

Protective roles of Cimin grape tissues on oxidative stress markers in the cellular system model

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Aim: To analyze the phenolic composition of the Cimin grape, which is 1 of 2 grape varieties with a protected geographical indication in Turkey and is used locally to treat the symptoms of some disorders such as impotence and cardiovascular diseases, and to investigate its antioxidant potency against oxidant mediators in the models of intra- and extracellular surroundings.

Materials and methods: Cimin grape samples were classified into 5 groups according to the grape's tissues and the extraction solvents used. Free radical scavenging (DPPH) and antilipid peroxidation product [thiobarbituric acid reactive substance (TBARS) and conjugated diene (CD)] levels of the grape tissues were extrapolated from the measurement of total phenolic and individual monomeric flavanol contents in each extract.

Results: The seed methanolic extract had the highest total amount of flavanols, with the amount of catechin at 4.034 mM. The DPPH activities of the seed extracts were 2- to 10-fold higher than those of the other samples. The seed extract-treated erythrocyte and unfractionated human plasma also showed lower TBARS and CD values. In addition, regeneration of glutathione was more obvious in grape seed extracts than in the rest of the tissues.

Conclusion: The underlying mechanism of these changes can be related mainly to increased antioxidant status. Cimin grape consumption may have beneficial effects on health maintenance.

Key words: Cimin grape, flavanols, glutathione, reactive oxygen species, thiobarbituric acid reactive substance

1. Introduction

The effects of reactive oxygen species (ROS) on cellular and extracellular components of organisms have been investigated extensively in recent years (1). ROS such as hydroxyl radical, superoxide anion, peroxynitrite, and hydrogen peroxide are generated in biological systems by aerobic metabolism, or by exogenous factors including drugs, pollution, and UV light. ROS react with most cellular components including lipids, proteins, carbohydrates, and nucleic acids (2,3). Oxidative stress, the imbalance between the formation and manifestation of ROS, and the ability of a biological system to detoxify the reactive intermediates or to repair the damage causes pathophysiological events involving atherosclerosis, cancer, and autoimmune disorders (2).

In order to counteract the aforementioned effects of ROS, cells employ complex redox mechanisms, both enzymatic (superoxide dismutase, glutathione reductase, catalase) and nonenzymatic (polyphenols, tocopherols,

ascorbic acid, glutathione) (4). Moreover, foods, especially fruits and vegetables, and beverages also have a crucial role in protecting the physiological redox balance (5). The average human diet has a rich mixture of a great variety of nutrients with antioxidant properties. Among these nutrients, polyphenolics, including flavonoids (catechin, quercetin, and proanthocyanidins), which are abundant in plentiful supply in fruits and plants such as oranges, garlic, teas, and grapes, play an important role in the protection of the redox balance (6).

Flavan-3-ols [(epi)catechin and its gallyl forms] are the building blocks of most proanthocyanidins, which are widely present in grapes, plums, and cranberries (7). The amounts of monomeric and polymeric forms of flavonoids in grapes are higher than those of other fruits, such as grapefruits, oranges, peaches, and pears. The primary protective effect of these phenolic antioxidants in the fruits is the trapping and stabilization of free radical species by inhibiting enzymes or chelating metals involved in their generation (8).

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Erythrocytes have been commonly used as a cellular model in the investigation of in vitro free radical-induced oxidative stress because of their accessibility, easy preparation, high membrane concentration of polyunsaturated fatty acids (PUFAs), rich oxygen supply associated with redox active hemoglobin molecules, and inability to replace damaged components. Free hemoglobin subjected to H_2O_2 undergoes heme degradation, and the iron cations are released in this reaction, which catalyzes the production of ROS and lipid peroxidation (3). In addition to this 'intracellular' erythrocyte model, there is an 'extracellular' in vitro/ex vivo system in which ROS activities can be measured as antioxidant supplementations that provide protection against lipid peroxidation in unfractionated human plasma (UHP) (9).

