Intake of Purple Grape Juice as a Hepatoprotective Agent in Wistar Rats

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ABSTRACT Grape juice is a source of polyphenols, as catechin, anthocyanidins, resveratrol, and others. Some health benefits have been attributed to these compounds (e.g., antioxidant and antitumorigenic properties). In this study, we investigated the possible antioxidant activity of two different grape juices: organic purple grape juice and conventional purple grape juice. The antioxidant activity of both grape juices was evaluated by an animal model of three groups: control and organic and conventional juices. After 30 days, all animals were sacrificed, and blood and liver were collected to evaluate lipid peroxidation level (thiobarbituric acid-reactive substances [TBARS] assay), protein oxidative level (carbonyl assay), and catalase (CAT) and superoxide dismutase (SOD) activities. The group treated with organic grape juice showed the highest SOD and CAT activities in both plasma and liver when compared with the conventional and control groups (P < .05). In plasma, we observed a positive correlation among SOD and CAT activities, resveratrol, and all anthocyanin contents, suggesting that these polyphenols may be, at least in part, responsible for this increased antioxidant defense. The grape juices were capable of reducing carbonyl and lipid peroxidation levels in plasma and liver. However, in plasma, the organic group showed lower carbonyl and TBARS levels when compared to the conventional grape juice group (P < .05). Our findings suggest that the intake of purple grape juice, especially of organic juice, induces a better antioxidant capacity when compared to conventional juice and that this may be an important issue for further investigations in the area of biochemical functional foods.

KEY WORDS: • antioxidant • hepatoprotective • oxidative stress • phenolic content

INTRODUCTION

The liver regulates many important metabolic functions. Hepatic injury is associated with distortion of these metabolic functions.⁴ Additionally, the liver is the key organ of metabolism and excretion, and it is continuously and variably exposed to xenobiotics because of its strategic placement in the body. Thus, liver diseases remain one of the more serious health problems. The CCl⁴⁻-induced hepatotoxicity model is frequently used for investigating hepatoprotective effects of drugs and plant extracts.²,³

CCl⁴⁻-induced toxicity is a well-characterized murine model for the study of oxidative damage in vivo. The toxicity of CCl⁴ results from its reductive dehalogenation by the liver cytochrome P450 enzyme system into the trichloromethyl free radical, which readily interacts with molecular oxygen to form trichloromethyl peroxyl radicals.⁴ Both radicals are able to attack proteins and lipids or still abstract hydrogen atoms from an unsaturated lipid, leading to membrane lipid peroxidation, cellular dysfunction, and, finally, to cell necrosis.⁵

In a recent study, grape leaf extracts were able to reduce the damage caused by CCl⁴.⁶ The possible hepatoprotective activity of purple grape juices, either organic or conventional, has not been reported so far.

Grape juice is a very rich source of polyphenols, such as flavonoids, tannins, and resveratrol.⁷ Although there are studies reporting that Vitis vinifera grape juices show antioxidant activity,⁸–¹⁰ there is no reference in literature about this on Vitis labrusca cultivars. Presently, there is an increasing interest in a healthier and more environmentally friendly production method for fruits. Organic production is a cultivation method characterized by restrictions against the use of synthetic pesticides and fertilizers, as well as of genetic engineering.¹¹

Given these considerations, the aim of the present study was to investigate the beneficial effects of two different purple grape juices—organic and conventional—in reducing the damage to liver and the oxidative stress in plasma and liver, using the well-established murine model.
MATERIALS AND METHODS

Grape juices

The grape juice samples used in this study were produced from *V. labrusca*, variety Bordo, vintage 2005. The organic juices were produced with organically cultivated grapes (no pesticides) and were obtained from Cooperativa Aecia (Antonio Prado, RS, Brazil), which received the ECOVIDA certificate, a guarantee of organic food production. The conventional juices were obtained from Vinhos Monte Reale (Flores da Cunha, RS, Brazil). Both grape juices were kindly donated by these wineries.

Phenolic compounds

Total phenolic content was measured by using the modification of Singleton et al.\textsuperscript{12} of the Folin-Ciocalteu colorimetric method. High-performance liquid chromatography (HPLC) analysis was used in order to quantify the presence of individual phenolic compounds. Prior to HPLC analysis, 5 mL of each sample was filtered through a cellulose membrane with a 0.20-mm diameter. The equipment used in the analysis consisted of a chromatographic system of liquid gradient, LC-DAD Series 1100 chromatograph (Hewlett-Packard, CA, USA), with a detector system of diode array. A Zorbax 300 SB C18 pre-column (12 mm × 4.6 mm, 5 μm particle size) and C18-ODS column (150 mm × 4 mm, 5 μm particle size) were used in the equipment.

