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Antioxidant effect of grape molasses in rat heart tissues

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Abstract

Grape molasses, which is rich in mineral substances such as flavonoids, polyphenol, antioxidants, iron, calcium, phosphorus, potassium, and magnesium is a natural nutrient, that is concentrated with boiling without adding any additive. In this study, we aimed to investigate the effects of molasses and resveratrol diets on oxidative stress parameters in cardiac damage induced with 7,12-dimethylbenz(a)anthracene (DMBA). A total of 42 Wistar albino female rats were divided into six groups with seven in each. Control group was administered subcutaneous injection of 1 mL mixture including 20 mL sesame oil + 30 mL Dimethyl Sulphoxyde (DMSO); DMBA group received subcutaneous injection of 10 mg/Kg DMBA on the 0th and 7th days. DMBA + Molasses group received DMBA application and feed containing 20 % molasses; DMBA + Resveratrol group received subcutaneous injection of DMBA + 10 mg/Kg resveratrol a day. Molasses group received feed with 20 % molasses, and Resveratrol group received subcutaneous injection of 10 mg/Kg resveratrol a day. There was a statistically significant difference between the DMBA group and DMBA + Molasses, control, resveratrol and molasses groups in terms of nitric oxide activity. There was a statistically significant difference between the DMBA group and DMBA + Resveratrol, DMBA + Molasses, control, molasses and resveratrol groups in term of malondialdehyde (MDA) activity. There was a statistically significant difference between DMBA groups, and DMBA + Molasses, DMBA + Resveratrol, and control groups in terms of glutathione (GSH) activity. Based on these results, it could be said that grape molasses could provide protection against oxidative stress as resveratrol, decreasing the risk of damage by free radicals.

Keywords: Grape molasses, resveratrol, oxidative stress, DMBA, heart

Introduction

Today, while industrial developments greatly facilitate our lives, it cannot be ignored that it increases environmental pollution. Industrial pollutants negatively threaten our health through their negative effects on air, water, soil, and foods.

Polycyclic aromatic hydrocarbons (PAHs) are released to nature during coke production, heating of residences and natural events such as forest fires and volcanoes, due to incomplete burning of fossil fuels, oils, tobacco and organic substances. Atmospheric transportation and accumulation of PAHs are commonly seen in the ecosystem [1]. PAHs that in the procarcinogen class are involved among carcinogens because of the suppression and inhibition of the immune system, their immunotoxic effects and formation of free radicals [2]. A PAH derivative, 7,12-dimethylbenz(a)anthracene (DMBA) is among the oldest atmospheric pollutants with its mutagenic, cytotoxic, carcinogenic and immunosuppressive damage by playing an active role in the formation of free radicals [3].

Free radicals are unstable and highly reactive molecules, that are formed by endogenous or exogenous sources in the cells, and carry uncompensated electrons on them. Free radicals include different species such as superoxide radicals, hydrogen peroxide, singlet oxygen, nitric oxide (NO) and peroxynitrite. The most important radicals in biological systems are oxygen-induced reactive oxygen species (ROS). Antioxidant defense system decelerates or eliminates ROS-induced damage. However, oxidative stress occurs as a result of increased reactive oxygen species due to different reasons as well as the antioxidant mechanism that remain insufficient [4].

Resveratrol (3,5,4′-trihydroxystilbene) is a polyphenolic phytoalexin found in grapes, wine, peanuts, and blueberries, which shows antioxidant, anti-ischemic, anti-atherosclerotic, anti-hypertensive effects, anti-inflammatory, and anticarcinogenic properties. Its heart-protective effect has been named as French

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paradox due to it's in vivo and in vitro antioxidant and free radical scavenging [5,6]. Resveratrol is primarily found in grape skin at a concentration of 50-100 µg/g. Grape molasses, which is rich in mineral substances such as flavonoids, polyphenol, antioxidants, iron, calcium, phosphorus, potassium, and magnesium is a natural nutrient, that is concentrated with boling without adding any additive [7]. Resveratrol content of grape molasses obtained from different grape species has been reported between 0.02-0.12 mg/L [8]. It has been reported that, in addition to radical scavenging capacity, molasses has a protective effect against DNA oxidative stress owing to its rich content. In addition, it has been shown that, besides antioxidant and antimutagenic features, molasses can exhibit biological activities such as in vitro anti-inflammation [9]. In our study, we aimed to investigate the heart protective effects of resveratrol and grape molasses on PAH derivative 7,12-dimethylbenz (a) anthracene (DMBA) toxicity in rat cardiac tissues.