Grapes are extensively consumed, either fresh or in the forms of grape juice, dried grape, table grapes, and wine. Turkey is the fifth largest grape producer in the world with many varieties (10). The Cimin grape variety is grown only in Turkey and mainly in the Üzümlü district of Erzincan Province; it is also popularly known as Erzincan grape. Cimin grape, generally considered unsuitable for wine-making, has a short shelf-life, very soft seed, and superfine skin with a dark color. Its seed paste has been widely used by regional people to treat impotence, cardiovascular disorders, diabetes, and indigestion (11). Cimin grapes are 1 of the only 2 grape varieties that has obtained a protected geographical indication and designation of origin in Turkey. We did not find any scientific report about the phenolic composition and biological activity of the Cimin grape. The aim of this study is to investigate the phenolic contents and the radical scavenging activity in aqueous and methanolic extracts of the different tissues of the Cimin grape and to compare the antioxidative effects of these extracts in human erythrocyte and plasma exposed to in vitro oxidative stress.

2. Materials and methods

2.1. Chemicals

Phenolic standards, (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epigallocatechin-3-gallate, (-)-epicatechingallate, gallic acid, and Folin-Ciocalteu reagent were obtained from Sigma-Aldrich (St Louis, MO, USA). Thiobarbituric acid (TBA), trichloroacetic acid (TCA), copper(II) chloride, sodium azide, hydrogen peroxide, 2,2-diphenyl 1-picrylhydrazyl (DPPH), and 5,5-dithiobis 2-nitrobenzoic acid (DTNB) were purchased from Merck (Darmstadt, Germany).

2.2. Preparation of grape extracts

Red grape samples (*Vitis vinifera*, variety Cimin) were collected from a cultivated field in the Üzümlü district of Erzincan, Turkey, in September 2010. The grapes were harvested at optimum maturity. Their seeds and skins were

manually separated when fresh, air-dried, and ground in 10 s to a fine powder by using a grinder. The extracts of skins and seeds were obtained by treating 2 g of the dry powder with 150 mL of absolute methanol in a Soxhlet apparatus for 2 h. The aqueous extracts were obtained by treating 2 g of the dry powder with 150 mL of water at 25 °C for 18 h in a shaker bath. The extracts were then concentrated by rotary vacuum evaporation and lyophilized. In parallel, grape pulps were squeezed through sterilized linen; freshly prepared and hand-squeezed grape juices were filtered through a 0.45-mm filter (Millipore, Molsheim, France) and pasteurized at 85 °C. Consequently, 5 different types of grape samples were obtained: methanolic extracts of seeds (GSeM) and skin (GSKM), aqueous extracts of seeds (GSeA) and skins (GSKA), and grape juice (GJ). All the extracts were kept at -20 °C until analysis.

2.3. Estimation of total phenolics

Total phenolic contents in the extracts were spectrophotometrically determined at 700 nm by Folin-Ciocalteu reagent (12) and expressed as catechin (mM) equivalents (CE).

2.4. Estimation of flavan-3-ols

The flavan-3-ol constituents were determined by HPLC-PDA system (Thermo Separation Products, Waltham, MA, USA) as described in ISO14502-2 (13). For the separation, a Luna C_{18} reverse-phase column (4.6 mm × 250 mm) packed with 5- μ m-diameter particles (Phenomenex, Torrance, CA, USA) operating at 35 °C was used. The eluent was formed from A) C_2H_3N : H_2O in EDTA (20 mg/L) : acetic acid (9:89:2) and B) C_2H_3N : H_2O in EDTA (20 mg/L) : acetic acid (80:18:2), and the flow rate was 1 mL/min. The elution profile was performed as follows: 0–10 min, 0% B; 10–25 min, 32% B; 25–35 min, 32% B and 35–45 min, 0% B. Chromatograms were recorded at 278 nm, and major flavanol constituents in the extracts were identified and quantified by using the standards and their calibration curves.

