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Ziziphus Jujube'nin Melanom Hücreleri Üzerindeki Potansiyel Antioksidan Etkileri

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Öne Çıkanlar:

- Ziziphus Jujube hidrodistilasyon ile ekstrakte edildi
- Ziziphus Jujube ekstraktı melanoma hücrelerine (G361) uygulandı
- Ziziphus Jujube ekstraktının GSH, NO, TAS ve TOS üzerine etkileri araştırıldı

Anahtar Kelimeler:

- Antioksidanlar
- Melanoma
- Oksidatif Stres
- *Ziziphus Jujube*

ÖZET:

Hücresel redoks homeostazı, iç veya dış stres faktörlerine maruz kaldığında tehlikeye girerek oksidanların üstünlüğüne neden olur. Bu reaktif oksijen türlerinin diyabet ve kanser gibi birçok ciddi hastalığın etiyolojisinde kritik rol oynadığı bilinmektedir. Melanom, cilt kanserinin en ölümcül şeklidir ve oranları şu anda diğer önlenebilir kanserlerden daha hızlı artmaktadır. Melanom, pigment üreten hücreler olan melanositlerde genetik mutasyonların ortaya çıkmasından kaynaklanır. *Ziziphus Jujube* Mill., Asya'nın tropik/subtropikal iklime sahip bölgelerinde yetişir ve binlerce yıldır temel geleneksel halk ilacı olarak kullanılmaktadır. Bu çalışma, *Ziziphus Jujube*'nin melanom üzerindeki potansiyel antioksidan etkilerini belirlemek amacıyla yapılmıştır. Hücrelerin proliferasyonu, MTT analizi (kolorimetrik) ile ölçüldü. Melanom hücreleri, %100 konsantrasyonda *Ziziphus Jujube* esansiyel yağı ile üç saat muamele edildi. Total antioksidan ve oksidan kapasite, glutatyon ve nitrik oksit seviyeleri spektrofotometrik olarak belirlendi. *Ziziphus Jujube*, hücre çoğalmasını önlemiş ve toplam antioksidan kapasitesini artırmıştır. Sonuçlarımız, *Ziziphus jujube*'nin melanom hücrelerinde antioksidan seviyelerindeki düşüşü ve nitrik oksit seviyelerinin yükselmesini engellediğini göstermiştir. Ayrıca, *Ziziphus Jujube* uygulaması GSH düzeylerini iyileştirmiştir. Özetle, *Ziziphus Jujube'*nin melanom hücrelerinde potansiyel antioksidatif ve antiproliferatif etkiler gösterdiği sonucuna varılabilir. Son araştırmalar, *Ziziphus Jujube* meyvesinin tıbbi kullanımı için ayrıntılı bir etnofarmakolojik yaklaşım sunmaktadır.

The Potential Antioxidant Effects Of *Ziziphus Jujube* On Melanoma Cells

Highlights:

- Ziziphus Jujube was extracted by hydrodistillation
- Ziziphus Jujube extract was applied to melanoma cells (G361)
- The effects of Ziziphus Jujube extract on GSH, NO, TAC and TOC were investigated

Keywords:

- Antioxidants
- Melanoma
- Oxidative stress
- *Ziziphus jujube*

ABSTRACT:

Cellular redox homeostasis is compromised when exposed to internal or external stress factors, resulting in the superiority of the oxidant. It is known that these reactive oxygen species play a critical role in the etiology of many serious diseases like diabetes and cancer. Melanoma is the deadliest form of skin cancer and its rates are currently increasing faster than other preventable cancers. Melanoma is caused by the appearance of genetic mutations in pigment-producing cells, melanocytes. *Ziziphus jujube* Mill. spreads in regions of Asia with tropical/subtropical climates and has been used for thnds of years as basic traditional folk medicine. This study was carried out to determine the potential antioxidant effects of *Ziziphus jujube* on melanoma. Survival of cells was measured by MTT assay (colorimetric). Melanoma cells were treated three hours with 100% concentration *Ziziphus jujube* essential oil. Total antioxidant and oxidant capacity, glutathione, and nitric oxide levels were determined by spectrophotometrically. *Ziziphus jujube* prevented cell proliferation and increased total antioxidant capacity. Our results indicated that *Ziziphus jujube* inhibited the decrease in antioxidant levels and elevation of nitric oxide levels in melanoma cells. Also, the *Ziziphus jujube* administration improved GSH levels. Briefly, it can be concluded that *Ziziphus jujube* showed potential antioxidative and antiproliferative effects in melanoma cells. Recent research provides a detailed ethnopharmacological approach for the medicinal use of *Ziziphus jujube* fruit.