Material and Methods

Chemicals, animals, and diets

Rats used in this study was supplied from the Inonu University Experimental Animals Production and Research Center. The study was conducted in accordance with the principles of Inonu University Experimental Animals Ethics Committee (2011/A-106).

Female rats of 18 weeks, weighed 205±13 grams were kept in the housing cages, drinking water was refreshed daily, and the cases were cleaned with one-week intervals. The rats were harbored in ventilated rooms at 24-27oC with 12 hours dark/light cycle. A total of 42 rats were fed with standard pellet feed except the molasses groups.

Preparation of Resveratrol, DMBA

110 mg resveratrol was dissolved in 110 mL DMSO, and prepared for injection on the day of application. A mixture was prepared with 65 mg DMBA dissolved in 65 mL sesame oil. All chemicals and reagents used were of analytical grade and were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Bidestilled water was used in all the studies.

Preparation of Grape Molasses

Red grapes molasses used in the study was obtained from Arapgir district of Malatya province. Feeds containing 20% molasses were prepared and given to the rats ad libitum.

Experimental Design

Control Group (n=7): Rats in the control group received 1 mL subcutaneous injection from the mixture of 20 mL sesame oil + 30 mL DMSO.

DMBA Group (n=7): Rats in the DMBA group received subcutaneous injection of 10 mg/Kg DMBA mixture on the 0th and 7th days.

DMBA + Molasses Group (n=7): 10 mg/Kg was taken from the prepared DMBA mixture and subcutaneously injected on the 0th and 7th days. Feed containing 20% molasses was administered daily.

DMBA + Resveratrol Group (n=7): Resveratrol mixture was subcutaneously injected as 10 mg/Kg/day. Prepared DMBA mixture was subcutaneously injected on the 0th and 7th days.

Molasses Group (n=7): Feed containing 20% molasses was regularly left into the cages daily.

Resveratrol Group (n=7): Prepared resveratrol mixture was subcutaneously injected as 10 mg/Kg/day.

Preparation of the Tissues for Analysis

The rats were sacrificed on the 10th day of the study under general anesthesia. Heart tissues were labeled, individually wrapped with aluminum foils, and kept in a deep freezer at -70oC until the analysis.

Tissue Homogenization

2 mL Tris-HCl buffer was added on tissue samples weighed approximately 200 mg, and the mixture was homogenized at a rate of 16000 revolutions per minute. Following homogenization, tissue tubes were centrifuged at 4000 rpm and +4oC for 10 minutes. Supernatant portions were then put into Eppendorf tubes and kept in the freezer until the analysis.

Estimation of oxidative stress markers

Measurement of reduced glutathione

Total sulfhydryl content was determined with spectrophotometric measurement of the absorbance of yellow product formed as a result of the reaction with Ellman's Reagent 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB), at 412 nm [10].

Measurement of lipid peroxidation

The measurement of MDA, which is a marker of lipid peroxidation, based on spectrophotometric evaluation of the absorbance of pink-red color formed as a reaction with 2-Thiobarbituric acid (TBA) at 95oC, at 532 nm [11].

Measurement of nitric oxide

The level of nitric oxide (NO), which was formed with nitric oxide synthase (NOS) activity in the medium, was determined by spectrophotometric measurement of the color compound formed as a result of a reaction with Griess reactive by reducing fro nitrate to nitrite, at 545 nm [12].

Statistical Analysis

Homogeneity of the data was evaluated with a web-based free program, and it was found that the data showed no Homogeneity [13]. In order to make multiple comparisons within groups Kruskal-Wallis analysis was used. Comparisons between the groups were performed by using Conover test. Both analyses were done by using a web-based application [14].

Results

There was no statistically significant difference in GSH activity between the control, molasses and resveratrol groups ($p>0.05$). No significant difference was found between DMBA+Molasses and DMBA+Resveratrol groups, while there was a statistically significant difference between DMBA and DMBA+Molasses, DMBA+Resveratrol and Control groups in terms of GSH activity ($p<0.05$) Figure 1.