2.5. DPPH radical scavenging activity

Antiradical activities of the extracts of grape tissues were measured by utilizing the DPPH• method (14) and expressed as SC_{50} (mg/mL), the concentration necessary for a 50% scavenging of DPPH•. Lower absorbance of the reaction mixture indicates higher DPPH• radical scavenging activity. Distilled water was used as a negative control and catechin was used as a positive control.

2.6. Preparation of hemolysates from human erythrocytes

Peripheral venous blood samples were obtained from healthy volunteers (aged 23–31 years, mean: 26) and collected in ethylenediaminetetraacetic acid (EDTA)-containing tubes. For packing the erythrocytes, erythrocytes were washed 3 times with phosphate-buffered saline (PBS) to remove platelets and buffy coat after the

pooled blood was centrifuged at $3000 \times g$ at $4\text{ }^{\circ}\text{C}$ for 10 min. Packed erythrocytes were gently resuspended in PBS following the final wash. Erythrocyte suspensions were hemolyzed by adding ice-cold distilled water to obtain a target hemoglobin (Hb) concentration of 3 g/dL. The amount of Hb was determined according to the method of Drabkin et al. (15).

2.7. Experimental oxidative stress

For H_2O_2 -induced oxidative stress experiments with a cellular system, hemolysates were divided into 7 groups: control group (hemolysate and PBS), H_2O_2 -exposed group (hemolysate exposed to 10 mM H_2O_2 and PBS), and coinubation groups (hemolysate exposed concurrently to 10 mM H_2O_2 and methanolic and aqueous extracts of grape tissues, in concentrations equivalent to 100 μM catechin in PBS). For Cu^{+2} -induced oxidative stress, similarly, diluted plasma was exposed to Cu^{+2} (50 μM final concentration) with and without the extracts (in final concentrations equivalent to 50 μM catechin).

2.8. Estimation of glutathione (GSH) in erythrocyte hemolysate

GSH level in hemolysates was determined by DTNB test (16) and the results were expressed as mM/g Hb. The measured absorbance in samples was converted to concentrations using molar absorptivity coefficient of GSH ($\epsilon_{412} = 13.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$).

2.9. Estimation of lipid peroxidation products in erythrocyte hemolysates and plasma

Malondialdehyde (MDA) reacts with TBA in an acidic medium. The level of thiobarbituric acid reactive substances (TBARS) was determined as a measurement of oxidative stress in the erythrocyte and plasma for all fractions. The analyses of the erythrocyte TBARS were performed according to the method of Stocks et al. (17) with some modification, and the concentrations were expressed as nmol TBARS/g Hb. Grape extracts (100 μM) were added to 1 mL of erythrocyte hemolysate, and lipid peroxidation was induced by the addition of H_2O_2 at a final concentration of 10 mM. The absorbance of the chromogenic product (TBARS) was measured at 532 nm. The 1,1,3,3-tetraethoxypropane was dissolved in water and used to obtain the standard curve for TBARS.

The inhibition of plasma (UHP) lipid peroxidation was determined according to the method of Durak et al. (18) with a slight modification. Oxidative stress levels were then spectrophotometrically measured as TBARS concentration, as described by Wills et al. (19) TBARS levels were expressed as nmol/mL. At the same time, conjugated diene (CD) formation in UHP was measured in diluted plasma. After being diluted 150-fold, UHP was individually preincubated with each extract for 15 min, and the oxidation was started by adding Cu^{+2} as described by Spranger et al. (20). Measurements were performed in

a spectrophotometrical cuvette at $37\text{ }^{\circ}\text{C}$ by continuous recording with absorbance readings taken automatically every 6 min for 12 h against a reagent blank (without extract). There were 3 indexes indicating susceptibility of UHP to oxidation used: lag time (t-Lag), maximal rate of diene production (MDR) using the molar absorbance coefficient for conjugated dienes ($\epsilon_{234} = 29,500$), and the total amount of dienes produced (MDC).

