Purple grape juices prevent pentyleneetetrazol-induced oxidative damage in the liver and serum of Wistar rats

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Oxidative damages in hepatocytes may be caused by epilepsy and/or anticonvulsant drugs. Epilepsy is one of the most common neurological disorders, characterized by recurrent seizures, which may increase the content of reactive oxygen species. Organic and conventional grape juices are rich in polyphenols, compounds with important antioxidant activity. It is hypothesized that organic and conventional purple grape juices may have protective effect against oxidative damage induced by pentyleneetetrazole (PTZ) (a standard convulsant drug) in the liver and serum of Wistar rats. Animals (n = 16 in each group) received, by gavage, saline, organic grape juice or conventional grape juice (10 μL/g of body weight) for 17 days. Subsequently, half of the rats in each group received PTZ (60 mg/kg). After 30 minutes, the animals were euthanized by decapitation. Liver and blood samples were isolated to evaluate oxidative parameters (lipid and protein oxidation, nitric oxide metabolites, antioxidant defenses, and protein sulfhydryl content). The results of this study showed that although organic juice contains higher polyphenol content than conventional juice, both juices conferred protection against lipid and protein oxidative damage and limited the increase in PTZ-induced nitric oxide metabolite content in the liver and serum. In addition, both juices inhibited the PTZ-induced reduction in enzymatic antioxidant defenses (superoxide dismutase and catalase activities) and sulfhydryl protein content in the liver and serum. In summary, both organic and conventional grape juices were able to reduce oxidative damage induced by PTZ in the liver and serum of Wistar rats.

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1. Introduction

Fulminant liver failure is a rare complication of grand mal seizures with a high mortality, the prognosis being largely determined by the combination of the hepatic and neurologic insults [1]. Epilepsy is a neurologic disease that is characterized by recurrent seizures and affects more than 50 million people worldwide [2]. Recurrent and prolonged seizures can increase the content of reactive oxygen species (ROS) in the body and lead to hepatic oxidative damage by lipid peroxidation in hepatocytes [3,4]. The liver is among the tissues that act as organs of detoxification, protecting the body from dietary, environmental, and metabolic chemicals and toxins [4]. The mechanisms of acute liver failure resulting from epilepsy are poorly defined and appear to be multifactorial, including hypoxia and steatosis [1,3]. These processes are associated with oxidative stress [5,6], a condition characterized by an increase in the generation of free radicals and/or a decrease in the antioxidant defense systems [7].

Another concern associated with epilepsy is the side effects related to the drugs used for treating the disease. In general, anticonvulsant drugs are associated with a variety of side effects, including chronic toxicity [8], and can cause or exacerbate free radical-mediated damage [9]. Studies have shown that many anticonvulsant drugs, such as valproate, carbamazepine, phenytoin, lamotrigine, and phenobarbital are metabolized in the liver and may elevate liver enzyme levels, which may result in hepatic toxic effects or another type of liver injury [1,10]. In addition, large doses or combinations of these drugs often deplete hepatic stores of enzymatic antioxidant defenses and leave hepatocytes much more vulnerable to oxidative stress [3].

Moreover, epileptic patients present an increase in lipid oxidative damage and a reduction in the activity of antioxidant enzymes in the serum or plasma [11], showing that this disease causes a systemic imbalance.

Grape juice is widely sold throughout the world and is an easily accessible food for consumption by the population [12]. Both organic (free of pesticides and genetic engineering) and conventional (traditionally cultivated) juices may be found in the market. It has already been shown that organic grape juices present higher polyphenol content than their conventional counterparts [13]. Polyphenols demonstrate important antioxidant activity, which may protect the body against oxidative damage generated by ROS [14].

