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PROTECTIVE EFFECT OF ÖKÜZGÖZÜ (*VITIS VINIFERA* L. CV.) SEED EXTRACT AGAINST HYDROXYL RADICAL INDUCED DNA DAMAGE

Mihdiye PİRİNÇÇİOĞLU[1], Göksel KIZIL[1], Bircan ÇEKEN TOPTANCI[1], Gültekin ÖZDEMIR[2], Murat KIZIL[1]

[1]Dicle University, Faculty of Science, Department of Chemistry, Diyarbakir, Turkey
[2] Dicle University, Faculty of Agriculture, Department of Horticulture, Diyarbakir, Turkey

Corresponding author email: gozdemir@dicle.edu.tr

Abstract

Grape is one of the most commonly consumed fruits in the world. It has various biological functions, due to its potential of rich polyphenol ingredients, most of which are contained in its seeds (70%) and skin (30%).The aim of this study was to investigate the protective role of Öküzgözü cv. grape seed extracts against hydroxyl radical that induced oxidative DNA damage. The results revealed that the presence of various concentrations of grape seed extract in the reaction mixture significantly inhibited DNA damage induced by reactive oxygen species (ROS). In conclusion, the results demonstrated that Öküzgözü cv. grape seed extracts protected DNA against hydroxyl radical that induced oxidative damage and the extract could be used as a valuable food supplement or a nutraceutical product.

Key words*: grape, Öküzgözü, DNA damage, hydroxyl radical.*

INTRODUCTION

The opinion of cancer prevention through antioxidant intervention is due to the fact that fruits, vegetables and plants contain antioxidants and are associated with low cancer rates in those who consume them (Collins, 2005).

The protective effect of plants against DNA damage and protein oxidation can be demonstrated *in vitro*. A growing number of natural components of food, particularly fruits and vegetables are regarded as possible antioxidants with a role in protecting the cell against free radicals damage and chemicals, which can generate the oxidative forms (Collins et al., 2001). Several substances in plants express cytotoxic and genotoxic activities and shown correlation with the incidence of tumours (Srividya et al., 2013). Therefore, understanding of the health benefits and/or potential toxicity of these plants is important.

Grape is one of the most widely grown fruit crops throughout the world. Grape is the world's largest fruit crop, with more than 61 million metric tons, cultivated mainly as *Vitis vinifera* for wine production (Arvanitoyannis, 2006). *Vitis vinifera* (common grape vine) is a species of *Vitis*, native to the Mediterranean region, central Europe, and southwestern Asia, from Morocco and Portugal north to southern Germany and east to northern Iran (Gazioğlu Sensoy, 2012).

Turkey, today, is the fifth largest producer of grapes and is becoming one of the most important wine producers in the world. Öküzgözü is a Turkish grape variety and Turkish wine is produced from this grape. Öküzgözü is a rounded, dark red grape and is the largest among the grape varieties grown in Turkey.

Previous reports show that the leaves, fruits and juice of *V. vinifera* L. have a hepatoprotective effect on acetaminophen induced hepatic DNA damage, apoptosis and necrotic cell death (Pirinççioğlu et al., 2012).

The antioxidant activity of grape seed extract which were prepared using various solvents, such as acetone, ethyl acetate, methanol and mixtures of different solvents, such as ethyl acetate (EtOAc) and water were evaluated by using a β-carotene-linoleate model system and linoleic acid peroxidation method. At 100 ppm concentration, various extracts showed 65–90% antioxidant activity (Jayaprakasha et al., 2001).

In another study anti-hyperglycaemic and antioxidant effect of grape seed extract was investigated in normal and streptozotocin-induced diabetic Wistar rats.
The results showed that oral administration of grape seed extract (100 mg/kg/day) reduced the levels of lipid peroxides and carbonylated proteins and improved the antioxidant activity in plasma and hepatic tissue in rats treated with grape seed natural extract as compared with the diabetic control rats.
These results suggested that the grape seed extract enhanced the antioxidant defence against reactive oxygen species produced under hyperglycaemic conditions, hence protecting the liver cells (Chis et al., 2009).
The main objective of this study was to investigate the protective role of Öküzgözü cv. grape seed extracts against hydroxyl radical that induced oxidative DNA damage.