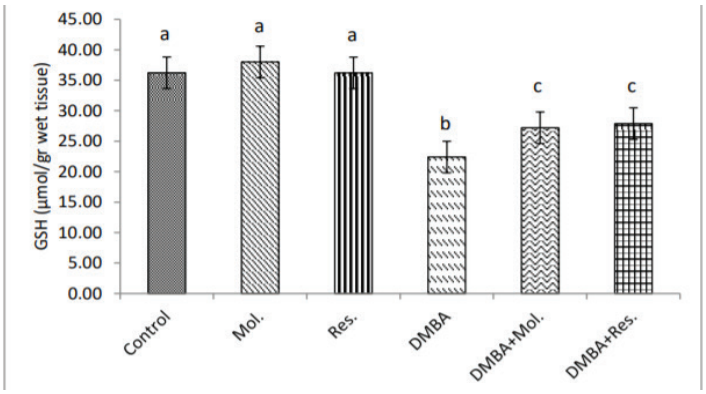

Figure 1. Comparison of GSH results. Different letters indicate the statistical difference between the groups. a,b,c: Statistically significant differences between the groups ($p<0.05$)

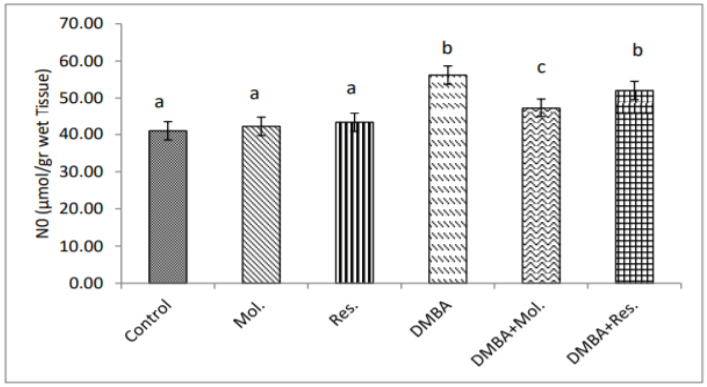

Figure 2. Comparison of NO results. Different letters indicate the statistical difference between the groups ($p<0.05$). a,b,c: Statistically significant differences between the groups ($p<0.05$)

There was no significant difference between DMBA and DMBA+Resveratrol groups in NO activity, while there as a statistically significant difference between DMBA and DMBA+Molasses, Control, Resveratrol and Molasse groups in terms of NO activity ($p<0.05$). No statistically significant difference was found between the control group, and Resveratrol and Molasses groups ($p>0.05$). There was no statistically significant difference between DMBA and DMBA + Resveratrol Groups in terms of NO levels ($p>0.05$) (Figure 2).

There was a statistically significant difference between the DMBA group, and DMBA+Resveratrol, DMBA+Molasses, Control, Molasses and Resveratrol groups in terms of MDA activity ($p<0.05$). The difference between the control group, and Molasses and Resveratrol groups in terms of MDA activity was not statistically significant ($p>0.05$) (Figure 3). Comparison of oxidative stress parameters within groups was given in Table 1.

Table 1. Comperation of oxidative stress markers. Different letters in the same column indicate the statistical difference between groups ($p<0.05$).

	N0 (µmol/gr wet Tissue) Median (min.-max.)	MDA(nmol/gr wet tissue) Median (min.-max.)	GSH (µmol/gr wet tissue) Median (min.-max.)
Control	40.98 (34.06-45.28)a	47.3 (40.04-49.2)a	27.79 (26.23-48.8)a
Mol.	44.17 (37.33-56.83)a	40.7 (37.57-43.56)a	37.95 (29.02-39.82)a
Res.	46.17 (44.7-51.73)a	43.23 (39.93-48.51)a	36.21 (33.08-48.62)a
DMBA	52.15 (35.34-65.28)b	54.12 (46.53-60.06)b	25.3 (20.83-29.26)b
DMBA+Mol.	45.77 (32.36-71.04)c	48.18 (35.64-53.79)c	28.78 (21.92-38.91)c
DMBA+Res.	46.17 (39.68-58.89)b	49.17 (38.94-57.09)c	27.89 (23.95-40.04)c