In this context, we hypothesized that organic and conventional grape juices may have protective effect against oxidative damage induced by pentylenetetrazole (PTZ) (the most commonly used convulsant chemical agent [8]). Therefore, the objective of this study was to determine the oxidative markers for lipids and proteins, nitric oxide metabolites (NOx) content, superoxide dismutase (SOD), and catalase (CAT) enzymatic activities, and sulfhydryl protein content in the liver and serum of Wistar rats after the treatment with organic or conventional grape juices and PTZ.

2. Methods and materials

2.1. Chemicals

2,4-Dinitrophenylhydrazine, 5,5’-dithiobis (2-nitrobenzoic acid), thiobarbituric acid, and PTZ were obtained from Sigma-Aldrich, St. Louis, MO, USA. All other reagents (Merck, Darmstadt, Germany and Hexapur, CA, USA) and solvents (Nuclear, Diadema, SP, Brazil) were of analytical grade.

2.2. Grape juices

The purple grape juice samples used in this work were from Vitis labrusca grapes of the Bordeaux variety harvested in 2009. The organic juice was from Cooperativa Aecia Agricultores Ecologistas Ltda (Antonio Prado, RS, Brazil), and it was certified by Rede de Agroecologia ECOVIDA. The conventional juice was obtained from Vinicola Perini Ltda (Farroupilha, RS, Brazil). The main characteristics of each purple grape juice are shown in Table 1. Carbohydrates, lipids, proteins, and ascorbic acid determinations were performed according to the Association of Official Analytical Chemists [16]. Total phenolic content was measured using Folin-Ciocalteau colorimetric method [17], and major compounds were measured by high-performance liquid chromatography as previously described [15].

2.3. Animals

Forty-eight male Wistar rats (3 months old, weighing 300 ± 50 g) from the breeding colony of Centro Universitário Metodista do IPA (Porto Alegre, Brazil) were used in the experiments. The animals were handled under standard laboratory conditions consisting of a 12-hour light/dark cycle and maintained at a fixed temperature (23°C ± 2°C). Rats were allowed free access to water and AIN-93G growth

<p>| Table 1 – Main chemical characteristics of organic and conventional purple grape juices. |</p>
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Organic juice</th>
<th>Conventional juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energetic values (kcal)</td>
<td>82.85 ± 0.57</td>
<td>81.63 ± 0.55</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>17.50 ± 0.01</td>
<td>17.50 ± 0.28</td>
</tr>
<tr>
<td>Lipid (%)</td>
<td>1.25 ± 0.09</td>
<td>1.07 ± 0.06</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>0.38 ± 0.03</td>
<td>0.50 ± 0.01 a</td>
</tr>
<tr>
<td>Ascorbic acid (mg/%)</td>
<td>45.34 ± 1.64</td>
<td>26.71 ± 1.65 a</td>
</tr>
<tr>
<td>Total phenolic content (mg catechin/%)</td>
<td>146.32 ± 1.01</td>
<td>125.76 ± 1.71</td>
</tr>
<tr>
<td>Anthocyanins (mg/L)</td>
<td>340.76 ± 0.47</td>
<td>255.03 ± 0.35 a</td>
</tr>
<tr>
<td>Catechins (mg/L)</td>
<td>33.68 ± 0.01</td>
<td>14.06 ± 0.01 a</td>
</tr>
<tr>
<td>Gallic acid (mg/L)</td>
<td>5.30 ± 0.01</td>
<td>8.27 ± 0.01 a</td>
</tr>
<tr>
<td>Procyanidins (mg/L)</td>
<td>14.47 ± 0.31</td>
<td>15.21 ± 0.27</td>
</tr>
<tr>
<td>Resveratrol (mg/L)</td>
<td>0.22 ± 0.41</td>
<td>0.15 ± 0.01 a</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE of determinations in duplicate. Carbohydrates, lipids, proteins, ascorbic acid, total phenolic content, gallic acid, and resveratrol are data from Rodrigues et al [15].

a Significant difference from organic grape juice (Student t test, P < .05).
purified diet recommended by the American Institute Nutrition [18]. All experimental procedures were performed in accordance with the Brazilian Society of Neurosciences and Behavior. The study was approved by the Research Ethics Committee of Centro Universitário Metodista do IPA, number 298/2009.