In order to quantify the resveratrol compound, we used a mobile phase of ultrapure water and acetonitrile (75:25 vol/vol) (pH 3.0), at a constant flow of 1.0 mL/minute for 20 minutes, in a controlled-temperature room at 20°C. The peak was detected at 306 nm, and the amount of sample injected was 20 μL.\textsuperscript{13}

In order to determine cyanidin-3-glucoside, delphinidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside, a mobile phase with solvents A (ultrapure water, formic acid, and acetonitrile [87:10:3 by volume]) and B (ultrapure water, formic acid, and acetonitrile [40:10:50 by volume]), at a constant flow of 0.8 mL/minute, in a controlled-temperature room at 25°C, was applied. The peak was detected at 518 nm, and the amount of sample injected was 50 μL. The elution conditions were 50–60% (30 minutes), 60–100% (30 minutes), and 100–50% (10 minutes).\textsuperscript{14}

Animals

Twenty-four male Wistar rats (60 days old, weighing 200 ± 50 g) from our breeding colony were used in the experiments. The animals were handled under standard laboratory conditions of a 12-hour light/dark cycle and fixed temperature (25 ± 2°C). Food and water were available *ad libitum*. All experimental procedures were performed in accordance with the U.S. National Institutes of Health’s *Guide for the Care and Use of Laboratory Animals* with the approval of the local ethics committee.

Treatment

The animals were randomly allocated into one of the three experimental groups (n = 8): group 1 served as the control and received vehicle saline, and conventional or organic purple grape juices were given to groups 2 and 3, respectively. The doses of purple grape juice were determined by calculating the amount of juice that would be consumed daily in average by a 70-kg male human.\textsuperscript{15} Juices were administered to the rats (7 μL of grape juice/g of body weight) twice a day. During the experiment, the amount was adjusted according to the animals’ weight. Before sacrifice, the animals’ blood was collected and kept in heparin-coated tubes. On day 30, half of the animals received a single intraperitoneal CCl₄ (3 mL/kg) dose. The animals that received CCl₄ or only vehicle (mineral oil [control]) were killed 6 hours later by decapitation. Liver samples were isolated and stored at −70°C until analysis.

Oxidative stress analyses

We used the thiobarbituric acid-reactive species (TBARS) output during an acid-heating reaction as an index of lipid peroxidation, which is widely adopted as a sensitive method for the measurement of lipid peroxidation, as previously de-

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**Table 1. Total Phenolic Content and Levels of Resveratrol and Anthocyanins (Cyanidin, Delphinidin, Peonidin, and Malvidin) in Organic and Conventional Grape Juices**

<table>
<thead>
<tr>
<th>Grape juice</th>
<th>Organic</th>
<th>Conventional</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenolic compounds (mg of catechin/mL)</td>
<td>262.50 ± 0.70*</td>
<td>119.59 ± 3.53</td>
</tr>
<tr>
<td>Resveratrol amount (ppm)</td>
<td>0.213 ± 0.005*</td>
<td>0.075 ± 0.010</td>
</tr>
<tr>
<td>Cyanidin (ppm)</td>
<td>11.79 ± 0.42*</td>
<td>0.76 ± 0.04</td>
</tr>
<tr>
<td>Delphinidin (ppm)</td>
<td>26.30 ± 1.15*</td>
<td>4.10 ± 0.40</td>
</tr>
<tr>
<td>Peonidin (ppm)</td>
<td>19.21 ± 1.43*</td>
<td>8.59 ± 0.82</td>
</tr>
<tr>
<td>Malvidin (ppm)</td>
<td>232.46 ± 4.25*</td>
<td>95.26 ± 1.95</td>
</tr>
</tbody>
</table>

Data are mean ± SD values.

*Statistically different between the two grape juices (P < .05).
scribed. In brief, the samples were mixed with 10% trichloroacetic acid and 0.67% thiobarbituric acid and then heated in a boiling water bath for 15 minutes. TBARS were determined by absorbance at 535 nm.

The oxidative damage to proteins was assessed by determining carbonyl groups based on the reaction with dinitrophenylhydrazine, as previously described. In brief, proteins were precipitated by addition of 20% trichloroacetic acid and redissolved in dinitrophenylhydrazine, and the absorbance was read at 370 nm.

