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Celtis australis Exhibits Cytotoxic Effects by Inducing DNA Damage in Human Ovarian Cancer Cells

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Abstract: Native to the Mediterranean and southwest Asia, *Celtis australis* is used in folk medicine to treatment of many diseases such as peptic ulcer, diarrhea, dysentery, pain, and colic. However, there are very few studies on the effects of this species on cancer. We report for the first time the cytotoxic and genotoxic properties of *C. australis* on the human ovarian cancer cell line A2780. In the study, *C. australis* extract prepared in ethanol/phosphate buffer was applied to A2780 cells. The change in viability level in A2780 cells after treatment was determined by MTT assay and DNA damage was determined by single-cell gel electrophoresis (Comet) analysis. Our results showed that plant extract application at doses above 200 µg/mL significantly decreased A2780 cell viability ($p<0.05$). The IC50 value for A2780 cells of *C. australis* was determined as 251.43 µg/mL. Comet analysis results revealed that 50 µg/mL and above dose applications increased the level of DNA damage ($p<0.05$). These results suggest that *C. australis* mediates cell death by inducing DNA damage in A2780 cancer cells.

Celtis australis İnsan Over Kanseri Hücrelerinde DNA Hasarını İndükleyerek Sitotoksik Etki Sergiler

Anahtar Kelimeler
Over kanseri, *Celtis australis*, Sitotoksisite, DNA hasarı

Öz: Akdeniz ve güneybatı Asya'ya özgü olan *Celtis australis*, halk tıbbında yaprak ve meyveleri peptik ülser, ishal, dizanteride, ağrı ve kolik gibi birçok hastalığın tedavisinde kullanılmaktadır. Buna karşın bu türün kanser üzerine etkilerini konu edinen oldukça az sayıda çalışma vardır. Bu çalışmada *C. australis*'in insan over kanseri hücre serisi A2780 üzerine sitotoksik ve genotoksik özelliklerini ilk defa rapor ediyoruz. Çalışmada *C. australis*'ın etanol/fosfat tamponu içerisinde hazırlanan özütü A2780 hücrelerine uygulandı. Uygulama sonrası A2780 hücrelerinde canlılık düzeyi değişimi MTT analiziyle, DNA hasarı ise tek hücre jel elektroforezi (Comet) analizliyle belirlendi. Sonuçlarımız 200 µg/mL üzeri dozlarda bitki özütü uygulamasının A2780 hücre canlılığını anlamlı düzeyde azalttığını gösterdi ($p<0.05$). *C. australis*'ın A2780 hücreleri için IC50 değeri 251,43 µg/mL olarak belirlendi. Comet analiz sonuçları 50 µg/mL ve üzeri doz uygulamalarının DNA hasar düzeyini arttırdığını ortaya koydu ($p<0.05$). Bu sonuçlar *C. australis*'ın A2780 kanser hücrelerinde DNA hasarını indükleyerek hücre ölümüne aracılık ettiğini göstermektedir.

1. INTRODUCTION

Cancer is an important public health problem that negatively affects human health and quality of life and is increasing day by day. It is characterized by abnormal growth of cells in any tissue of the body as a result of genetic alteration and spread to different regions [1]. Ovarian cancer is the eighth leading cause of cancer-related death in women worldwide. It is the fifth most common cause of cancer-related death in women in Australia, North America and Western Europe [2]. In Turkey, ovarian cancer is women's the 7th most common cancer type [3]. According to World Health Organization data, 295,414 people were diagnosed with

ovarian cancer in 2018 and 184,799 people died from ovarian cancer that year [4].

Medicinal plants, which have been used for therapeutic purposes from the past to the present, have been instrumental in the discovery and development of many drugs in modern pharmacy [5, 6]. *Celtis australis*, also known as the hedgehog in our country, grows wild in temperate Mediterranean regions (e.g. southern Europe, northern Africa) and Southeast Asia. It has been reported that *C. australis* is an important source of fiber, protein, vitamins and also important bioactive compounds such as lutein, β-carotene, zeaxanthin and tocopherols in fruit samples [7]. It also contains rare flavonoids (such as flavone O-glycosides and flavone C-glycosides) and important metabolites such as tannins and saponins [8, 9]. Due to these, it is reported to exhibit strong antimicrobial and antioxidant effects [7]. Moreover, considering the cytotoxic effects of these compounds, it can be assumed that the extract prepared from the fruits and leaves of *C. australis* may have cytotoxic potential.