2.4. Experimental design

The animals were randomly allocated to 1 of 3 experimental groups (n = 16 per group): group 1 served as control and received saline solution, groups 2 and 3 were given, by oral gavage, organic, and conventional grape juices (10 μL/g of body weight), respectively, once a day for 17 days, as previously described [15]. The doses of purple grape juice were determined by calculating the amount of juice consumed on average by a 70-kg human male, that is, around 500 mL/d [19]. Experiments were carried out between 8:00 AM and 1:00 PM in a noise-free room. On the 18th day, half of the rats in each group (n = 8) received a single intraperitoneal dose of PTZ (60 mg/kg of body weight) that was dissolved in sterile isotonic saline. The other rats (negative control) received saline solution (intraperitoneal). After 30 minutes, the animals were euthanized by decapitation, and liver and blood samples were collected. The livers were washed with cold phosphate-buffered saline buffer (isotonic solution) until all blood was removed. Serum samples were obtained by centrifugation of the blood (5 minutes at 3000g). The liver and serum samples were stored at −80°C until analysis. Before each assay, the livers were homogenized in phosphate-buffered saline (pH 7.4) using a ground-glass-type Potter-Elvehjem homogenizer and centrifuged for 5 minutes. This supernatant was used in all assays. All processes were carried out under cold conditions (around 2°–8°).

2.5. The protective effects of grape juice in the liver and serum

To examine the effects of grape juice, lipid and protein oxidative damage, NOx content, and enzymatic (SOD and CAT) and nonenzymatic (sulfhydryl protein) antioxidant defenses were evaluated. The formation of thiobarbituric acid reactive species (TBARS) during an acid-heating reaction was used as an index of lipid peroxidation, as previously described [20]. The results were expressed as nanomoles of malondialdehyde (MDA) per milligram of protein. The oxidative damage to the proteins was assessed by determining the number of carbonyl groups based on the reaction with dinitrophenylhydrazine (DNPH), as previously described [21]. The results were expressed as nanomoles of DNPH per milligram of protein. The NOx production was determined based on the Griess reaction [22]. Nitric oxide measurements are very difficult to assess in biological specimens; tissue nitrite was estimated as an index of nitric oxide production [23]. The results were expressed as milligrams per millimeter of sodium nitroprusside per milligram of protein. The SOD activity was assayed by measuring the inhibition of adrenaline auto-oxidation, as previously described [24]. The results were expressed as U SOD/mg of protein. One unit was defined as the amount of enzyme that inhibits the rate of adrenochrome formation in 50%. The CAT activity was assayed by measuring the rate of decrease in hydrogen peroxide (H2O2) absorbance at 240 nm. The results were expressed as mmol H2O2/min/ mg of protein [25]. The protein sulfhydryl content was evaluated by the 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) method and the results were expressed as nanomoles of DTNB per milligram of protein [26]. The protein concentration was measured by the Bradford method [27], using bovine serum albumin as a standard.

3. Results

In this study, the effects of organic and conventional grape juice on the liver and serum of Wistar rats treated with PTZ were evaluated. It was observed that all PTZ-treated animals presented tonic-clonic seizures (data not shown). Grape juices alone do not cause seizure. In addition, PTZ treatments induced an increase in lipid peroxidation (TBARS), protein damage (carbonyl protein content), and NOx levels, in both the liver and serum of rats, when compared with the saline group. In addition, the SOD and CAT activities and the sulfhydryl protein content were reduced in the liver and serum of rats treated with PTZ (Tables 2 and 3). Conversely, organic and conventional grape juice treatments did not induce oxidative damage or an increase in NOx content and decrease in sulfhydryl protein content, and the endogenous antioxidant defense levels in the liver (Table 2) and serum (Table 3) of rats were maintained. A pretreatment with organic or conventional grape juice protected against PTZ-induced oxidative damage to lipids and proteins and the increased NOx content in the liver and serum of rats. Moreover, both grape juices prevented the decrease in the SOD and CAT activities and the reduction in the sulfhydryl protein content induced by PTZ in the liver and serum of rats (Tables 2 and 3).

