Antioxidant and Antigenotoxic Activities of Purple Grape Juice—Organic and Conventional—in Adult Rats

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ABSTRACT Oxidative damage to biomolecules occurs by the accumulation of molecular damage due to free radicals and/or a diminution of antioxidant protection. The aim of this study was to evaluate the protection of organic and conventional purple grape juices in brain, liver, and plasma from adult Wistar rats (7 months old) against the oxidative damage provoked by carbon tetrachloride (CCl4). Adult rats were divided into three groups (control, conventional purple grape juice, and organic purple grape juice). Half of the rats received CCl4, and the other half received the vehicle (vegetable oil). The chemical analytical determination showed that the highest levels of total phenolic, resveratrol, and catechins were seen in organic purple grape juices. Considering the treatment groups, it was observed that in all tissues (brain structures and liver) and plasma, CCl4 treatment increased the lipid peroxidation (LP) levels. Both grape juices were capable to reduce LP levels in cerebral cortex and hippocampus; however, in the striatum and substantia nigra only the organic grape juice reduced LP level. CCl4 caused an increase in catalase activity in cerebral cortex, hippocampus, and substantia nigra and in superoxide dismutase activity in substantia nigra. This increase was reduced by both juices in substantia nigra and hippocampus structures (P < .05). In the alkaline version of the comet assay performed on whole blood, it was observed that CCl4 was capable of inducing mainly DNA damage class 4 and 3 frequencies, which was significantly reduced in groups that received both purple grape juices. This implies that both grape juices have an important antigenotoxic activity.

KEY WORDS: • DNA damage • grape juice • oxidative stress

INTRODUCTION

Epidemiological studies have suggested an inverse relation between the consumption of polyphenol-rich foods and beverages and the risk of degenerative diseases, particularly cancers and cardiovascular diseases, mainly by their antioxidant capacity.1,12 Some researches have also shown that diets rich in fruits and vegetables are beneficial in both forestalling and reversing the deleterious effects of aging on neuronal communication and behavior.3–5 In these studies, the protection observed may be the result of the antioxidant and anti-inflammatory properties of the polyphenolic compounds found in these fruits and vegetables.6 Grape juice is a very rich source of polyphenols, such as flavonoids, tannins, and resveratrol,7 showing important antioxidant activity in vitro.7

However, the question whether properties demonstrated in in vitro studies are relevant to protect against oxidative damage in vivo remains. Indeed, in previous studies,8,9 we observed that chronic intake of grape juice—organic and conventional—was able to reduce oxidative stress damage in brain tissues (striatum and substantia nigra), liver, and plasma in young rats (2 months old), using carbon tetrachloride (CCl4) as the oxidative damage inducer. CCl4-induced toxicity is a well-characterized murine model for oxidative damage in vivo. The toxicity of CCl4 results from its reductive dehalogenation by the cytochrome P450 enzyme system into a trichloromethyl free radical, which readily interacts with molecular oxygen to form the trichloromethyl peroxyl radical. Several studies have demonstrated that the liver is not the only target organ of CCl4. CCl4 has been reported to cause lipid peroxidation (LP) in other organs such as kidney, heart, and brain.10,11 Aging is a process that accumulates oxidative damage leading to DNA, lipid, and protein damages. Such free radical-mediated damages are prevalent during aging and are associated with diseases like Alzheimer’s and Parkinson’s.12,13 In this study, we chose rats older than those of the first studies8,9 to verify if grape juice protection is also present in older individuals. Consequently, the aim of the present study was to investigate the beneficial effects of two different purple grape juices—organic and conventional—in reducing oxidative stress generated by CCl4 in 7-month-old rats.
MATERIALS AND METHODS

Grapes and grape juices
The grape juices used in this work were elaborated with grapes of the Vitis labrusca Bordo variety. Organic grape juice was produced with grapes cultivated without pesticides,7 certified by ECOVIDA, and obtained from the Cooperativa Aecia (Antonio Prado, Rio Grande do Sul, RS, Brazil). Conventional juice was produced with grapes cultivated using traditional methods7 and obtained from Vinhos Monte Reale (Flores da Cunha, RS, Brazil). Throughout the tests, we observed the expiry dates of the juices and always used the same trademarks.