Antioxidant enzyme assays were performed in tissue homogenates, as previously described. Catalase (CAT) activity was assayed by measuring the rate of decrease in H$_2$O$_2$ absorbance at 240 nm. Superoxide dismutase (SOD) activity was assayed by measuring the inhibition of adrenaline autooxidation at 480 nm, as previously described.

Statistical analyses

Biochemical data are expressed as mean ± SEM values, and analysis of variance and Tukey’s test were performed using the SPSS (Chicago, IL) version 12.0 package. All tests were performed in duplicate. Pearson’s correlation coefficient was used to test the correlation between polyphenol content and the assays.

RESULTS

Table 1 shows the content of phenolic compounds in the two types of purple grape juice used in this study. The two types present a statistical difference in the content of total phenolic compounds (P < .05), especially in resveratrol amount; the organic purple grape juice had higher amounts in both parameters. Important differences could be observed between both grape juices with regard to content of anthocyanins (malvidin, cyanidin, delphinidin, and peonidin); we also observed that the organic juice is richer in the amount of all phenolics than the conventional juice (Table 1).

In this study, we have demonstrated that grape juice, especially the organic one, was capable of altering oxidative parameters in plasma. It was observed that the animals that received organic grape juice showed lower plasma lipid peroxidation levels when compared to conventional grape juice and control groups (P < .05) (Fig. 1A). We found a negative correlation between lipid peroxidation (TBARS) and total phenolic content (r = −0.511) and resveratrol (r = −0.546), cyanidin (r = −0.604), peonidin (r = −0.512), delphinidin (r = −0.593), and malvidin (r = −0.526) con-
Cytotoxic and proinflammatory effects of 

The result of the assay, polyphenol content showed an important correlation; we also observed a negative correlation between carbonyl content and polyphenol total amount ($r = 0.779$) and resveratrol ($r = -0.679$), cyanidin ($r = -0.604$), peonidin ($r = -0.698$), delphinidin ($r = -0.629$), and malvidin ($r = -0.692$) contents ($P < .05$ for all comparisons). When analyzing the activities of antioxidant enzymes, we observed that the group treated with organic grape juice had higher SOD and CAT activities as compared to the conventional grape juice and control groups. We observed a positive correlation between the SOD and CAT activities ($r = 0.707; P < .01$). We observed a positive correlation between SOD and CAT activities and total phenolic content ($r = 0.773$ and 0.578, respectively) and also between resveratrol ($r = 0.775$ and 0.602), cyanidin ($r = -0.775$ and 0.632), peonidin ($r = 0.687$ and 0.578), delphinidin ($r = 0.766$ and 0.629), and malvidin ($r = 0.721$ and 0.588) contents ($P < .01$ for all comparisons). This suggests that these polyphenols may be responsible for this increased antioxidant defense. The SOD/CAT ratio of the organic group presented the lowest level when compared to conventional juice and control groups ($P < .05$) (Fig. 1C). This ratio showed a negative correlation with the content of phenolic compounds; we observed this correlation with resveratrol ($r = -0.621$), cyanidin ($r = -0.608$), peonidin ($r = -0.609$), delphinidin ($r = -0.619$), and malvidin ($r = -0.615$) ($P < .05$ for all comparison).

CCL_4 damage was quantified through the lipid peroxidation detection assay, and the level of lipid peroxides was significantly increased in the liver of rats after the CCL_4 injection ($P < .05$) (Fig. 2). However, after treatment with organic grape juice these levels decreased significantly ($P < .05$) when compared to conventional grape juice and control treatments (Fig. 2). This could be explained by the phenolic content; we observed a negative correlation between liver lipid peroxidation and total phenolic content ($r = -0.511$) and resveratrol ($r = -0.546$), cyanidin ($r = -0.604$), peonidin ($r = -0.512$), delphinidin ($r = -0.593$), and malvidin ($r = -0.526$) contents ($P < .05$ for all comparisons).

Figure 3 shows the capacity of CCL_4 to induce protein oxidative damage in liver when compared to control ($P < .05$). A significant attenuation of the oxidative damage induced by the CCL_4 injection can be observed in the groups that were given grape juice ($P < .05$), but the group that received conventional grape juice showed lower values (higher protection against damage) when compared to the group that
received organic grape juice ($P < .05$) (Fig. 3). However, in the groups that received vehicle in addition to grape juice, the organic grape juice group showed a more significant decrease when compared to the conventional grape juice group, but both grape juices provided protection when compared to the vehicle-only group ($P < .05$) (Fig. 3).