4. Discussion

Recurrent and prolonged seizures and anticonvulsant drugs may increase the concentration of ROS in the body, which may result in substantial deleterious effects on an individual’s health. The liver, in particular, is an organ that is sensitive to oxidative damage [4]. Seizures may lead to lipid peroxidation and decreased antioxidant defense mechanisms in hepatocytes [3,4]. These types of damage may lead to epilepsy.
complications, such as fulminant liver failure [3]. Furthermore, anticonvulsant drugs may elevate liver enzyme levels, to deplete hepatic enzymatic antioxidant defenses, which may result in hepatotoxic effects or liver injury[1,3,10]. Epileptic patients also present alterations in the redox status of their serum samples [11]. Grape juices are foods rich in polyphenols [13], compounds with important antioxidant activity [14]. In this study, the possible protective effects of organic and conventional grape juice on the liver and serum of Wistar rats treated with PTZ were evaluated. The dose of PTZ used (60 mg/kg of body weight) is between half of the effective dose to cause seizures (33 mg/kg) and the median lethal dose (75 mg/kg) [23].

The results show that seizures induced by PTZ lead to an increase in lipid and protein damage and NOx content and to a decrease in SOD and CAT activities and sulfhydryl protein content in the liver and serum of rats. These results are consistent with previous data of rats treated with PTZ [3,4,28,29]. Several studies have shown that PTZ may trigger a variety of biochemical processes, including proteolysis and the release of ROS[8,29,30], such as superoxide and NOx [30], which can lead to lipid and protein damage and a decrease in antioxidant defenses and sulfhydryl protein content [4,31,32]. Moreover, the increased oxidative stress observed in the liver of rats could be due to the activation of glutamate receptors in the liver. The activation of type-5 metabotropic glutamate receptors contributes to the development of hypoxia-induced liver cell injury [33].

Superoxide dismutase and CAT are the main enzymes in the enzymatic antioxidant defense system, which is responsible for protecting against the increase in ROS production [30].

### Table 2 – Determination of TBARS, carbonyl protein, NOx content, SOD, CAT, and sulfhydryl protein in the liver of rats treated with organic or conventional grape juices in PTZ-induced seizures.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (saline)</th>
<th>PTZ (60 mg/kg)</th>
<th>Organic grape juice (10 μL/g)</th>
<th>Organic grape juice + PTZ</th>
<th>Conventional grape juice (10 μL/g)</th>
<th>Conventional grape juice + PTZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (nmol of MDA/mg of protein)</td>
<td>0.44 ± 0.06a</td>
<td>0.62 ± 0.03b</td>
<td>0.29 ± 0.02c</td>
<td>0.32 ± 0.03c</td>
<td>0.28 ± 0.02c</td>
<td>0.24 ± 0.01c</td>
</tr>
<tr>
<td>Carbonyl protein (nmol of DNP/ mg of protein)</td>
<td>1.37 ± 0.09a</td>
<td>2.53 ± 0.08b</td>
<td>1.30 ± 0.16a</td>
<td>1.06 ± 0.21a</td>
<td>1.21 ± 0.23a</td>
<td>1.40 ± 0.19a</td>
</tr>
<tr>
<td>NOx content (mg/mL of sodium nitroprusside/mg of protein)</td>
<td>1.26 ± 0.07a,b</td>
<td>3.37 ± 0.09a</td>
<td>1.22 ± 0.11a</td>
<td>1.53 ± 0.03c</td>
<td>1.23 ± 0.05c</td>
<td>1.51 ± 0.10c</td>
</tr>
<tr>
<td>Superoxide dismutase (U SOD/mg of protein)</td>
<td>2.48 ± 0.26a</td>
<td>1.07 ± 0.15b</td>
<td>2.05 ± 0.45a</td>
<td>2.21 ± 0.49a</td>
<td>1.90 ± 0.01a</td>
<td>2.02 ± 0.17a</td>
</tr>
<tr>
<td>Catalase (mmol H2O2/min/mg of protein)</td>
<td>73.15 ± 8.33a</td>
<td>42.88 ± 1.22b</td>
<td>85.24 ± 6.79b</td>
<td>82.22 ± 8.43a</td>
<td>68.89 ± 2.46e</td>
<td>77.55 ± 1.38e</td>
</tr>
<tr>
<td>Sulfhydryl protein (nmol DTNB/mg of protein)</td>
<td>0.33 ± 0.04a</td>
<td>0.17 ± 0.01b</td>
<td>0.39 ± 0.04a</td>
<td>0.30 ± 0.01c</td>
<td>0.24 ± 0.01d</td>
<td>0.29 ± 0.01c</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE (n = 8 per group) of determinations in triplicate. Different superscript letters between groups denote significant differences (Tukey post hoc test, P < .05).