Phenolic compounds
Total phenolic content was quantified using the Singleton-Rossi modification of the Folin-Ciocalteau colorimetric method.14 High-performance liquid chromatography analysis was used to measure the presence of phenolic compounds. Before high-performance liquid chromatography analysis, 5 mL of each sample was filtered through a cellulose membrane with a 0.20-mm-diameter pore size. The equipment used in the analysis consisted of an LC-DAD Series 1100 (Agilent Technologies, Palo Alto, CA, USA) liquid chromatographic system with a diode array detector system.

Resveratrol analysis. In order to quantify resveratrol, 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.15

Analysis of catechins. In order to determine catechins [(+)-catechin and (−)-epicatechin] we used a mobile phase with solvent A (50 mmol/L ammonium hydroxide diphosphate, pH 2.6), solvent B (20% solvent A and 80% acetonitrile), and solvent C (0.2 mol/L o-phosphoric acid, pH 1.5) at a constant flow of 0.5 mL/minute, in a controlled-temperature room at 40°C. The peak was detected at 204 nm, and the amount of sample injected was 5 μL. The elution conditions were as follows: 100% solvent A for 5 minutes, 96% solvent A and 4% solvent B for 10 minutes, 92% solvent A and 8% solvent B for 10 minutes, 8% solvent B and 92% solvent C for 20 minutes, 30% solvent B and 70% solvent C for 5 minutes, 40% solvent B and 60% solvent C for 5 minutes, 80% solvent B and 20% solvent C for 5 minutes, and 100% solvent A for 5 minutes.16

Animals
Twenty-four male Wistar rats (7 months old) 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 NIH Guide for the Care and Use of Laboratory Animals with the approval of the local ethics committee.

Chronic intake
The animals were randomly allocated into one of the three experimental groups (n = 8). Group 1 served as control and received saline, whereas groups 2 and 3 were given conventional and organic purple grape juice, respectively. The doses of purple grape juice were determined by calculating the daily average amount of juice consumed by a 70-kg human male. As a reference, we used a study with humans who received 480 mL/day.17 The amount of juice was administered to the rats according to their body weight. The rats received by oral gavage 7 μL of grape juice/g of body weight, twice a day. On day 30, half of the animals received a single intraperitoneal dose of CCl4 (3 mL/kg). The animals that received CCl4 (positive control) or those that received only vegetable oil (vehicle) (negative control) were killed 6 hours later by decapitation. The dose of CCl4 was previously chosen based on pilot experiments, which indicated that this dose demonstrated significant increase in protein and lipid oxidative damage and animals’ survival of 100%.18

The whole blood was collected with heparin. One part was separated for the comet assay, the other part was centrifuged, and the plasma was isolated and stored until analysis (thiobarbituric acid-reactive species [TBARS], catalase [CAT], and superoxide dismutase [SOD] activities). Brain structures (cerebral cortex, hippocampus, striatum, and substantia nigra) and liver were isolated and stored at −70°C until analysis (TBARS, CAT, and SOD activities).

Oxidative stress parameters
As an index of LP, we used the formation of TBARS during an acid-heating reaction, which has been widely adopted as a sensitive method for measuring LP, as previously described.19 Antioxidant enzyme assays were performed in plasma and tissue homogenates, as previously described.19 CAT activity was assayed by measuring the rate of decrease in H2O2 absorbance at 240 nm, and the results were expressed in units of CAT/g of protein.20 SOD activity was assayed by measuring the inhibition of adrenalin autoxidation, as previously described, and the results were expressed in units of SOD/mg of protein.21