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INTRODUCTION

Antioxidants allow aerobic organisms to withstand daily oxidative stress attacks, resisting the negative effects of oxygen-free radicals generated by metabolic activities in the body. Antioxidants prevent or eliminate oxidative stress-related diseases by resisting the worsening effect of reactive oxygen species (ROS). Antioxidants destroy free radicals and play an important role in the optimal protection of cellular functions. Whenever antioxidant capacity is altered by oxidative stress, lipid peroxidation, and DNA damage may occur (Papageorgiou et al., 2005).

Many plant species have been used in the treatment of various diseases since past years. These plants, which contain bioactive compounds, are considered as an alternative treatment source in cancer studies in order to develop new therapeutic agents due to factors such as their affordable cost, less side effects and less toxic effects. *Ziziphus jujube*, is a member of the *Rhamnaceae* family, blooms in May, June, and July, mostly grown in Europe, Asia, Australia, and Northern China. Its fruits are in the form of a 2 cm palm and sweet mucilage and are defined as the "fruit of life"(Hürkan, 2019). The essential nutrients in *Z. jujube* include carbohydrates, protein, lipid, moisture, and ash. Also, it includes polysaccharides, phenolics, vitamin C, triterpenic acids, and flavonoids. Recent *Z. jujube* studies demonstrated their beneficial actions, like the antioxidant, anticancer, anti-inflammatory, hepatoprotective, and gastrointestinal protective activities (Chen et al., 2013; Gao et al., 2013) .

Experimental evidence has revealed the effect of free radicals in tumor onset and promotion. This suggests that continuous free radical production may play an important role in maintaining the transformed state of tumor cells (Ottino and Duncan, 1997). Melanoma is a cutaneous tumor characterized by abnormal proliferation of melanocytes that is commonly seen on the skin, with high metastatic potential and the main cause of death in skin cancer (Siwak et al., 2005; Volkovova et al., 2012). The mechanism of molecular and cellular events underlying human melanoma progression is still unknown. Therefore, more information is needed on genetic changes that trigger the onset and progression of melanoma. Keeping above in view, the present study was aimed at investigating the effect of essential oil of *Ziziphus jujube* against skin cancer cell lines.

MATERIALS AND METHODS

The essential oil of *Z. jujuba* was extracted by hydrodistillation of 2 kg of the fruits using Clevenger-type apparatus. Each distillation was carried out for a period of three hours. Human melanoma cells (G361) were obtained from American Type Culture Collection (ATCC, Manassas, VA) and incubated with 5% CO_2 at 37 °C and kept in Dulbecco's Modified Eagle's Medium including streptomycin (100 µg/ml), fetal bovine serum (10%), glutamine (2 mM), penicillin (100 U/ml). Cells (0.4×10^5) were coated in 24-well plates and treated 3h with 100 µM essential oil of *Ziziphus jujube*. The supernatant was replaced with a fresh medium after the incubation.

Cell viability was measured by colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Hansen et al., 1989) based on quantifying the conversion of the tetrazolium salt to its formazan product to determine the mitochondrial activities of viable cells with MTT reagent (Fluka, USA). Melanoma cells (2×10^6 cells cultured in 2 mL culture medium each well) were placed three times in a 12- well plate and cultured at diversified times containing the specified doses of the agent at 37 °C. MTT (5 mg/ml; 1 ml/well) was administered to the well plates for an extra 2 h for each experimental time. Only the viable cells produced a dark blue formazan product. This product was dissolved in acidic isopropanol (1 ml/well), and measured by ELISA reader (µQuant-USA) at 570 nm to determine its absorbance. Cell viability was calculated by normalizing optical densities (OD) to negative control.

GSH levels were analyzed according to reaction with 5,5-dithiobis-2-nitrobenzoic acid resulting in the formation of a product that has a maximal absorbance at 410 nm (Sedlak and Lindsay, 1968). Nitric oxide levels in cell homogenates were measured by a method based on Griess reaction.

The total antioxidant level of the samples was analyzed according to the ABTS (dark blue colored radical) reduction capacity of antioxidants at 660 nm. The total oxidant level was measured by colorimetric methods at 530 nm.

Statistical analysis

Statistical analyses were performed with the one-way analysis of variance (ANOVA) and post hoc Duncan tests procedures of SPSS statistical software package. The results are expressed as average ±SE and $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

We analyzed the effects of ZJ essential oil on skin cancer cells viability using MTT assay. The administration for 3 h and 100% concentration of ZJ oil reduced cell viability in melanoma cells significantly (Figure 1).

