and cell growth was 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 normal embryonic cell line (HFFF2) were treated with different concentrations (0.0001-100 mg/ml) of aqueous and n-hexane extracts of *P. dactylifera* L. The cell lines were observed by an inverted microscope and it was

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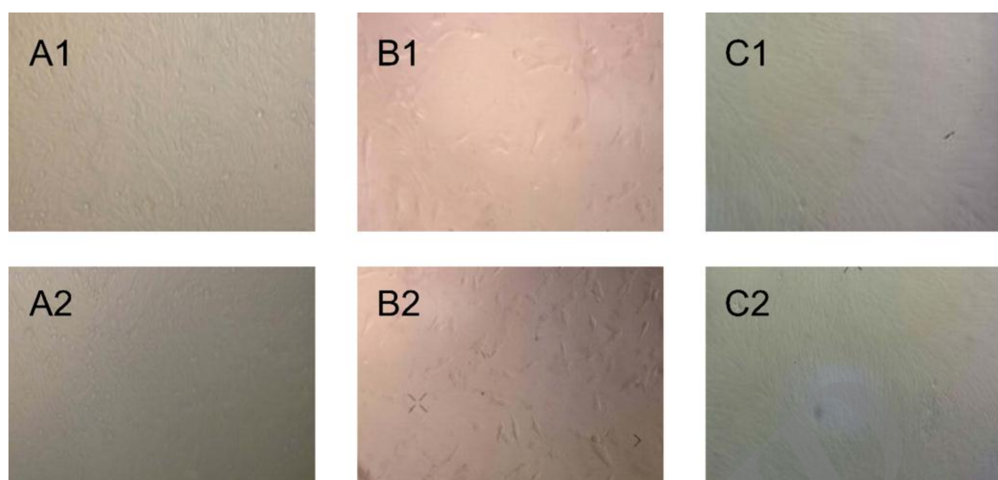

Figure 1. Determination of cell viability by an inverted Microscope. The cell lines viability was detected by an inverted microscope (Axiovert 25 Inverted Microscope) just before MTT assay. A1: Aqueous extract-treated A2780, A2: N-hexan extract-treated A2780, B1: Aqueous extract-treated, B2: N-hexan extract-treated A172, C1: Aqueous extract-treated HFFF2, C2: N-hexan extract-treated HFFF2.

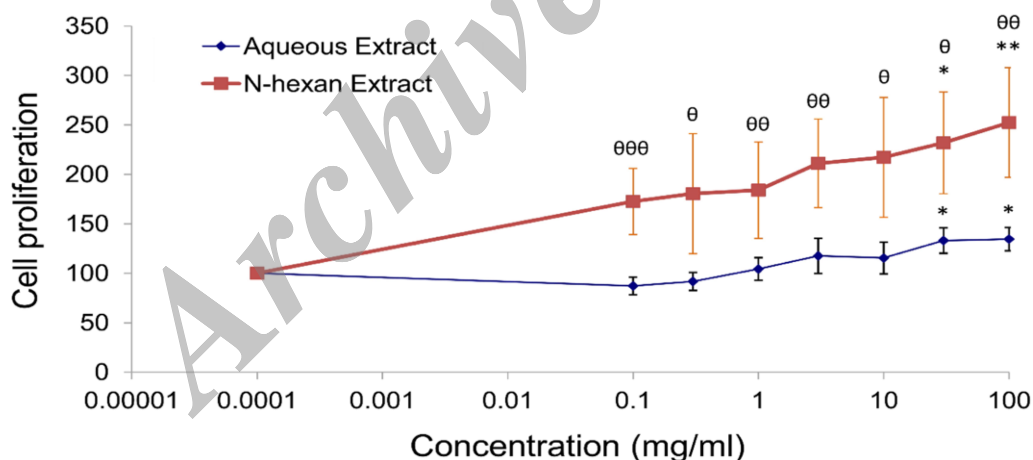

Figure 2. Effects of *P. dactylifera* L. aqueous and n-hexane extracts on cell proliferation of A2780. MMT assay was used for cell viability detection. Values are presented as mean±SD. *$p < 0.05$, **$p < 0.01$ compared with control; **θ** $p < 0.05$, **θθ** $p < 0.01$; **θθθ** $p < 0.001$ compared with aqueous extracts.