Comet assay
The standard protocol for preparation and analysis of the comet assay followed the procedure of Tice et al.22 The slides were prepared by mixing 5 μL of whole blood from all groups of rats with 90 μL of low melting point agarose (0.75%). The mixture (cells/agarose) was added to a fully frosted microscope slide coated with a layer of 300 μL of normal melting agarose (1%). After solidification, the coverslip was gently removed, and the slides were placed in lysis solution (2.5 M NaCl, 100 mM EDTA, and 10 mM Tris [pH 10.0–10.5] with freshly added 1% Triton X-100 and...
10% dimethyl sulfoxide) for a minimum of 1 hour and a maximum of 7 days. Subsequently, the slides were incubated in freshly prepared alkaline buffer (300 mM NaOH and 1 mM EDTA, pH 12.6) for 20 minutes. The DNA was electrophoresed for 20 minutes at 25 V (0.90 V/cm) and 300 mA; the buffer was neutralized with 0.4 M Tris (pH 7.5). Finally, the DNA was stained with silver nitrate.

The slides were coded for blind analysis. In order to ensure adequate electrophoresis conditions and efficiency, negative and positive controls (human blood) were used for each experiment. Images of 100 randomly selected cells (50 cells from two replicated slides) were analyzed from each animal. Cells were also scored visually according to tail size into five classes, from no tails (0), to maximally long tails (4), resulting in a single DNA damage score for each subject and, consequently, for each study group. The results were expressed in damage class frequency (%) and damage index.

Statistical analysis

The statistical analysis was done by analysis of variance and Tukey’s test using the SPSS version 12.0 package (SPSS, Chicago, IL, USA). All tests were performed in duplicate. Pearson’s correlation coefficient was used to test the correlation between polyphenol content and the results of the assays.

RESULTS

As described in Table 1, the organic grape juice shows higher levels of total phenolic compounds, resveratrol, and catechin than the conventional purple grape juice ($P < .05$). It was observed that CCl$_4$ induced an increase in LP in all tissues (Table 2) when compared to the control group ($P < .05$). The chronic treatment with both grape juices reduces peroxidation levels induced by CCl$_4$ in the cerebral cortex and hippocampus (Table 2). In substantia nigra and striatum, only the organic grape juice was capable of reducing the CCl$_4$-induced peroxidation levels. In plasma (Table 2), only the conventional grape juice group showed this decrease. Treatments with only the grape juices showed reduced TBARS levels in cerebral cortex when compared to the control (saline). In substantia nigra, only the organic grape juice was able to reduce TBARS levels, and in plasma, only the conventional one did so (Table 2).

CCl$_4$ induces a reduction in SOD activity in cerebral cortex, hippocampus, plasma, and liver (Table 3). However, in substantia nigra, this activity was increased when compared to the control ($P < .05$). SOD activity depletion was restored by both grape juices in hippocampus; however, only the conventional grape juice restored this activity in plasma. In the striatum, CCl$_4$ did not alter the values when compared with the control; however, when comparing the CCl$_4$ group with the grape juice groups it was observed that both grape juices reduced SOD activity. Considering only the vehicle groups, both grape juices increased SOD activity in substantia nigra and liver. However, in hippocampus and cerebral cortex only the conventional group increased this enzyme activity, whereas in the striatum and plasma the organic group showed the highest level ($P < .05$) (Table 3).

The group that received CCl$_4$ showed a significant reduction in CAT activity in striatum and plasma (Table 4). However, in plasma, only the organic grape juice was capable of increasing CAT activity. In cerebral cortex, substantia nigra, and hippocampus, CCl$_4$ produced an increase in CAT activity. In substantia nigra, both grape juices were able to reduce this increase, but the organic juice performed better, i.e., reduced it more. In hippocampus, only the organic juice was able to reduce this increment.