A few number of studies report the cytotoxic effect of *Celtis* species against cancer cells. It has been reported that a new flavonoid C-Glycoside isolated from *C. australis* and *C. occidentalis* leaves exhibits strong antioxidant effects and cytotoxic effects on different cancer cell lines [10]. Acquaviva *et al*. have shown that the extract obtained from *C. aetnensis* caused cell damage and inhibited viability by interfering with the oxidant/antioxidant cell balance in Caco-2 human colon carcinoma cells [11]. In addition, local people in South Africa use the sun-dried bark and roots of *C. africana* in the treatment of cancer [12, 13]. However, most other studies have focused on content analysis of these genres. This study aimed to elucidate the *in vitro* effects of *C. australis* on ovarian cancer, an important malignancy in women. In this context, the cytotoxic activity and genotoxic effect of *C. australis* extract against A2780 cell lines were evaluated.

2. MATERIAL AND METHOD

2.1. Preparation of Plant Extract

Ripe fruits of *C. australis* were extracted in 80% ethanol/phosphate buffer at a ratio of 1:10 (g/mL). The solvent of the extract was evaporated in a rotary evaporator (Buchi R100, Switzerland) and the total volume was made up to 10 mL with phosphate buffer. The stock solution was sterilized by filtration through a 0.22 µm filter and stored at 4°C for the duration of the experiment.

2.2. Cell Culture Studies

Human ovarian cancer cell line A2780 was used in the study. Cells were cultured in RPMI-1640 medium (10% FBS, 1% penicillin-streptomycin solution, 1% non-essential amino acid solution). Cell flasks were maintained at 37°C (Thermo Forma II CO_2 Incubator, USA) with 5% CO_2 throughout the experimental process. Confluent cells were removed with trypsin/EDTA solution and cell counting was performed. 96-well plates were used for cytotoxic assays. Approximately 15,000 cells were seeded in each well. The next day, the medium in the well was aspirated and a new medium was added. The treatment groups were treated with different doses (50-600 µg/mL) of the prepared plant extract for 24 hours. At the end of the period, cell viability levels in the groups were determined by MTT assays [14]. The absorbance values obtained from the wells were ratioed to the control absorbance value and percent viability values were calculated.

2.3. Genotoxicity Analysis

In the study, DNA damage effects of plant extract were determined by single-cell gel electrophoresis (Comet) analysis [15]. For analysis, cells were seeded in 6-well plates. Cells were treated with 50, 100, and 200 µg/mL doses of the plant extract for 24 hours. The collected cell samples were then washed with phosphate buffer and resuspended. Approximately 10,000 cells were mixed with low-melting agarose and this mixture was dropped onto agarose-coated slides and coverslips were closed. The preparations were kept at 4°C for 10-15 minutes and the coverslips were separated. The slides were then kept in lysis solution for 1 hour and placed in a horizontal gel electrophoresis tank. Samples were run at 25 volts (maximum 300 mA) for 25 minutes. After electrophoresis, the slides were washed 3 times for 5 minutes with a neutralization buffer. Finally, ethidium bromide solution was added to the slides and DNA damage images were recorded under a Zeiss Axio Scope.A1 fluorescence microscope. Analyses were performed using Tritek Comet Score software. This program analyzes images using the background of the image taken as a reference and uses pixel changes/intensity to determine the level of DNA damage. Tail length (tail length; TL), tail moment (tail moment; TM), tail intensity (tail intensity; TI). and %DNA tail ratio (%DNA tail) parameters were determined for at least 100 random cells from each group.

2.4. Statistical Analysis

Sigma Plot 12 package program was used in the analysis. The normal distribution of the analyzed variables was examined by the Shapiro-Wilk test before comparisons were made between groups. Kruskal Wallis H test was used to determine the differences between groups and Dunn's test was used for multiple comparisons. Statistically, $p<0.05$ was considered statistically significant. After MTT assays, the IC50 of the plant extract for A2780 cells was calculated using AAT Bioquest IC50 Calculator [16].

3. RESULTS

3.1. Cell Viability Level

The viability change in the A2780 cell line treated with different concentrations of *C. australis* plant extract is

shown in Figure 1. 24 hours after treatment, *C. australis* 400 and 600 µg/mL doses significantly reduced cell viability compared to the control group ($p<0.05$). Furthermore, the IC50 value on *C. australis* A2780 cells was determined as 251.43 µg/mL.