2.10. Statistical analysis

All the measurements were performed as triplicates for each set of grape part extracts. Results were expressed as mean \pm SD. Numeric data were tested for distribution characteristics by the Kolmogorov-Smirnoff test. One-way analysis of variance (ANOVA) was performed and means were compared using Dunnett's test. Statistical significance was considered as $P < 0.05$. SPSS 16.0 was used in all statistical analyses.

3. Results

The phenolic content of the grape was estimated since it is believed to be responsible for most of the antioxidant properties. Figure 1 shows total phenolic substances (TPS) as mM catechin equivalent. Individual monomeric flavanol (IMF) contents of the different extracts of grape tissues are listed in Table 1. TPS was the highest in GSeA, followed by GSeM. The catechin was the most abundant among monomeric flavanols, followed by epicatechingallate, in all the extracts. The catechin content of GSeM was more predominant than those of the other extracts. TPS and IMF contents of GJ and the extracts of grape skin were lower than those of the grape's seed.

DPPH assay as a free radical-scavenging activity has been widely used to evaluate the antioxidant capacity of

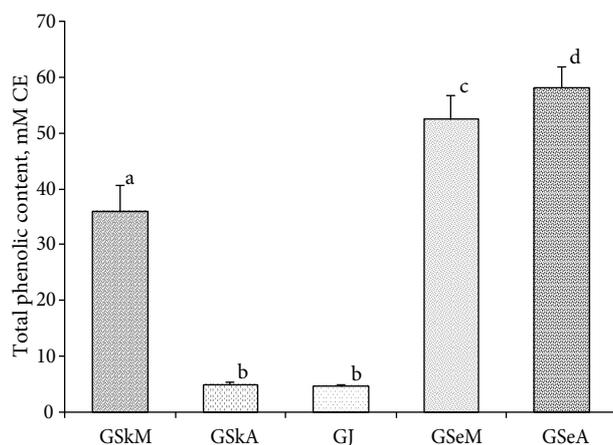


Figure 1. Total phenolic substances of the aqueous and methanolic extracts of grape tissues given as mM catechin equivalent. Values with different superscripts differ significantly at $P < 0.001$ according to ANOVA and means compared using Dunnett's test.

Table 1. Individual monomeric flavanol (IMF) composition of grape tissues.

Extract types	IMF (μM)				
	C	EC	ECG	EGCG	EGC
Methanolic extract of grape skin (GSkM)	7	340	3.10	11	186
Aqueous extract of grape skin (GSkA)	20	2	0.07	1.70	3
Grape juice (GJ)	11	6.5	0.11	12	18
Methanolic extract of grape seed (GSeM)	4034	<0.01	4.70	28	1004
Aqueous extract of grape seed (GSeA)	596	6.80	0.13	6.5	171

C: Catechin, EC: epicatechin, EGC: epigallocatechin, EGCG: epigallocatechin-3-gallate, ECG: epicatechingallate.

various plants. Our results obtained from the grape tissues are listed in Table 2. The lowest SC_{50} values were found for both the extracts of the grape seed, whereas GSkA and GJ showed the highest SC_{50} values, indicating lower activity. The DPPH free radical-scavenging activity in the extracts of the grape seed was almost 9-fold higher than that of the aqueous skin extract and the grape juice.

Red blood cells (RBCs) are susceptible to oxidative damage because of the high concentrations of PUFAs in their membranes. The addition of H_2O_2 into reaction mixture significantly increased the level of TBARS. Table 3 indicates that TBARS values of RBC hemolysate resulted directly from membrane lipid peroxidation after an incubation period of 45 min and H_2O_2 -induced oxidative stress, with and without the extracts of the grape. GSeA and the methanolic extracts of the grape in comparison to H_2O_2 -induced RBC hemolysates without the extract showed a significant decrease in lipid peroxidation. The methanolic extracts of the grape, rich in flavanol contents, have a more protective effect than those of aqueous extracts of the grape. On the other hand, GJ and GSkA,

with lower levels of flavanol contents, failed to show the same protective effect. Treatment of RBC hemolysates with grape extracts as mentioned above reduced the TBARS production by almost 15% compared with the control group. GSH level in hemolysates was established and is shown in Table 3. The grape extracts could not protect the whole GSH reserve of the cell. Each extract of grape seed as compared with other extracts, nonetheless, induced a significant increment in GSH levels of erythrocytes.