### Table 1. Total Phenolic Content and Levels of Resveratrol and Catechins in Grape Juices (Organic and Conventional)

<table>
<thead>
<tr>
<th>Grape juice</th>
<th>Total phenolic compounds (mg of catechin/mL)</th>
<th>Resveratrol (ppm)</th>
<th>Catechins (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic</td>
<td>$271.5 \pm 12.02$</td>
<td>$0.214 \pm 0.0023$</td>
<td>$36.58 \pm 0.020$</td>
</tr>
<tr>
<td>Conventional</td>
<td>$120.67 \pm 1.87^* $</td>
<td>$0.073 \pm 0.0021^* $</td>
<td>$24.13 \pm 0.001^* $</td>
</tr>
</tbody>
</table>

* $P < .05$ between juices.

### Table 2. TBARS Level in Cerebral Cortex, Substantia Nigra, Hippocampus, Striatum, Liver, and Plasma of Rats Treated with Conventional and Organic Grape Juices

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cerebral cortex</th>
<th>Substantia nigra</th>
<th>Hippocampus</th>
<th>Striatum</th>
<th>Liver</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>$4.55 \pm 1.58$</td>
<td>$2.06 \pm 0.04$</td>
<td>$1.91 \pm 0.10$</td>
<td>$1.43 \pm 0.08$</td>
<td>$2.55 \pm 0.19$</td>
<td>$69.06 \pm 5.07$</td>
</tr>
<tr>
<td>CCl$_4$</td>
<td>$7.46 \pm 2.56^*$</td>
<td>$2.53 \pm 0.73^*$</td>
<td>$14.00 \pm 0.10^*$</td>
<td>$2.82 \pm 0.77^*$</td>
<td>$5.27 \pm 1.19^*$</td>
<td>$80.55 \pm 6.69^*$</td>
</tr>
<tr>
<td>Conventional</td>
<td>$1.41 \pm 0.62^+$</td>
<td>$3.02 \pm 1.09^*$</td>
<td>$2.90 \pm 0.41^+$</td>
<td>$2.90 \pm 0.74^*$</td>
<td>$6.27 \pm 1.09^*$</td>
<td>$51.03 \pm 2.06^+$</td>
</tr>
<tr>
<td>Conventional + CCl$_4$</td>
<td>$0.98 \pm 0.04^+$</td>
<td>$2.81 \pm 0.44^*$</td>
<td>$7.85 \pm 1.56^+$</td>
<td>$2.12 \pm 0.50^+$</td>
<td>$4.10 \pm 2.38^*$</td>
<td>$62.06 \pm 7.68^+$</td>
</tr>
<tr>
<td>Organic</td>
<td>$2.62 \pm 0.74^+$</td>
<td>$2.10 \pm 0.48^+$</td>
<td>$2.45 \pm 0.36^+$</td>
<td>$1.62 \pm 0.18^+$</td>
<td>$5.70 \pm 1.23^+$</td>
<td>$71.82 \pm 6.00^+$</td>
</tr>
<tr>
<td>Organic + CCl$_4$</td>
<td>$3.81 \pm 0.64^+$</td>
<td>$2.17 \pm 0.45^+$</td>
<td>$3.85 \pm 0.54^+$</td>
<td>$1.30 \pm 0.27^+$</td>
<td>$6.33 \pm 1.01^+$</td>
<td>$82.75 \pm 0.65^+$</td>
</tr>
</tbody>
</table>

Control rats received saline. Data are mean ± SD values.

* $P < .05$, different from control; † $P < .05$, different from CCl$_4$; ‡ $P < .05$ different between grape juice treatments.
When considering the vehicle groups, it was observed that both grape juices increased CAT activity in cerebral cortex and substantia nigra when compared to the control ($P < .05$); in plasma, the organic grape juice was capable of increasing this enzyme activity. However, in striatum, both grape juices reduced CAT activity (Table 4).

In striatum, we observed a negative correlation between CAT activity and total phenolic content and resveratrol ($r = -0.492$ and $r = -0.328$, $P < .05)$, respectively. We also observed a significant increase of SOD/CAT ratio in liver of rats that received CCl$_4$. However, in this tissue, only the organic grape juice decreased these levels (Table 5). In cortex, both grape juices were capable of reducing this parameter when compared with the vehicle or CCl$_4$ groups.