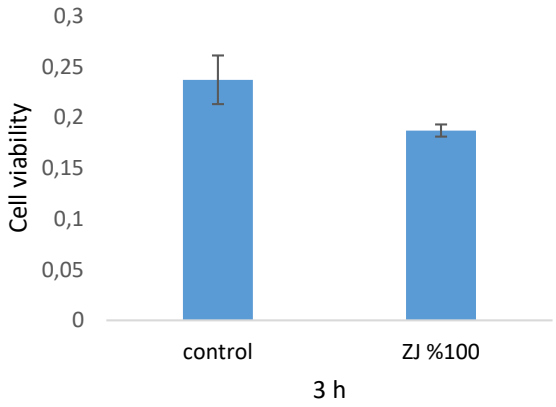

Figure 1. Effects of ZJ (100% concentration) on cell viability in melanoma cells (3h)

At the end of 3 hours, samples taken from cell media, NO oxide levels approximately 20% decreased in ZJ treated samples (Figure 2). GSH levels increased 2,5 times after 100% concentration ZJ administration for 3 hours (Figure 3). In the lysate samples obtained at the end of the study, in melanoma cells, the application of ZJ essential oil was found to decrease the TOC value by 7% (Figure 4), whereas ZJ essential oil increased the decreased TAC levels by 3% (Figure 5).

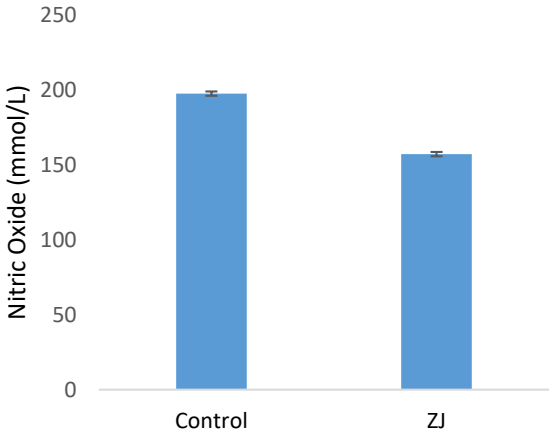

Figure 2. Effects of ZJ (100% concentration) on nitric oxide level in melanoma cells

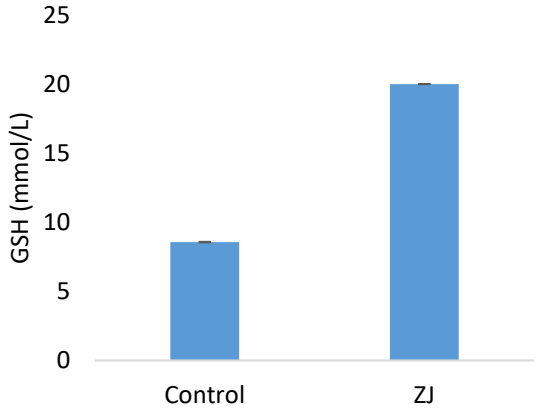

Figure 3. Effects of ZJ (100% con.) on glutathion level in melanoma cells (3 h).

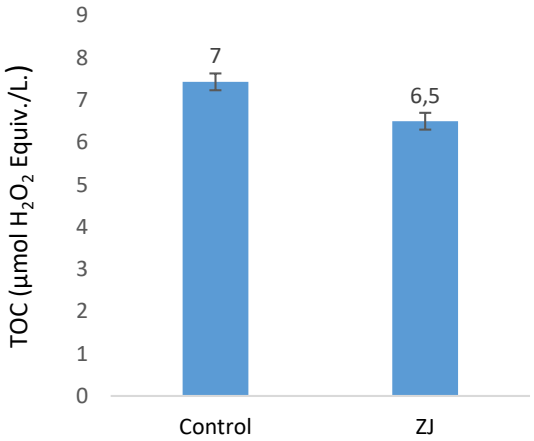

Figure 4. Effects of ZJ (100%) on TOC level in melanoma cells (3 h)

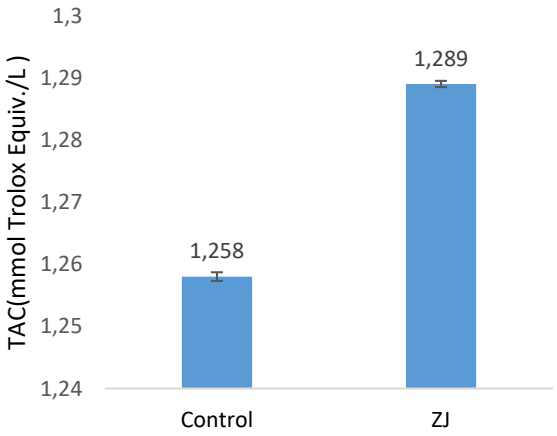

Figure 5. Effects of ZJ (100%) on TAC level in melanoma cells (3 h)