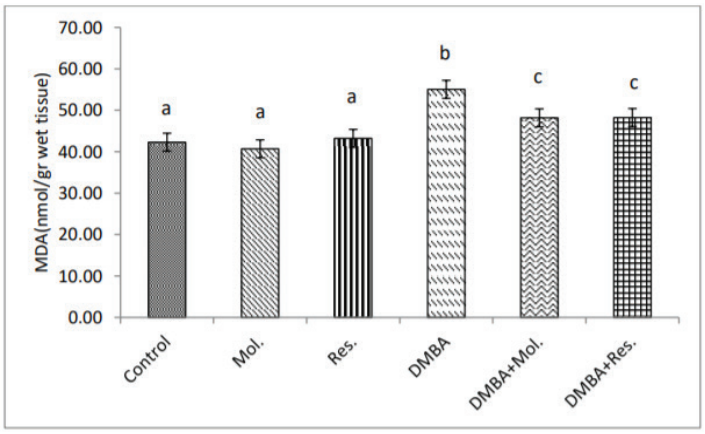

Figure 3. Comparison of MDA results. Different letters indicate the statistical difference between the groups ($p<0.05$). a,b,c: Statistically significant differences between the groups ($p<0.05$)

Discussion

Grapes dietary components including grape seed extracts and grape juice are promising for alternative medicine with anticarcinogenic properties as phyto-foods. It is known that, their mechanism of action is mostly based on decreasing oxidative stress, which plays an active role in the pathogenesis of cardiovascular disease and cancer [6].

In the present study, we investigated the effects of resveratrol and grape molasses on oxidative stress parameters in cardiac tissues of rats with PAH derivative 7,12-Dimethylbenz[a]anthracene (DMBA) induced toxicity. Radical scavenging capacity and protective effect of molasses, which is rich in minerals and antioxidants, against oxidative stress in cardiac tissue were compared with the effects of resveratrol.

It has been reported in studies conducted on rats that, grape powder inhibited DMBA induced epidermal hyperplasia, proliferation, and inflammation in mice [15]. In addition, it has been reported that resveratrol decreased the level of malondialdehyde and oxidative damage caused by hypertension [16], diet with phytochemically enriched grape powder decreased cardiac fibrosis and diastolic dysfunction, and increased cardiac glutathione levels [17], improved arterial relaxation and vessel coherence, and reduced cardiac hypertrophy [18]. Effects of grapes and grape juice diets on Cd-induced oxidative damage in plasma and liver tissues [19], and CCl4 induced oxidative damage in brain and liver tissues of rats [20] have been studied. It has been reported that resveratrol

decreased blood pressure and glucose, LDL cholesterol and triglyceride levels, and increased HDL cholesterol rat groups with Type II diabetes and renovascular hypertension [21]. The mentioned last three studies have reported an increase in antioxidant activities and a decrease in the levels of thiobarbituric acid reactive substances (TBARS) in the groups administered grapes diet. Marques et al. showed that acute supplement of trans-resveratrol provided improvement in the endothelial function without changing blood pressure in female patients with a high level of LDL cholesterol [22]. Resveratrol therapy has been shown to statistically significantly decrease systolic blood pressure, fasting glucose and total cholesterol values in obese patients [23]. In our study, groups that received resveratrol or molasses combined with DMBA, showed a significant decrease in NO and MDA levels and an increase in GSH level. This shows the antioxidant properties of resveratrol and molasses.

The amount of malondialdehyde which is among by-products of lipid peroxidation is a marker of the level of oxidative stress. MDA activity in rats' cardiac tissue was found as statistically significant in the DMBA group, compared to DMBA+Molasses, DMBA+Resveratrol, Control, Molasses and Resveratrol groups ($p<0.05$). There was no significant difference between DMBA+Resveratrol and DMBA+Molasses groups ($p>0.05$) (Figure 3). In addition, no statistically significant difference was found between the Control group, Resveratrol and Molasses groups in terms of MDA levels ($p>0.05$) (Table 1). Significantly higher MDA level in the DMBA group compared with all other groups is a marker of the damage. Significant decrease in malondialdehyde level by the administration of molasses and resveratrol diets to DMBA groups, suggests that food supplements provided oxidant / antioxidant balance, which improves oxidative damage.