In the comet assay, the internal controls (human blood) showed low levels of damage in the negative control (class 1, 12%; class 0, 88%) and high damage in positive control (methyl methanesulphonate) (4%, 15%; class 3, 22%; class 2, 32%; class 1, 19%; and class 0, 19%). As shown in Figure 1, CCl$_4$ was capable of inducing DNA damage, identified by the increase of damage index and also damage class 4 and 3 frequencies when compared with the control group, which received only the oil. In the groups that received grape juice, a significant reduction of damage class 4, 3, and 2 frequencies was observed. However, this reduction was more significant in the organic group.

### DISCUSSION

Polyphenol-rich food with health benefits has become a more common element in food marketing. In a recent study, purple grape juice was the beverage that showed the highest antioxidant properties in vitro, higher than, e.g., orange, açai, and apple juices. The antioxidant activity was also observed in healthy individuals who received 100 mL of purple grape juice/day for 14 days. These individuals showed an increase in plasma antioxidant activity. Grape juice benefits were also demonstrated in a previous study, with younger rats (2 months); we observed that chronic treatment with grape juice was able to reduce LP and protein oxidation levels in liver, plasma, and brain structures after CCl$_4$ injection. Indeed, it was observed that the juice was capable of reducing LP in the plasma and substantia nigra more significantly than the conventional juice. This was also observed in protein oxidation, where the organic juice showed better results in the liver and striatum. These protection activities—observed in young rats—could indicate a neuro- and hepatoprotective action of the purple grape juice because the CCl$_4$ damage was reduced after grape juice intake.

Considering that aging is a process where there is an accumulation of oxidative damage and that oxidative stress is considered a risk factor and a contributor to age-related...
increase of oxidized lipids and proteins, this study evaluated the in vivo antioxidant and antigenotoxic effects of grape juice on the rat model of CCl4-induced toxicity, using 7-month-old rats, older than the previous study with 2-months-old rats.

The findings of the present study conducted with older rats suggest a reduction of LP in the brain structures and plasma in groups that received the purple grape juices. However, we observed that the organic grape juice was more efficient than the conventional one. The explanation could be the higher total phenolic, resveratrol, and catechin contents (Table 1) of this juice. These compounds have been reported in many studies for their beneficial properties.

Although the LP decrease was observed in some structures, it is important to highlight the reduction observed in the hippocampus because this structure is one of the brain regions with an ability to generate neurons (neurogenesis), a feature that decreases with aging.

When comparing the results obtained in this study with those from assays performed with younger rats, we observe that the LP levels in older (7-month-old) rats were higher than those observed in younger rats. Moreover, it can also be seen that, in young rats, both grape juices were capable of reducing LP induced by CCl4. These differences might be explained by an inefficient endogenous antioxidant system as found in aging rats and by the difference in the brain tissues, because the brain is vulnerable to oxidative damage due, among other reasons, to the high utilization of oxygen and the large amount of easily oxidizable polyunsaturated fatty acid. Although we did not find any correlation between TBARS level and polyphenol content, we may attribute the decrease in the peroxidation level to these compounds, which are present in both juices, but especially in the organic one.

According to Park et al., healthy men who received grape juice (480 mL) daily for 8 weeks showed depletion of free radical levels. Recently, a similar study was conducted in which either grape juice (10 mL/kg/day) or z-tocopherol (400 IU/day) was given for 2 weeks to healthy subjects who were otherwise on a flavonoid-restricted diet. In both treatments, serum oxygen radical antioxidant capacity and low-density lipoprotein oxidation resistance were increased, implying that the grape juice had a possible free radical scavenging effect. More specifically, grape juice supplementation reduced protein oxidation more efficiently than z-tocopherol supplementation. The authors suggested that grape juice supplementation provided a strong antioxidant activity. Moreover, in another study, purple grape juice was capable of reducing LP induced by cadmium in older (13-month-old) rats.