MATERIALS AND METHODS

Plant Material

Fresh Öküzgözü cv. grape samples were harvested at optimum maturity from Elazığ (Sün Village).
After removing the seeds by squeezing the berry gently by hand, grapes (2 kg) were washed and the seeds were removed from the pulp by squeezing the fruit gently (Figure 1).

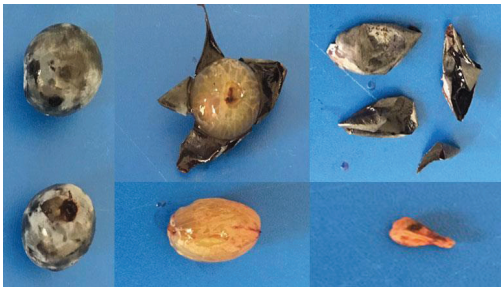

Figure 1. Öküzgözü cv. grape berry

Seeds were dried at room temperature and powdered in a blender. Powdered seeds were extracted with petroleum ether (250 mL) at 60 ^{o}C for 6h and ethyl acetate:methanol:water (150:75:25; 250 mL) for 8 h in a Soxhlet extractor, respectively. Finaly, ethyl acetate extract was filtered under pressure, then the filtrate was frozen and lyophilised in a lyophiliser (Martin Christ, 0.21 mm Hg, 80 °C) for 24 h. The lyophilised powder of seeds (4.5 g) was stored at -20°C until use (Baydar et al., 2007).

DNA cleavage protective effect of Öküzgözü cv. grape seed extract

DNA damage protective effect of Öküzgözü cv. grape seed extractagainst hydroxyl radical that induced DNA damage was investigated on pBluescript M13 (+) plasmid DNA. Plasmid DNA was isolated by Qiagene plasmid miniprep kit (Kızıl et al., 2003). Plasmid DNA was oxidized with H_2O_2 + UV treatment in presence of seed extract and checked on 1% agarose after modification (Attaguile et al., 2000). In brief, the experiments were performed in a volume of 10 µL in a microcentrifuge tube containing 200 ng of plasmid DNA in phospate buffer (7.14 mmol phospate and 14.29 mmol NaCl), pH 7.4. H_2O_2 was added at a final concentration of 2.5 mmol/L with and without 1 µL of (100, 200, 300, 400 µg/mL) seed extract. The reactions were initiated by UV irradiation and continued for 5 min on the surface of a UV transilluminator (8000 µW cm^{-1}) at 300 nm at room temperature. After irradiation, the reaction mixture (10 µL) with gel loading dye was placed on 1% agarose gel for electrophoresis. Electrophoresis was performed at 40 V for 3 h in the presence of ethidium bromide (10 mg/mL). Untreated pBluescript M13+ plasmid DNA was used as a control in each run of gel electrophoresis along with partial treatment, i.e., only UV treatment and only H_2O_2. Percent inhibition of the DNA strand scission was calculated as follows.

$$\text{Inhibition (\%)} = I\text{-}[(S_{m+a}\text{-}S_c)/(S_m\text{-}S_c)]$$

where S_{m+a},is the percentage remaining supercoiled after treatment with mix plus agent, S_c, is the percentage remaining supercoiled in control untreated plasmid and S_m, is the percentage remaining supercoiled with mix without agent (Fukuhara et al., 1998).

Densitometric analysis of treated and control pBluescript M13+ plasmid DNA

Gel was scanned on Gel documentation system (Gel-Doc-XR, BioRad, Hercules, CA, USA).

Bands on the gels were quantified discovery series Quantity One programme (version 4.5.2, BioRad Co.).

RESULTS AND DISCUSSIONS

When DNA was exposed to H_2O_2 and irradiated with UV light, H_2O_2 will generated to hydroxyl radicals, then the supercoiled form of DNA would cleave. Figure 2 shows the quantified band intensity for the sc-DNA (form I), oc-DNA (form II) and l-DNA (form III) and the electrophoretic pattern of DNA after UV-photolysis of H_2O_2 (2.5 mM) in the absence and presence of the seed extract of *V. vinifera* L. (100, 200, 300, 400, 500 µg/mL). DNA derived from pBluescript M13+ DNA plasmid showed two bands on agarose gel electrophoresis (lane 1), the faster moving band corresponded to the native form of supercoiled circular DNA (scDNA) and the slower moving band was the open circular form (ocDNA). The UV irradiation of DNA in the presence of H_2O_2 (lane 2) resulted in the cleavage of scDNA to linear form (linDNA), indicating that $^{\cdot}OH$ radical generated from UV photolysis of H_2O_2 produced DNA strand scission. The addition of extract (lanes 6-10) to the reaction mixture suppressed the formation of linDNA and induced a partial recovery of scDNA. In fact, the intensity of scDNA bands scanned from the agarose gel electrophoretic patterns was 45.69, 51.07, 61.77 and 68.07 % for plasmid DNA treated with H_2O_2 in the presence of 100, 200, 300, 400, and 500 µg/mL extract, respectively, as compared with the untreated plasmid DNA. The inhibition activities of *V. vinifera* L. seed extract on DNA damage were found to be between 18.10-62.69% at the concentrations rate of 100-500 µg/mL.