Nitric oxide is formed with synthesizing of L-Arginine, which is a semi-essential amino acid, by nitric oxide synthase enzymes (eNOS, iNOS and nNOS). Elevated NO level accompanied by oxidative stress can trigger the formation of ONOO-, which is a potent oxidant. Therefore, they can react with proteins, lipids and nucleic acids, causing lipid peroxidation and amine nitration [24]. Humans with atherosclerosis, diabetes or hypertension often show impaired NO pathways [25]. There was no significant difference between DMBA and DMBA+Resveratrol groups in NO activity, while there as a statistically significant difference between DMBA and DMBA+Molasses, Control, Resveratrol and Molasses groups in terms of NO activity ($p<0.05$). No statistically significant difference was found between the control group, and Resveratrol and Molasses groups ($p>0.05$). There was no statistically significant difference between DMBA and DMBA + Resveratrol Groups in terms of NO levels ($p>0.05$) (Figure 2) (Table 1). Significant increase in the DMBA group supports the formation of NO with oxidative stress. Statistically lower levels of NO in DMBA+Molasses group compared to DMBA and DMBA + Resveratrol groups suggest that molasses were more efficient compared to resveratrol in providing oxidant / antioxidant balance.

It has been reported that, resveratrol increases the expressions of superoxide, glutathione and catalase, and ROS inactivation in cardiac tissues of rats [26]. It has been reported in a study with rats that resveratrol therapy may have a protective effect against cardiac hypertrophy [27]. It has been found that grapes extract and resveratrol therapy decreases the levels of cardiovascular risk markers in cardiovascular diseases [28], and can provide cardiovascular benefits by inhibition of atherothrombotic signals in blood mononuclear cells [29]. It has been reported that no any change was observed in the markers affecting cardiovascular health, such as inflammation and plasma markers as a result of resveratrol and placebo administration in the group consisting of obese male and female patients [30]. Similar to these results, resveratrol treatment is lowered MDA and NO levels and increased GSH levels compared to DMBA received groups in this study.

It has been reported that resveratrol therapy increased the levels of endogenous antioxidants and inhibited lipid peroxidation in heart, kidney and brain tissues, improved cardiovascular function in rats with induced hypertension [31], while resveratrol therapy increased survival and weight loss, improved cardiac and endothelial functions, and lowered mitochondrial lipid peroxidation in rats with cardiac hypertrophy and hypertension [32]. Ahmet et al. reported that prolonged diet resveratrol supplement decreased cardiovascular structural and functional impairment in the model of rats with chronic heart failure [33]. It has been stated that administration of lyophilized grape powder in the diet resulted in a significant decrease in lipoprotein metabolism, oxidative stress, and inflammatory markers, and positively affected the key risk factors of coronary heart disease in women in the pre- and postmenopausal period [34]. It was found that grape juice increased serum antioxidant capacity, and protected LDL against oxidation [35]. It has been reported that resveratrol supplement decreased oxidative stress, might be helpful in the prevention of developing atherosclerosis [36], and supported the immunomodulatory effect in patients with Type 2 diabetes mellitus [37]. It was found that resveratrol therapy might have the antiatherosclerotic effect, lowered LDL-cholesterol level [38], increased cardiac energy metabolism in heart failure [39] and increased energy metabolism of the skeletal muscle and exercise performance in heart failure [40].

In the present study, GSH activity was statistically significant in cardiac tissue of DMBA+Resveratrol and DMBA+Molasses groups compared to the DMBA group ($p<0.05$) . No significant difference was found between DMBA+Resveratrol and DMBA+Molasses groups in MDA activity ($p>0.05$) (Figure 1) (Table 1). Similarly to the studies in the literature, the antioxidant effect of resveratrol was demonstrated in our study, and it was concluded that grape molasses might have an antioxidant effect equivalent to resveratrol.

Based on these results; it can be said that grape molasses can provide protection against oxidative stress just as resveratrol, reducing the risk of free radical damage. In conclusion; we think that the use of grape molasses, which is a local product can be helpful especially in persons with cardiovascular disorders.

Conflict of interest
The authors declare that there are no conflicts of interest.

Financial Disclosure
All authors declare no financial support.

Ethical approval
The study was approved by Board of Ethics in Animal Experiments of Inonu University.

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