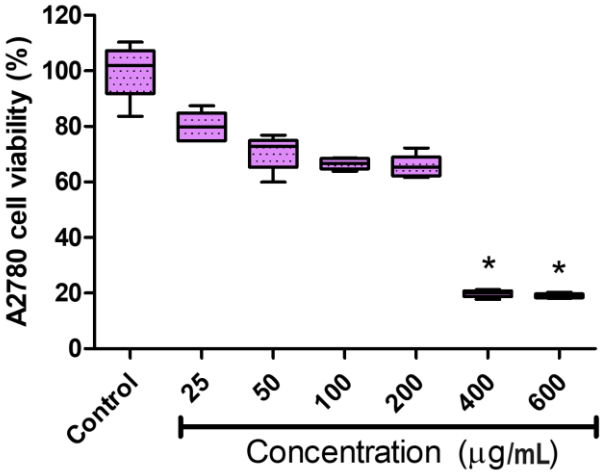

Figure 1. Viability change (%) in A2780 cell line 24 hours after plant extract treatment. Box plots are expressed as median (min.–max.). *p<0.05 compared to control.

3.2. Impact on DNA Damage

After MTT analysis, doses with at least 70% viability were applied to the cells for Comet analysis. The results of Comet analysis after the treatments are summarized in the Table 1. Accordingly, %DNA tail and TI parameters determined after the treatments were higher in the treatment groups compared to the control group ($p<0.05$). On the other hand, no difference was observed between the groups in the other two parameters (TL and TM). These results indicate that the plant extract causes DNA damage and decreases cell viability. Microscope images of the study are presented in Figure 2.

Table 1. Comet analysis results

	%DNA tail	TL	TI	TM
Control	4.03 (2.83 - 5.03)	1.00 (0.00 - 2.00)	30405.00 (21320.00 - 37024.00)	0.01 (0.00 - 0.06)
50 µg/mL	4.61 (3.12 - 9.01)*	0.00 (0.00 - 3.00)	34439.00 (22192.50 - 84088.00)*	0.00 (0.00 - 0.13)
100 µg/mL	10.89 (3.84 - 43.72)*	0.00 (0.00 - 8.75)	99552.50 (25614.25 - 510185.75)*	0.00 (0.00 - 1.33)
200 µg/mL	11.11 (3.58 - 88.37)*	1.00 (0.00 - 48.50)	75139.00 (30188.50 - 705810.00)*	0.02 (0.00 - 31.43)

Values expressed as median (25% - 75%). *p<0.05 compared to the control group.

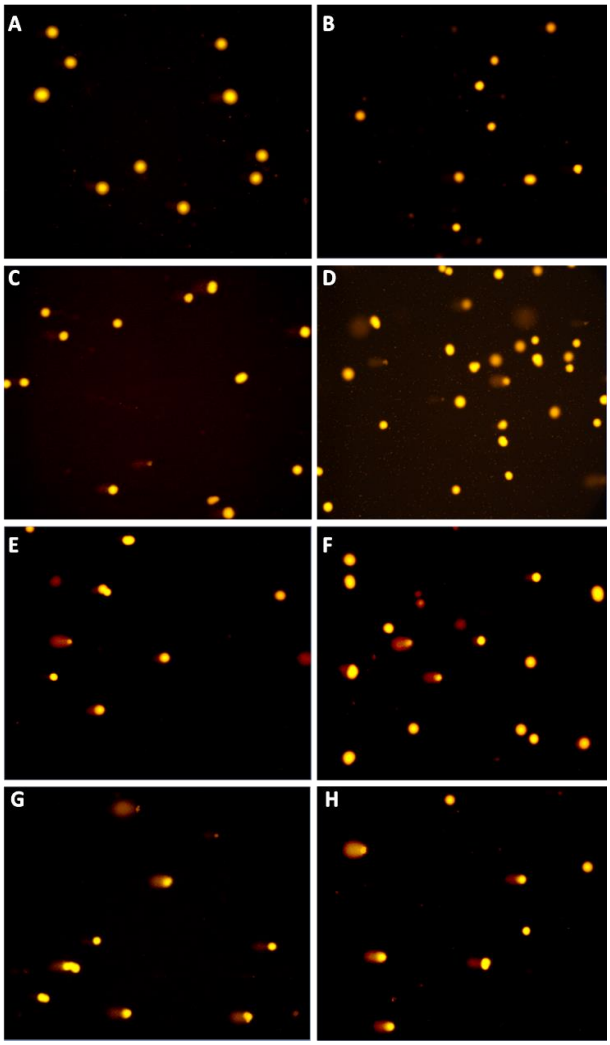

Figure 2. DNA damage image after plant extract application to A2780 cell line. (A,B: control group, C,D: 50 µg/mL, E,F: 100 µg/mL and G,H: 200 µg/mL plant extract treated groups. X100).