To further confirm the protective effect of the grape tissues, we measured the formation of TBARS and CDs by copper-induced oxidation in UHP as an extracellular environment. The protective benefits of the extracts, compared to UHP without the extracts (control group), on the kinetics of copper-induced diene-formation are given in Table 4. The longest lag time for CD formation was observed in UHP with GSeA, followed by GSkM. GSkA had the shortest lag time. Similarly, lower levels in maximal formation rate and concentration of CD in UHP were observed in the extracts of GSeM, GSeA, and GSkM. Additionally, TBARS results obtained by Cu^{+2} -induced oxidative stress in UHP are given in the same table. Again, both extracts of the grape seed were rich in catechins, compared to all of other groups, and exhibited the most preventative effects against oxidative stress. In Figure 2, the kinetic data of Cu^{+2} -induced UHP oxidation in all study groups show that their absorbance values and slopes were significantly higher than those of the control group. In light of these results, both extracts of grape seed and methanolic extract of grape skin apparently have the best protective effects against oxidative stress on either cellular model system.

4. Discussion

In the present study, the flavan-3-ol composition of the grape tissues was initially analyzed. In vitro protective effects of the tissues of the Cimin grape were then tested against oxidative stress, through the level of lipid peroxidation product evaluated in RBC hemolysates as an intracellular model. The beneficial effects of the grape

Table 2. DPPH free radical-scavenging values[‡] of the tissues of grape and standard.

Sample types	SC_{50} (μM catechin [§])
Standard (catechin)	$56 \pm 1^{\text{a}}$
GSkM	$142 \pm 13^{\text{b}}$
GSkA	$910 \pm 35^{\text{c}}$
GJ	$617 \pm 38^{\text{d}}$
GSeM	$74 \pm 2^{\text{e}}$
GSeA	$83 \pm 8^{\text{e}}$

[‡]: Values are expressed as mean \pm S.D, n = 3.

[§]: Molar concentrations of total polyphenols in catechin molecular weight equivalent.

^{a, b, c, d, e}: Values in the column with different superscripts differ significantly at $P < 0.01$.

Table 3. TBARS and GSH levels[‡] in the H₂O₂-treated erythrocytes with and without different extracts.

Samples	TBARS		GSH	
	(nmol/g Hb)	% Decrease [§]	(μmol/g Hb)	% Increase [§]
Erythrocyte	38 ± 7 ^{a*}		3.47 ± 0.66 ^{d*}	
Erythrocyte + H ₂ O ₂	680 ± 74 ^d		0.15 ± 0.11 ^a	
Erythrocyte + H ₂ O ₂ + standard (catechin)	404 ± 34 ^{b**}	40.6	0.49 ± 0.11 ^c	227
Erythrocyte + H ₂ O ₂ + GSkM	601 ± 56 ^c	11.6	0.18 ± 0.10 ^a	20
Erythrocyte + H ₂ O ₂ + GSkA	677 ± 40 ^d	0.4	0.15 ± 0.07 ^a	0
Erythrocyte + H ₂ O ₂ + GJ	678 ± 62 ^d	0.3	0.19 ± 0.18 ^a	27
Erythrocyte + H ₂ O ₂ + GSeM	591 ± 59 ^c	13.1	0.35 ± 0.11 ^b	133
Erythrocyte + H ₂ O ₂ + GSeA	622 ± 77 ^c	8.5	0.33 ± 0.16 ^b	120

[‡]: Values are expressed as mean ± SD, n = 10.