Figure 4 shows the effects of grape juice treatment on CAT and SOD and on the ratio of both enzymes’ activities in liver. Modifications in CAT activity in the liver were observed between the control and CCl$_4$-treated groups, with the CCl$_4$ group showing higher values ($P < .05$) (Fig. 4A). However, a significant increase in CAT activity was also observed in the organic grape juice group when compared to the conventional grape juice and control groups that had both received vehicle in addition ($P < .05$). We observed a correlation between CAT activity and total phenolic content ($r = 0.372$) and resveratrol ($r = 0.380$), cyanidin ($r = 0.376$), delphinidin ($r = 0.381$), peonidin ($r = 0.373$), and malvidin ($r = 0.376$) contents ($P < .01$ for all comparisons). SOD activity was reduced in CCl$_4$-treated rat liver ($P < .05$) (Fig. 4B). We observed that both juices reduced SOD activity in liver when compared to the control group that received vehicle only ($P < .05$) (Fig. 4B). The liver SOD/CAT ratio decreased significantly when compared to the control and conventional groups (Fig. 4C).

**DISCUSSION**

In our study, we observed that chronic treatment with grape juice was able to reduce the lipid peroxidation level in liver and plasma after CCl$_4$ injection, whereas the organic juice induced a more significant reduction than the conventional juice. These protection activities could indicate a hepatoprotective action of grape juice since the CCl$_4$ damage was smaller after grape juice intake.

Orhan et al.$^6$ showed that the ethanol extract of *V. vinifera*—despite not having measured its active compounds in their study—was capable of inducing a possible hepatoprotective action. They believed that it could be due to (1) inhibiting the cytochrome P450-dependent oxygenase activity, (2) preventing lipid peroxidation, and (3) stabilizing the hepatocyte membrane, induced by polyphenolic compounds.

We also observed that CCl$_4$ injection increased lipid peroxidation level 6 hours after a single intraperitoneal injection, suggesting that this agent makes a good topic for investigating the antioxidant effect of chronic treatment with grape juice. The phenolic content of the juices could be a possible explanation for this effect. Organic grape juice showed higher contents of total phenolic compounds and other phenolics, such as resveratrol and anthocyanins, associated with antioxidant activities.$^9,^{20–23}$

Phenolic compounds are secondary metabolites produced and accumulated in plant tissues. Depending on the presence of biotic and abiotic factors (e.g., phytopathogenesis, water availability), different amounts of these compounds in plant organs would result. Actually, organic farming is a small-scale practice, in which there is no use of chemical protective substances like pesticides or artificial fertilizers to promote plant growth. Since pesticides are not used, the plants are more susceptible to the action of phytopathogenic organisms, resulting in the production of larger amounts of phenolic compounds.$^{11}$ This study shows that the choice of agricultural practice used for grapes (organic vs. conventional) results in different amounts of total phenolic compounds, resveratrol, and anthocyanins. The correlations observed between amount of resveratrol and anthocyanins with antioxidant enzyme activity could help explain why some important studies attributed health benefits.$^9,^{20–23}$ to grape juice intake, as we have shown our study.

Our study further showed that especially after treatment with organic grape juice the liver SOD/CAT ratio was lower than that in the control group. This parameter is very important because as a result of an imbalance between these two enzymes oxidative stress may be induced, and it participates in some diseases.$^{24,25}$ SOD activity leads to the production of hydrogen peroxide, which can react with iron via the Fenton reaction to generate hydroxyl radicals, which are thought to be the most toxic oxygen molecules in vivo.$^{26}$ CAT could scavenge an excess of hydrogen peroxide, avoiding its potential role as an oxidative stress-facilitating molecule. Our results showed that grape juice treatment induced CAT activity in a different way than SOD, reducing the SOD/CAT ratio and suggesting a better antioxidant protective status once there would be no extra hydrogen peroxide to overcome Fenton chemistry.

In conclusion, based on earlier reports$^{27–29}$ providing evidence of antiplatelet and antioxidant benefits from grape consumption and on our results showing reduced oxidative stress in liver and plasma, it seems reasonable to recommend that moderate quantities of purple grape juice be regularly included in daily servings of fruits and vegetables in order to help maintain a healthy life by attenuating oxidative damage and providing hepatoprotective action, at least in some in the organs studied.

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