4. DISCUSSION AND CONCLUSION

Recent developments have led to the realization that free radical-induced lipid peroxidation and DNA damage are associated with major health problems such as cancer and aging. Plant-derived antioxidants are reported to be effective in protecting against these processes [17-19]. *C. australis* has various uses in folk medicine. Studies have shown that this species is a rich source of phytochemicals such as phenolic compounds, flavonoids and minerals [20, 21]. Most of the studies on *C. australis* have focused on revealing the phytochemical composition of the plant and determining its antioxidant activity. In this study, we present the first findings showing the cytotoxic and genotoxic effects of *C. australis* species on ovarian cancer A2780 cells.

Few studies have focused on the effects of *C. australis* on cancer cells. El-Alfy *et al*. evaluated the cytotoxic effects of ethanol and aqueous extracts of *C. australis* and *C. occidentalis* plants on human hepatocellular carcinoma (HEP-G2), leukemia carcinoma (CCRF-CEM), colon adenocarcinoma (COLO 205), ovarian adenocarcinoma (NIH: OVCAR-3) and gastric carcinoma (NCI-N87) cell lines. The results showed that COLO 205, HEP-G2 and NCI-N87 cell lines were the

cancer types most affected by the viability change after treatments. ED50 values for *C. australis* ovarian adenocarcinoma cells were reported as 77.65 ± 0.52 µg/mL (ethanolic extract) and 72.77 ± 0.48 µg/mL (aqueous extract) [10]. Some studies on other *Celtis* species support the cytotoxic activity of *C. australis*. Acquaviva *et al*. reported that *C. aetnensis* extract applied at doses of 5 µg/mL and above-induced apoptosis in Caco-2 cells and decreased cell viability. In addition, while an increase in reactive oxygen level was observed in cell lines after plant extract application, a decrease in HO-1 protein expression, which mediates cell survival, was detected [22]. In another study, the cytotoxic effect of two new triterpene ester isolates from *C. philippinensis* on Lu1 (human lung cancer), Col2 (human colon cancer), KB (human oral epidermoid carcinoma) and LNCaP (hormone-dependent human prostate cancer) was investigated. The results of the study revealed that these compounds exhibited significant cytotoxic effects in cell lines with ED50 values generally in the range of 5-15 µg/mL [23].

This study provides evidence that *C. australis* extract, which is considered a valuable medicinal plant species, exhibits strong cytotoxic and genotoxic effects against ovarian cancer cell line. Our results showed that *C. australis* extract significantly decreased cell viability in A2780 cells at doses above 200 µg/mL and caused DNA damage from 50 µg/mL. These results show that *C. australis* extract causes cell death by inducing DNA damage in A2780 cells. Our study supports the growing data in this field showing the anti-cancer effect of *Celtis* species. We can say that this species, which is widely used in traditional medicine practices, reveals these effects through the secondary metabolites it contains. In light of the study's findings, further investigation into the use of this plant is necessary as a supplementary natural product for cancer treatment and prevention in both *in vitro* molecular study and *in vivo* animal models.

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REFERENCES

[1] Jiramongkol Y, Lam EW. FOXO transcription factor family in cancer and metastasis. Cancer Metastasis Rev. 2020;39(3):681-709.

[2] Wild C, Weiderpass E, Srewart B. World cancer report. International agency for research on cancer: WHO; 2020.

[3] Kara F, Keskinkılıç B. Türkiye kanser istatistikleri 2017 Ankara: T.C. Sağlık Bakanlığı Halk Sağlığı Genel Müdürlüğü; 2021 [updated 17.11.2021. Available from: https://hsgm.saglik.gov.tr/depo/birimler/kanser-db/istatistik/Turkiye_Kanser_Istatistikleri_2017.pdf .

[4] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424.

[5] Romero-Sandoval EA, Fincham JE, Kolano AL, Sharpe BN, Alvarado-Vazquez PA. Cannabis for chronic pain: Challenges and considerations. Pharmacotherapy. 2018;38(6):651-62.