The free radical damages could be prevented or repaired by the antioxidant defense. In the human body, there are two major enzymes, SOD and CAT. SOD plays a key role in detoxifying superoxide anions, which otherwise damage the

<table>
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<tr>
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<th>Substantia nigra</th>
<th>Hippocampus</th>
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<th>Liver</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>38.20 ± 0.46</td>
<td>1.63 ± 1.09</td>
<td>23.22 ± 3.33</td>
<td>6.29 ± 2.59</td>
<td>8.64 ± 1.89</td>
<td>7.75 ± 0.35</td>
</tr>
<tr>
<td>CCl4</td>
<td>9.30 ± 0.62*</td>
<td>2.73 ± 0.67</td>
<td>20.1 ± 0.62</td>
<td>10.22 ± 2.80</td>
<td>17.40 ± 2.22*</td>
<td>7.54 ± 1.19</td>
</tr>
<tr>
<td>Conventional</td>
<td>0.15 ± 0.02s†</td>
<td>13.63 ± 1.92</td>
<td>27.0 ± 0.12</td>
<td>11.50 ± 6.7</td>
<td>5.12 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>Conventional + CCl4</td>
<td>7.33 ± 1.35s†</td>
<td>10.05 ± 1.10</td>
<td>8.55 ± 2.94</td>
<td>11.50 ± 6.7</td>
<td>5.12 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>Organic</td>
<td>14.39 ± 1.35s†</td>
<td>4.26 ± 0.80</td>
<td>0.85 ± 0.15s†</td>
<td>10.31 ± 2.38</td>
<td>13.25 ± 3.88</td>
<td>42.18 ± 18.11s†</td>
</tr>
<tr>
<td>Organic + CCl4</td>
<td>3.80 ± 1.69s†</td>
<td>33.02 ± 9.41s†</td>
<td>2.80 ± 0.48s†</td>
<td>7.73 ± 3.17</td>
<td>3.26 ± 1.22s†</td>
<td>16.35 ± 1.62s†</td>
</tr>
</tbody>
</table>

Control rats received saline. Data are mean ± SD values.

*P < .05, different from control; †P < .05, different from CCl4; ‡P < .05 different between grape juice treatments.

FIG. 1. (A) DNA damage index and (B) damage class frequency in whole blood of rats treated with organic and conventional grape juices. *P < .05, different from control; **P < .05, different from CCl4. Control rats received saline.
cell membranes and macromolecules.\textsuperscript{36} CAT has the ability to detoxify \( \text{H}_2\text{O}_2 \) radicals. Release of \( \text{H}_2\text{O}_2 \) promotes the formation of numerous other oxidant species that greatly contribute to oxidative stress, leading to pathogenesis.\textsuperscript{37} Clinical studies demonstrated a reduction of SOD activity in Parkinsonism associated with other neurodegenerative disorders, showing the key role played by this enzyme in fighting free radicals produced in the brain.\textsuperscript{38,39} In our results, we observed that in some tissues (cerebral cortex, hippocampus, liver, and plasma) \( \text{CCl}_4 \) decreased SOD activity. According to Chang \textit{et al.},\textsuperscript{40} the decrease of SOD activity by \( \text{CCl}_4 \) could result from the consumption of SOD to compensate excessive peroxyl radicals derived from \( \text{CCl}_4 \)’s metabolism. In a further study, the authors showed that the decreased SOD activity in aged tissue was brought back to near normal levels upon grape seed extract administration, perhaps because this extract acts as a potent scavenger of superoxide radicals and metal chelator.\textsuperscript{41} The reduction caused by \( \text{CCl}_4 \) was increased by both grape juices in the hippocampus.