Thus, the identification of natural products able to provide protection against UV radiation-induced inflammatory responses and the generation of oxidative stress may have important human health implications. In fact, seed extract suppressed the formation of linDNA, generated by exposure of plasmid DNA to OH radical generated by H_2O_2 UV-photolysis, and induced a partial recovery of scDNA. DNA damage protecting activity of *V. vinifera* L. seed extract is corresponding to its antioxidant potential.

ROS-induced DNA damage can be described both chemically and structurally and shows a characteristic pattern of modification. It is well known that in various cancer tissues free radical-mediated DNA damage was found (Valko et al., 2001). The majority of these changes can be reproduced by ROS experimentally including the following: modification of all bases, production of base-free sites, deletions, frameshifts, strand breaks, DNA-protein cross-links, and chromosomal rearrangement. An important reaction involved in DNA damage involves generation of hyrodxyl radical, e.g., through Fenton chemistry (Brezova et al., 2003). Hydroxyl radical is known to react with all components of the DNA molecule: the purine and primidine bases as well as the deoxyribose backbone (Valko et al., 2004).

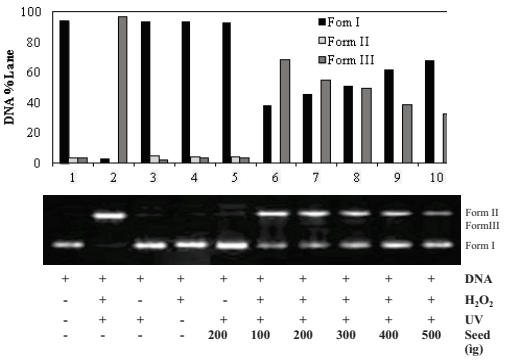

Figure 2.The quantified band intensity for the sc-DNA (form I), oc-DNA (form II) and l-DNA (form III) with Quantity One 4.5.2. version software and Electrophoretic pattern of pBluescript M13+ DNA oxidized with H_2O_2+UV treatment in presence of seed extract

Reaction vials contained 200 ng of supercoiled DNA (31.53 nM) in distilled water, pH 7). Electrophoresis was performed using 1% agarose at 40 V for 3 h in the presence of ethidium bromide (10 mg/mL).

Electrophoresis running buffer: TAE (40 mM Tris acetate, 1 mM EDTA, pH 8.2). Lane 1, control DNA; Lane 2, DNA + H2O2 (2.5 mM)+UV; Lane 3, DNA + UV; Lane 4, DNA + H2O2 (2.5 mM); Lane 5, DNA + Vv (200 µg/mL); Lane 6, DNA + Vv (100 µg/mL) + H2O2 (2.5 mM) + UV; Lane 7, DNA + Vv (200 µg/mL) +H2O2(2.5 mM) + UV; Lane 8, DNA + Vv (300 µg/mL) + H2O2 (2.5 mM)+UV; Lane 9, DNA + Vv (400 µg/mL) +

H2O2 (2.5 mM) + UV; Lane 10, DNA + Vv (500 µg/mL) + H2O2 (2.5 mM) + UV. Reactions were all performed at room temperature in phospahate buffer containing 100 mM sodium chloride.

CONCLUSIONS

The present study suggests that, grape seed extract is capable of suppressing DNA cleavage *in vitro*. Therefore, may be beneficial in the prevention of reactive oxygen species (ROS) related diseases, such as cardiovascular, inflammatory and cancer.

In conclusion, we still do not know how fruits, vegetables and plants protect against cancer, but it seems increasingly unlikely that it is simply because they contain high concentrations of antioxidants.

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