[6] Shaito A, Thuan DTB, Phu HT, Nguyen THD, Hasan H, Halabi S, Abdelhady S, Nasrallah GK, Eid AH, Pintus G. Herbal medicine for cardiovascular diseases: Efficacy, mechanisms, and safety. Front Pharmacol. 2020;11:422.

[7] Ota A, Visnjevec AM, Vidrih R, Prgomet Z, Necemer M, Hribar J, Cimerman NG, Mozina SS, Bucar-Miklavcic M, Ulrih NP. Nutritional, antioxidative, and antimicrobial analysis of the Mediterranean hackberry (Celtis australis L.). Food Sci Nutr. 2017;5(1):160-70.

[8] Spitaler R, Gurschler S, Ellmerer E, Schubert B, Sgarbossa M, Zidorn C. Flavonoids from Celtis australis (Cannabaceae). Biochem Syst Ecol. 2009;37(2):120-1.

[9] Zehrmann N, Zidorn C, Ganzera M. Analysis of rare flavonoid C-glycosides in Celtis australis L. by micellar electrokinetic chromatography. J Pharm Biomed Anal. 2010;51(5):1165-8.

[10] El-Alfy TS, El-Gohary HM, Sokkar NM, Hosny M, Al-Mahdy DA. A new flavonoid C-glycoside from Celtis australis L. and Celtis occidentalis L. leaves and potential antioxidant and cytotoxic activities. Sci Pharm. 2011;79(4):963-75.

[11] Acquaviva R, Sorrenti V, Santangelo R, Cardile V, Tomasello B, Malfa G, Vanella L, Amodeo A, Genovese C, Mastrojeni S, Pugliese M, Ragusa M, Di Giacomo C. Effects of an extract of Celtis aetnensis (Tornab.) Strobl twigs on human colon cancer cell cultures. Oncol Rep. 2016;36(4):2298-304.

[12] Krief S, Hladik CM, Haxaire C. Ethnomedicinal and bioactive properties of plants ingested by wild chimpanzees in Uganda. J Ethnopharmacol. 2005;101(1-3):1-15.

[13] Koduru S, Grierson DS, Afolayan AJ. Ethnobotanical information of medicinal plants used for treatment of cancer in the Eastern Cape Province, South Africa. Curr Sci. 2007;92(7):906-8.

[14] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65(1-2):55-63.

[15] Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res. 1988;175(1):184-91.

[16] Bioquest A. "Quest Graph™ IC50 Calculator." AAT Bioquest, Inc. 2021 [updated 14.04.2021. Available from: https://www.aatbio.com/tools/ic50-calculator.

[17] Shen CY, Jiang JG, Yang L, Wang DW, Zhu W. Anti-ageing active ingredients from herbs and

nutraceuticals used in traditional Chinese medicine: pharmacological mechanisms and implications for drug discovery. Br J Pharmacol. 2017;174(11):1395-425.
[18] Dhanjal DS, Bhardwaj S, Sharma R, Bhardwaj K, Kumar D, Chopra C, Nepovimova E, Singh R, Kuca K. Plant fortification of the diet for anti-ageing effects: A review. Nutrients. 2020;12(10):3008.
[19] Shin SA, Moon SY, Kim WY, Paek SM, Park HH, Lee CS. Structure-based classification and anti-cancer effects of plant metabolites. Int J Mol Sci. 2018;19(9):2651.
[20] Safari F, Hassanpour H, Alijanpour A. Evaluation of hackberry (Celtis australis L.) fruits as sources of bioactive compounds. Sci Rep. 2023;13(1):12233.
[21] Badoni R, Semwal DK, Rawat U, Rawat M. Chemical constituents from fruits and stem bark of Celtis australis L. Helvetica Chimica Acta. 2011;94:464-73.
[22] Acquaviva R, Sorrenti V, Santangelo R, Cardile V, Tomasello B, Malfa G, Vanella L, Amodeo A, Genovese C, Mastrojeni S. Effects of an extract of Celtis aetnensis (Tornab.) Strobl twigs on human colon cancer cell cultures. Oncol Rep. 2016;36(4):2298-304.
[23] Hwang BY, Chai H-B, Kardono LBS, Riswan S, Farnsworth NR, Cordell GA, Pezzuto JM, Douglas Kinghorn A. Cytotoxic triterpenes from the twigs of Celtis philippinensis. Phytochemistry. 2003;62(2):197-201.