### Table 3 – Determination of TBARS, carbonyl protein, NOx content, SOD, CAT, and sulfhydryl protein in the serum of rats treated with organic or conventional grape juices in PTZ-induced seizures.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (saline)</th>
<th>PTZ (60 mg/kg)</th>
<th>Organic grape juice (10 μL/g)</th>
<th>Organic grape juice + PTZ</th>
<th>Conventional grape juice (10 μL/g)</th>
<th>Conventional grape juice + PTZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (nmol of MDA/mg of protein)</td>
<td>0.22 ± 0.02a,c</td>
<td>0.28 ± 0.01b</td>
<td>0.20 ± 0.01a</td>
<td>0.22 ± 0.01a,c</td>
<td>0.19 ± 0.01a</td>
<td>0.23 ± 0.01c</td>
</tr>
<tr>
<td>Carbonyl protein (nmol of DNP/ mg of protein)</td>
<td>1.11 ± 0.11a</td>
<td>1.77 ± 0.05b</td>
<td>0.72 ± 0.04c</td>
<td>0.74 ± 0.05c</td>
<td>0.84 ± 0.08c</td>
<td>1.03 ± 0.03a</td>
</tr>
<tr>
<td>NOx content (mg/mL of sodium nitroprusside/mg of protein)</td>
<td>0.35 ± 0.04a</td>
<td>0.51 ± 0.02b</td>
<td>0.25 ± 0.07a</td>
<td>0.35 ± 0.01a</td>
<td>0.31 ± 0.01a</td>
<td>0.27 ± 0.01a</td>
</tr>
<tr>
<td>Superoxide dismutase (U SOD/ mg of protein)</td>
<td>0.64 ± 0.16a</td>
<td>0.17 ± 0.01b</td>
<td>0.66 ± 0.01a</td>
<td>0.66 ± 0.01a</td>
<td>0.66 ± 0.01a</td>
<td>0.58 ± 0.01a</td>
</tr>
<tr>
<td>Catalase (mmol H2O2/min/mg of protein)</td>
<td>11.56 ± 1.09a</td>
<td>6.69 ± 0.39b</td>
<td>11.41 ± 1.37a</td>
<td>10.87 ± 0.90a</td>
<td>10.94 ± 1.36a</td>
<td>10.65 ± 1.20a</td>
</tr>
<tr>
<td>Sulfhydryl protein (nmol DTNB/mg of protein)</td>
<td>0.11 ± 0.01a</td>
<td>0.07 ± 0.01b</td>
<td>0.11 ± 0.01a</td>
<td>0.12 ± 0.01a</td>
<td>0.12 ± 0.01a</td>
<td>0.10 ± 0.01a</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE (n = 8 per group) of determinations in triplicate. Different superscript letters between groups denote significant differences (Tukey post hoc test, P < .05).
anions in hydrogen peroxide and oxygen [34], and CAT may decompose hydrogen peroxide in water and oxygen [30]. A poor defense system allows the formation of superoxide anions and hydrogen peroxide. The superoxide radical can react with NOx, generating a highly reactive molecule, the peroxynitrite anion, which is able to induce lipid oxidation and inactivate several key sulfhydryl-bearing enzymes [35], depleting the sulfhydryl protein content.