Our current results show that after \( \text{CCl}_4 \) injection CAT activity was increased in the cerebral cortex, substantia nigra, and hippocampus. This increase was diminished in the substantia nigra in both purple grape juice groups. However, in the hippocampus only the organic juice was capable of reducing the activity of this enzyme. This could be explained by the higher content in phenolic compounds of this juice. When comparing the results from the study in young rats,\textsuperscript{8,9} we observe that SOD and CAT activities are higher in the older rats than in the younger ones of control groups (saline). This might be explained by the fact that the older rats have a greater oxidative damage, requiring a higher antioxidant activity than the younger ones. This was also observed in the prior study comparing only the vehicle group (which received oil), which showed that CAT activity was increased in the striatum and liver after chronic intake of purple grape juice, especially in the group that received organic juice. This suggests that a high content in phenolic compounds is capable of increasing the activity of the enzymatic antioxidant defenses. When comparing only the groups that received vehicle, the results of our study showed that both purple grape juices increased CAT activity in the cerebral cortex and substantia nigra. However, in liver, only the organic juice was capable of increasing the activity of this enzyme. This was also observed in young rats.\textsuperscript{9} This increase might be explained by the phenolic content and the ability to remove toxic reactive species present in a normal physiological function.\textsuperscript{42}

When analyzing the results of SOD and CAT activities, we observed that \( \text{CCl}_4 \) brought about a reduction in SOD activity and an increase in CAT activity. This imbalance might explain the increase of oxidative damage, proven by the high LP observed in older rats. The SOD/CAT ratio is a very important parameter because an imbalance between these two enzymes of the antioxidant defenses may induce an increase in oxidative stress level and thus lead to the development of many diseases associated with it.\textsuperscript{43} SOD activity leads to the production of \( \text{H}_2\text{O}_2 \), which reacts with iron to generate hydroxyl radicals via the Fenton reaction, which in turn are thought to be the most toxic oxygen molecules \textit{in vivo}.\textsuperscript{32} CAT could clean up an excess of \( \text{H}_2\text{O}_2 \), thus reducing its oxidative effects. The differences found among all structures could be explained, at least in part, by the fact that, with age, some structures show a greater accumulation of oxidative damage than others, particularly in the striatum, because this area is especially vulnerable to oxidative stress during aging. The reason behind it may be that accelerated oxidation of dopamine by monoamine oxidase in the nerve terminals of the striatal neurons increases \( \text{H}_2\text{O}_2 \) production in these neurons.\textsuperscript{44}

In a recent article, it was noted that the chronic intake of both purple grape juices, but especially the organic juice, reduced the SOD/CAT ratio—increased by the \( \text{CCl}_4 \) injection—in the striatum and substantia nigra.\textsuperscript{9} Indeed, our present study, this ratio decrease was observed in the cerebral cortex, substantia nigra, and liver. However, in liver, only the organic juice was capable of reducing the ratio, and in the substantia nigra, the organic juice also performed best. In agreement with other studies,\textsuperscript{3,45,46} we observed that the combinations of antioxidant polyphenolics found in fruits and vegetables may show efficacy in aging. These secondary compounds serve a variety of functions that enhance the plants’ survivability. Moreover, they may be responsible for the putative multitude of beneficial effects of fruits and vegetables on health-related issues.\textsuperscript{37}

Purple grape juices—organic and conventional—were capable of reducing DNA damage caused by \( \text{CCl}_4 \) in adult (7-month-old) rats. Indeed, our findings suggest that the purple grape juices were capable of reducing the high damage class frequency and damage index when compared with the control that received \( \text{CCl}_4 \). In this sense, Park \textit{et al.}\textsuperscript{17} showed by comet assay that purple \textit{Vitis vinifera} grape juice was capable of reducing DNA damage in healthy individuals. This protection—that the compounds present in grape juice can inhibit the oxidative damage to DNA—has already been reported by Ferguson.\textsuperscript{9} The comet assay conducted in other studies with fruits and their juices, \textit{e.g.}, orange juice, furnished evidence of important antigenotoxic activity in rat blood.\textsuperscript{48–50}

Although further research is necessary, our study shows that a supplementation with grape juice could at least help preventing or decreasing the damages caused by oxidative stress, especially those associated with aging, as Parkinson’s and Alzheimer’s diseases, and others.

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**AUTHOR DISCLOSURE STATEMENT**

No competing financial interests exist.

**REFERENCES**