[§]: Percentage changes between the values for H₂O₂-induced erythrocytes with and without the grape extract.

^{a, b, c, d}: Values in the same column with different superscripts differ significantly at P < 0.05. ^{*}: Statistically different from erythrocytes with H₂O₂, GSkA, and GJ at P < 0.005.

^{**}: Statistically different from erythrocytes with H₂O₂ and all other groups at P < 0.0001.

Table 4. TBARS levels[‡] and CD indexes[‡] in the Cu²⁺-induced lipid oxidation in UHP with and without different extracts.

Samples	TBARS (nmol/mL)	t-Lag (min)	MDC (nmol/mg protein)	MDR (nmol mg protein ⁻¹ min ⁻¹) × 10 ⁻²
UHP + Cu ⁺²	2.07 ± 0.31 ^b	60 ± 4 ^{a*}	7.13 ± 2.85	3.6 ± 1.4 [§]
UHP + Cu ⁺² + Trolox	1.28 ± 0.11 ^a	163 ± 2 ^{d*}	7.81 ± 0.51	2.5 ± 0.1
UHP + Cu ⁺² + GSkM	1.50 ± 0.21 ^a	161 ± 5 ^{d*}	5.71 ± 2.02	1.7 ± 0.4
UHP + Cu ⁺² + GSkA	1.47 ± 0.40 ^a	81 ± 2 ^{b*}	9.07 ± 1.56 ^f	4.1 ± 0.8 [§]
UHP + Cu ⁺² + GJ	2.03 ± 0.30 ^b	106 ± 7 ^{c*}	8.41 ± 1.43 ^f	3.7 ± 0.5 [§]
UHP + Cu ⁺² + GSeM	1.36 ± 0.16 ^a	99 ± 5 ^{c*}	5.72 ± 1.47	2.6 ± 0.5
UHP + Cu ⁺² + GSeA	1.47 ± 0.11 ^a	190 ± 20 ^{e*}	5.48 ± 1.55	1.5 ± 0.5

[‡]: Values are expressed as mean ± SD, n = 6.

^{a, b, c, d, e}: Values in the same column with different superscripts differ significantly at P < 0.05. ^f: Statistical difference between UHP with GSeM and GSeA and GSkM at P < 0.05.

[§]: Statistical difference between UHP with GSeA and GSkM and positive control, and GSkA and GJ, at P < 0.001.

^{*}: Values in this column with different superscripts are statistically different from each other at P < 0.001.

tissues were also compared in copper-induced oxidation of UHP used as an extracellular model.

Some plants and fruits have been shown to be rich sources of antioxidants. Antioxidants are involved in health maintenance and in retarding or reversing the processes of chronic degenerative diseases (21). Among dietary components, grapes, a good source of antioxidants, form the basis of discussions for the explanation of the phenomenon known as the 'French Paradox' (22). The paradox is about the observation that the incidence of cardiovascular diseases in France is relatively lower than that of other developed countries, despite a diet high in saturated fat. This has been attributed to a relatively higher

level of grape product (wine) consumption by the French. This is a remarkable example of the health benefits of grapes. The skin and seeds of the Cimin grape, like other *Vitis* varieties, contain many phenolic compounds, and therefore have protective effects. The results presented in this study demonstrate that the extracts of Cimin grape are potent scavengers of ROS. The DPPH scavenging activities and total phenolic contents of the grape samples were observed to be higher in the seed extracts. The seed extract also showed the most potent activity against oxidative stress in cellular and extracellular systems. Solvents used in the extraction process may potentially affect the determination of the flavonoid compositions of the sample