Ziziphus jujube is a potent medicinal herb traditionally used among thousands of medicinal plants. The antioxidative activities reported in this study approved that the therapeutic effects of *Ziziphus jujube* are much more (Preeti and Tripathi, 2014). Hung et al. (2012) reported that the polysaccharide obtained from *Z. Jujube* has an anti-proliferative effect on the melanoma cell, depending on the dose and time. In addition, this increased the activity of caspase-3 and caspase-9 and triggered the formation of the apoptotic body, allowing melanoma cells to remain in the G2 / M phase during the cell cycle.

Traditional/alternative medicine offers several medicinal plants to treat many diseases and to get rid of them. Goyal et al. (2011) in their study on *Z. Jujube* fruit, have found that it possesses components such as jujuboside, flavonoid, and terpenes, which have anti-inflammatory effects, possibly with their mechanism that inhibits nitric oxide expression. Ursolic acid, isolated from jujube fruit, has been proven by studies on ursolic acid induce apoptosis in four human liver cancer cell lines that decrease cell viability depending on concentration and time (Yan et al., 2010). In in vitro studies using melanoma (B16F-10), prostate cancer (DU145) and breast cancer (MCF-7) cell lines, ursolic acid has been reported to show anticancer activity at micromolar concentrations by suppressing the NF-κB-mediated activation of Bcl-2 in these cells (Zhang et al., 2006; Manu and Kuttan, 2008; Kassi et al., 2009). Patel et al. (2009) demonstrates that changes in antioxidant activities are associated with nitric oxide production in oral cancer and this may have an important role in oral carcinogenesis. Current findings reported that *Ziziphus jujube* fruits have an important potential in preventing antioxidant activity. Studies have found that *Ziziphus jujube* fruits have hydroxyl, superoxide, and total radical scavenging activity, and protect DNA from H_2O_2 + UV damage, also from inflammation and aging (Pahuja et al., 2011; Rajopadhye et al., 2016). Studies in China have shown that polysaccharides in the jujube plant stimulate immune function and improve antioxidant activity. The high rate of antioxidant substances in the jujube plant has been found to increase the activity of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and natural killer (NK) cells produced as a defense against free radicals (Chi et al., 2015). Yue et al. (2014) reported that *Ziziphus jujube* treatment, most probably due to jujubosides, flavonoids, and terpenes presence, increases glutathione levels significantly and decreases malondialdehyde levels. Also, in the last decade, the studies focus on revealing the antioxidative and neuroprotective characteristics of natural herbal products. As a result, many natural herbal products are reported to protect neuronal cells from death and apoptosis in various neurodegenerative disorders (Kaeidi et al., 2015).

The antioxidant molecules obtained from natural compounds are known to be potential therapeutic agents in preventing the development and spread of cancer cells by targeting intracellular reactive oxygen species (Fuchs-Tarlovsky, 2013). The jujube essential oil we used in our study decreased nitric oxide levels in melanoma cells compared to control levels. Again, the essential oils of this plant increased the GSH level in cancer cells, decreased TOS levels and increased TAS values. Therefore, in the light of the results obtained, jujube essential oil contributed to the increase of antioxidant effect in melanoma cells. Also, ziziphus jujube showed important cytotoxic activity in melanoma cells. It is thought that the underlying mechanism of this effect may be that cancer cells stimulate proliferation by producing ROS, whereas antioxidants show anti-cancer or cytotoxic activity by clearing these species (Sammar et al., 2019).

In summary, recent study concluded that *Ziziphus jujube* is precious fruit rich in bioactive compounds with potential human health benefits. However, more studies are needed to determine the useful intracellular effects of the active *Ziziphus Jujube* Mill, the effectiveness of its components, as well as the default mechanisms of action for the prevention of oxidative stress and cancer.

This study can be useful for predicting other medicinal uses and potential drug or food interactions and can be useful for people who live in areas where jujube fruits are common but have few health resources.

CONCLUSION

As a result, with the data obtained in our study, it was found that the short-term application of the essential oil obtained from the *Ziziphus jujube* plant to the cells showed antioxidant properties in

the in vitro melanoma model. However, in order to be able to say the antioxidant effect exactly, it is necessary to support studies with many parameters and in vivo studies to reveal oxidative stress and antioxidant properties

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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