observed that they were viable during the study

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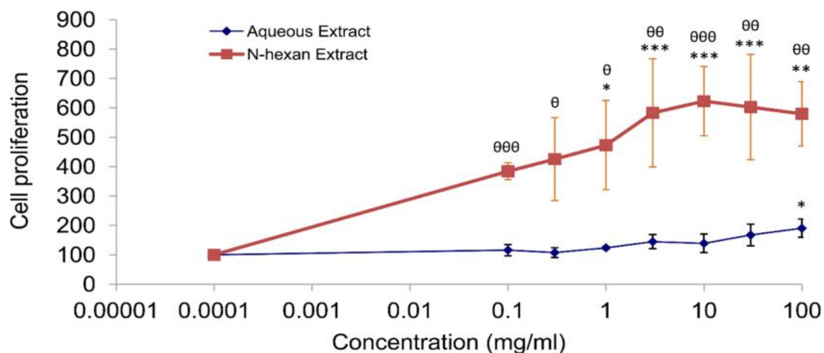

Figure 3. Effects of *P. dactylifera* L. aqueous and n-hexane extracts on cell proliferation of A172. MMT assay was used for cell viability detection. Values are presented as mean±SD. *$p < 0.05$, **$p < 0.01$, ***$p <$ 0.001compared with control; θ $p < 0.05$, θθ $p < 0.01$; θθθ $p < 0.001$ compared with aqueous extracts.

and before MTT assay (Figure 1). Cell viability was determined 5 days after the treatment. As indicated in figure 2, aqueous and n-hexane extracts of *P. dactylifera* L. had increasing effects on A2780 proliferation at different concentration in comparison with control one (without extract). However, the n-hexane extract acted more powerful than the aqueous extract in the elevation of A2780 proliferation. Aqueous and n-hexane extracts of *P. dactylifera* L. increased the proliferation of A172 cell line at different concentration in comparison to control

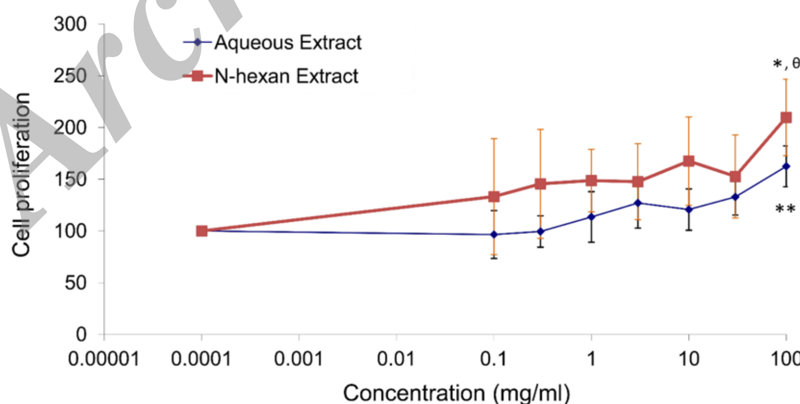

Figure 4. Effects of *P. dactylifera* L. aqueous and n-hexane extracts on cell proliferation of HFFF2. MMT assay was used for cell viability detection. Values are presented as mean±SD. *$p < 0.05$, **$p < 0.01$compared with control; **θ** $p < 0.05$ compared with aqueous extracts.

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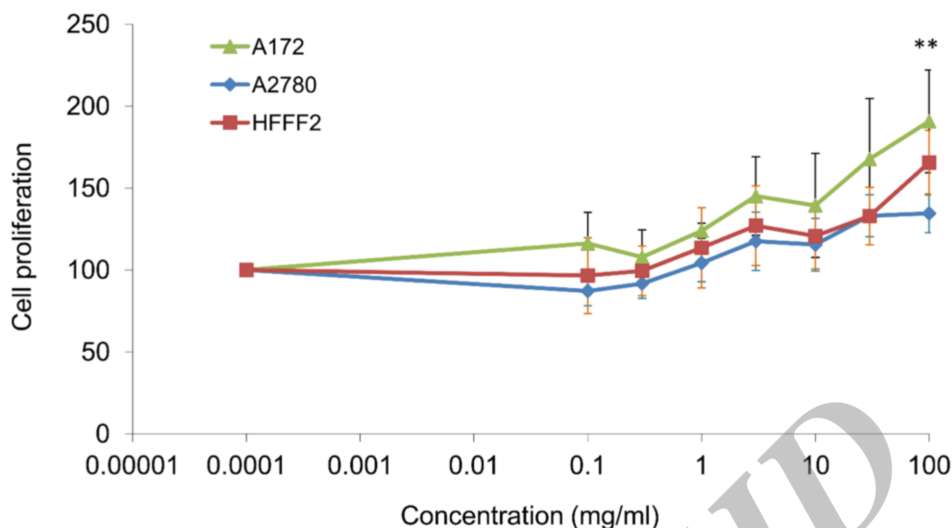