Treatment with organic or conventional grape juice (without PTZ treatment) was unable to induce oxidative stress in the liver and serum of rats, which is in agreement with data obtained by Dani et al. [36] in Wistar rats. Furthermore, pretreatments with organic or conventional grape juice completely protected against PTZ-mediated lipid and protein damage, the increase in NOx content, and the decrease in the antioxidants defenses and sulfhydryl protein content in liver and serum of rats. The hormone ghrelin [29] treatment was also able to reduce lipid damages and increase antioxidant enzymes activities in the liver of a PTZ epilepsy model in rats. However, ghrelin potently stimulates hunger and growth hormone secretion [29], and this may not be favorable for all patients. Similar results (reduce of the NOx content and increase antioxidant enzymes activities induced by PTZ in rats) were observed for high doses of topiramate plus vitamin E (50 mg/kg plus 150 mg/kg) treatment [30]. Grape juices dose was calculated by the daily amount of juice consumed on average by a 70-kg human male, and they present the same or better results than observed for ghrelin [29] and topiramate plus vitamin E [30].

It is possible that these effects are due to the high levels of polyphenols present in both juices. Phenolic compounds are secondary metabolites, which possess important antioxidant properties and are produced and accumulated in plant tissues. In this study, organic and conventional grape juices were studied. Organic farming does not use pesticides or synthetic fertilizers. Because pesticides are not used, plants are more susceptible to the action of phytopathogens, and this induces the organic plant to produce higher amounts of polyphenols as a means to defend itself [13,37]. In fact, the organic juice assayed in our work showed a higher content of total polyphenols, anthocyanins, catechins, and resveratrol than the conventional juice. However, both juices presented the same protective effect against oxidative damages induced by PTZ in both the liver and serum of rats. Manach et al. [38] showed that the absorption (milligrams per kilogram) of polyphenols, such as anthocyanins, catechins, and procyanidins, by the body is approximately at the scale of nanomoles or micromoles per liter. Nevertheless, even at this low concentration, it was possible to observe antioxidant, cardioprotective, anticancer, anti-inflammatory, and antimicrobial properties [39]. Besides, it was already shown that different concentrations of wine wastes [40], apple juices [41], the hormone ghrelin [29], and the monoterpene isopulegol [8] present the same biological results in rats or human tissues. The mechanisms of the antioxidant action of these compounds may include suppressing ROS formation by inhibiting enzyme depletion or by chelating trace elements involved in free radical production, scavenging ROS and up-regulating or protecting the antioxidant defenses [7]. However, considering that aging tends to increase oxidative stress, it would be interesting to study if the concentrations of polyphenols found in grape juices will efficiently protect from oxidative damage induced by seizures.

In conclusion, these data demonstrate that both organic and conventional grape juices present important hepatic and systemic protection effects against oxidative damages induced by PTZ in Wistar rats, confirming our initial hypothesis. Carrying out this study in an animal model may be considered a limitation due to the fact that it is not possible to extrapolate the results to humans. Therefore, further studies are necessary to determine whether grape juices may reduce the oxidative damage in epileptic patients using antiepileptic drugs. Although additional studies are needed, serum samples could be used for monitoring the oxidative damage in these individuals.

Acknowledgment

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