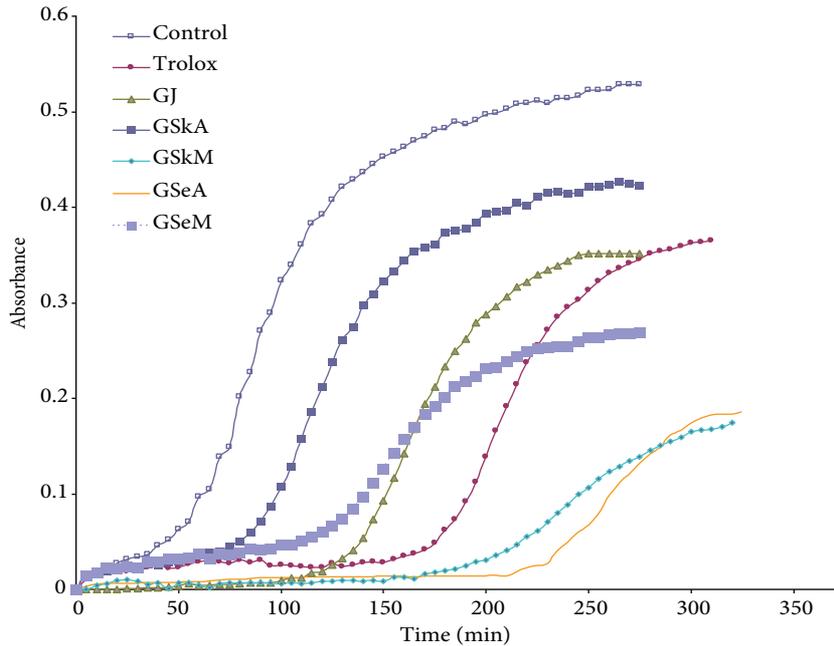


Figure 2. Kinetics of Cu^{2+} -induced oxidation of diluted UHP with and without the extracts and standard.

(23) and, therefore, water and methanol solvents were used to compare the efficiencies of extraction in this study.

Compositional analyses of grape tissues are important for the juridical and plant selection issues related with the grapes protected with a designation of origin. In general, flavan-3-ols [catechin (C), EC] and their gallyl derivatives (monomeric units of proanthocyanidin) are among the antioxidant compounds in grape extracts (24). In our study, the amount of C in the seed extracts was 120-fold higher than the sum in the extract of the skin and grape juice. The data for the C of the seed was in agreement with previous studies (7,9,25). The antioxidant activity of the grape seed extracts comes mainly from their flavonoid contents, mostly anthocyanins and flavanols. A positive correlation has been reported between the amount of C and radical scavenging activity (26). The lowest SC_{50} value was found in both types of seed extracts and the GSkM. GSkM contains more catechin derivatives with gallyl moiety. The flavan-3-ol content and its correspondence with radical scavenging potency in the grape seed extracts were in accordance with the findings of other authors (27,28). However, Bozan et al. (10) asserted that the higher antiradical activity of the extracts with lower C content may result from other flavonoid components of the extracts. Phenolic compounds scavenge free radicals by their aromatic hydroxyl groups, whose scavenging potential can be enhanced by electron donation from other constituents (29,30).

It has been known for a long time that flavan-3-ols can display antioxidant activity in numerous biological

systems. Erythrocytes are particularly sensitive to oxidative stress due to the presence of high levels of PUFAs in their membranes and high hemoglobin and oxygen contents (31). This system was therefore used to evaluate the antioxidant potential of grape extracts. The data obtained in the present study show that there is an inverse correlation between the flavan-3-ol and total phenolic contents of the grape and the TBARS levels in H_2O_2 -treated hemolysates. The extracts of grape seeds and GSkM brought about lower TBARS formation than that exhibited by GSkA and GJ. Since GSkM has higher acylated monomeric forms of flavan-3-ol than GSkA and GJ, it exhibits an important effect in prevention of membrane lipid peroxidation, as do the seed extracts. Epigallocatechin-3-gallate, which is acylated C, can act as an antioxidant by trapping peroxy radicals generated by the hydroxyl radical produced by H_2O_2 , which is able to easily penetrate the cellular membranes (31). This could explain the reason why the antilipid oxidation activity of GSkM extract is the same as those of GSeM and GSeA despite its containing very low C. The low amounts of flavanols in GSkA and GJ might be the reason why their TBARS are similar to those of the control lacking the extracts. The decrease in TBARS formation with the extract samples (seed, skin, and juice) compared to the control group without extract is almost 12%. A number of studies have demonstrated that the treatment of erythrocyte membrane with H_2O_2 results in peroxidation and disruptions of their structures and functions, including changes in ATPase activity and membrane fluidity (32,33). Many pathophysiological events such as inflammation,