Figure 5. Effects of *P. dactylifera* L. aqueous extract on cell proliferation of A2780, A172 and HFFF2. MMT assay was used for cell viability detection. Values are presented as mean±SD. **$p < 0.01$ compared with A2780.

during the exposure time. But the n-hexane extract has more activatory effects than the aqueous one on A2780 proliferation (Figure 3). Again, as shown in figure 4, both aqueous and n-hexane extracts of *P. dactylifera* L. exhibited activatory effects on normal cell line HFFF2 proliferation at different concentration. The n-hexane extract showed more increasing effects than the aqueous extract in HFFF2 proliferation. Both aqueous and n-hexane extracts of *P. dactylifera* L. had more activatory effect on A172 in comparison with other two cell lines (Figure 5 and 6).

Dates fruits are used as staple food in the Middle East for thousands of years. Various types of dates have shown medicinal value in various types of disease prevention and treatment. Dates and their constituents show a role in diseases prevention through anti-oxidant, anti-inflammatory, anti-bacterial activity. A recent report showed that aqueous extracts of dates have antioxidant, antimicrobial and anti-mutagenic activity [6, 14, 15]. The date fruits constituents have shown the antitumour activity but its exact mechanism of action and its constituents is not known exactly. Earlier studies reported that beta D-glucan isolated from dates

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has antitumour activity [16]. Study on animal model showed that glucans, constituents of date fruits exhibited a dose dependant anticancer activity with an optimum activity at a dose of 1 mg/kg in tumor [17].

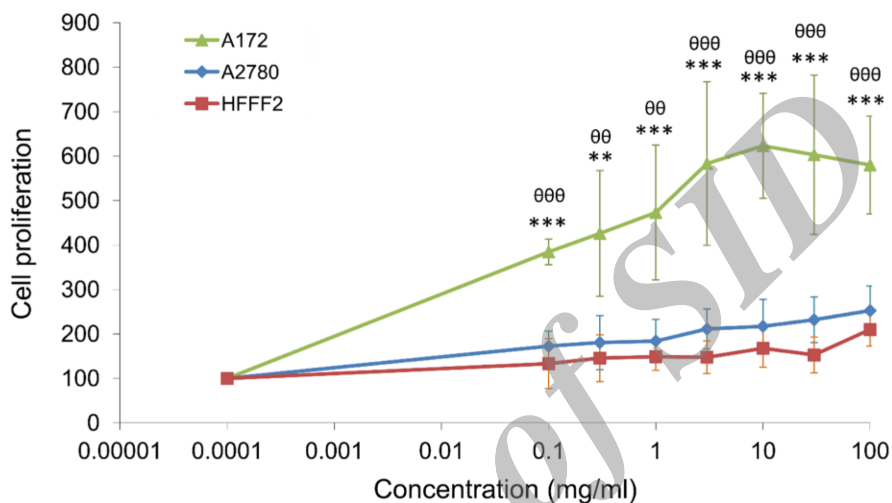

Figure 6. Effects of *P. dactylifera* L. n-hexane extract on cell proliferation of A2780, A172 and HFFF2. MMT assay was used for cell viability detection. Values are presented as mean±SD. ** $p < 0.01$, *** $p < 0.001$ compared with HFFF2; θθ $p < 0.01$; θθθ $p < 0.001$ compared with A2780.

As shown in Fig 2-4 aqueous and n-hexane extracts of *P. dactylifera* L. increased cell proliferation in human ovarian cancer cells (A2780), human glioblastoma (A172) and human fetus derived cells (HFFF2) in a dose dependent manner. However, the previous reports have been shown that *P. dactylifera* L. fruit has anticancer and antimutagenic effects. These differences can be explained by different properties of cell lines, different types of date fruits, and constitutes that fractionated in different solvents. In addition, the amounts of glucans that are responsible for antitumor effect of date may be low in date fruits of our study. We believe that further investigations are needed to clarify the exact role of *P. dactylifera* L. in cell proliferation and cancer. In this study, aqueous and n-hexane extracts of *P. dactylifera* L. showed more activatory effects on A172 cell line in comparison with A2780 and HFFF2. These differences in the activatory effects may

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

4. Conclusion

In this study, activatory effects of aqueous and n-hexane extracts of *P. dactylifera* were investigated in different cancerous (A2780 and A172) and fetus derived cell line (HFFF2). The aqueous and n-hexane extracts 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 and n-hexane extracts should be isolated and tested for the confirmation and validation of our findings.

Acknowledgments

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