aging, and ischemia/reperfusion are attributed to this peroxidation. MDA, which is a major content of TBARS in erythrocytes, reflects the extent of lipid peroxidation, and its cross-linking to membrane protein and phospholipids causes some disorders (21). Our results are consistent with the previous studies showing that the grape seed has higher antioxidant properties due to higher flavanol content (24).

The cellular damage resulting from oxidative stress in erythrocytes is due to the depletion of GSH, as well as the increase in lipid peroxidation (34). The maintenance of GSH level in an erythrocyte is important in the structural integrity and functional processes of its membrane (35). Ko et al. (34) had similar findings, showing that tea flavonoids have little effect on GSH levels of erythrocyte. In contrast, Carini et al. (25) showed that grape seed prevented the decrease of GSH levels in erythrocyte against UV-induced oxidative stress. The failure in the protection of erythrocyte GSH reserve in the experiment groups may be due to the insufficiency of polyphenolic compounds, because of consumption during lipid peroxidation.

Water-soluble extracts necessitate the use of UHP as an extracellular environment instead of the isolated low-density lipid (LDL) and high-density lipid (HDL). This enables the evaluation of the hydrophilic and hydrophobic compounds of UHP together against Cu^{+2} -induced oxidative stress. In addition, the results of previous studies (9,20), showing that long isolation procedures for lipoproteins impaired the antioxidant properties of hydrophilic flavonoid glycoconjugates in plant extracts, were another reason to select UHP for the extracellular model. Incubation of the highly diluted UHP with copper at 37 °C results in varying levels of lipid oxidations, such as dienic and 7-keto cholesterol hydroperoxide, as well as TBARS oxidation product (36). Total phenolic content of the grape extracts were negatively correlated with MDR, MDC, and t-Lag. The protective effect of plant extracts against oxidation has been observed in UHP (9) and

isolated LDL preparations (37) exposed to copper. When UHP is exposed to a strong oxidizing agent, plasma hydrophilic and lipophilic antioxidant contents including bilirubin, albumin, uric acid, ascorbate, and α -tocopherol are depleted, and subsequently the formation of lipid peroxides starts to rise (38). Our findings showing the protective effects of the extracts of Cimin grape tissues on UHP are in concordance with a previous report (38) that demonstrated the in vivo and in vitro inhibitory effects of grape skin on the formation of TBARS and CD in plasma. TBARS production in UHP was also suppressed by all of the samples except for GJ. The flavonoids of the grape tissues may retard lipid peroxidation by delaying the consumption of plasma antioxidants or by a chain-breaking action within the core of lipids in plasma. Both aqueous and methanolic extracts of the grape seed have beneficial antioxidant agents active under physiologic conditions, which suggest higher amounts of dietary consumption.

In conclusion, total and individual phenolic contents and radical scavenging activity of Cimin grape tissues in the different extraction media were comparatively studied. Initiation of lipid oxidation mediated by both H_2O_2 and Cu^{2+} was delayed by the addition of aqueous and methanolic extracts of Cimin grape tissues. In both models, the preventive effects of the grape seed extracts and the methanolic extract of the grape skin were more pronounced. In contrast, the grape juice and aqueous extract of the grape skin had no significant effect on the lipid peroxidation in these cellular systems. The results support the data generated by previous studies of grape in vitro. Further research on the bioavailability of grape part components and the susceptibility of erythrocytes or plasma lipids to oxidation in vitro in the presence of these components may offer a better understanding for large-scale human consumption.

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