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Neuroprotective and anticonvulsant effects of organic and conventional purple grape juices on seizures in Wistar rats induced by pentylenetetrazole

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ABSTRACT

Epilepsy is the most common neurological disorder worldwide. Studies have shown that recurrent seizures may increase the concentration of reactive oxygen species, which can lead to oxidative stress and neuronal damage. These seizures result in substantial deleterious effects on an individual's health. Organic and conventional grape juices are rich in polyphenols, compounds with important antioxidant activity. However, these juices could have differences in their polyphenol content. The aim of this study was to investigate the neuroprotective and anticonvulsant effects of organic and conventional grape juice treatments in Wistar rats against pentylenetetrazole (a convulsant drug)-induced damage. In addition, we evaluated potential behavioral changes in rats treated with the juices and the polyphenolic profile of those samples. Animals (n = 16 in each group) received treatment with saline, organic or conventional grape juice for 17 days. On the eighteenth day, behavioral changes were evaluated by an open field test. Afterwards, half of the rats from each group received pentylenetetrazole and were observed for 30 min to evaluate possible seizure characteristics. The animals were subsequently killed by decapitation and their hippocampus, cerebellum and cerebral cortex tissues were isolated. The results of this study showed that neither organic nor conventional grape juice altered the behavior parameters, and no statistical differences were observed in the seizure characteristics of the groups. Nevertheless, both juice types were able to protect from lipid and protein oxidative damage, decrease nitric oxide content and increase enzymatic (superoxide dismutase and catalase) and non-enzymatic (sulfhydryl protein) antioxidant defenses in brain tissues following pentylenetetrazole-induced seizures. In general, organic juice showed superior results in each test, probably due to its higher polyphenol content relative to conventional juice. These results indicate that grape juices can provide further insight into natural neuroprotective compounds and may lead to the development of new therapeutic strategies for epileptic patients.

1. Introduction

According to the World Health Organization (WHO, 2011), epilepsy is one of the most common serious neurological conditions, affecting more than 50 million people worldwide. Seizures are caused by sudden, excessive and recurrent electrical discharges from brain cells. Studies have shown that recurrent seizures may increase the concentration of reactive oxygen species (ROS), including superoxide anions, hydroxyl radicals and hydrogen peroxide, in the brain (Sudha et al., 2001; Xu and Stringer, 2008). The generation of ROS can predispose the brain to oxidative stress, and, consequently, to neuronal damage (Costello and Delanty, 2004). This predisposition, in turn, can lead to higher rates of other conditions, such as depression, anxiety, psychiatric disorders (Reilly et al., 2011), psychosocial issues and sudden death. Epilepsy increases a person's risk of premature death by approximately two to three times compared to the general population (Maldonado et al., 2010; WHO, 2011). Despite the existence of a large number of antiepileptic drugs, there is currently no cure for epilepsy, and treatment is limited (Wahab, 2010). More than thirty percent of patients with epilepsy have inadequate control of their seizures by drug therapy, but why this happens and whether it can be predicted remain unknown (Kwan and Brodie, 2000). Furthermore, antiepileptic drugs are associated with a variety of side-effects and chronic toxicity (Silva et al., 2009).

In recent years, a great deal of attention has been devoted to the consumption of polyphenols. These phytochemicals have

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antioxidant effects that may protect the body against the oxidative damage caused by ROS. Therefore, polyphenols have been linked to reductions in the risk of major chronic diseases, such as Parkinson's, Alzheimer's and other neurodegenerative diseases (Halliwell and Gutteride, 2007; Liu, 2003). Purple grape juice is a rich source of polyphenols, particularly anthocyanins, catechins and resveratrol (Dani et al., 2007). It is possible to find both organic (free of pesticides and genetic engineering) and conventional (traditional cultivation) juices. It has been already shown that organic grape juice contains more phenolic compounds than does conventional juice (Dani et al., 2007).

Pentylenetetrazole (PTZ) is the convulsant agent most commonly used in animal models for screening drugs for their potential anticonvulsant properties (Silva et al., 2009). The administration of this chemical convulsant leads to a decrease in γ-aminobutyric acid (GABA) function (inhibitory neurotransmission) and the stimulation and modification of either the density or sensitivity of different glutamate receptor subtypes (excitatory neurotransmission) (White et al., 2007). A growing body of evidence has suggested that ROS generation may underlie the neurotoxic effects of PTZ (Obay et al., 2008; Silva et al., 2009). In this context, the aim of the present study was to investigate the potential neuroprotective and anticonvulsant effects in Wistar rats of organic and conventional purple grape juice treatment against PTZ-induced damage. Furthermore, we evaluated the potential behavioral changes by an open field test of rats treated with the juices and measured the polyphenolic profile of these samples by liquid chromatography.

2. Materials and methods

2.1. Chemicals

Procyanidins B1 and B2, (+)-catechin, (•)-epicatechin, gallic acid, cyanidin-3-glucoside, delphinidin-3-glucoside, peonidin-3-glucoside, malvidin-3-diglucoside, malvidin-3-glucoside, trans-resveratrol, 2,4-dinitrophenylhydrazine, 5,5′-dithiobis(2-nitrobenzoic acid), thiobarbituric acid and pentylenetetrazole were obtained from Sigma–Aldrich. All other reagents (Merck and Hexapur) and solvents (Nuclear) were of analytical grade.

2.2. Grape juices

The purple grape juice samples used in this study were from *Vitis labrusca* grapes, Bordo variety, harvested in 2009. The organic juice was obtained from the Cooperativa Aecia Agricultores Ecologistas Ltda. (Antonio Prado, RS, Brazil) and was certified by Rede de Agroecologia ECOVIDA, while the conventional juice was obtained from Vinícola Perini Ltda. (Farroupilha, RS, Brazil). The main characteristics of each grape juice are shown in Table 1.

2.3. Animals and treatments

Forty-eight male Wistar rats (90 days old, weighing 250 ± 50 g) from the breeding colony of the Centro Universitário Metodista were used in these experiments. The number of animals was determined by a statistical F test – MANOVA ($F = 3.21$, $\alpha = 0.05$, power = 90%). The animals were handled under standard laboratory conditions consisting of a 12-h 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 Brazilian Society of Neurosciences and Behavior. The study was approved by the Research Ethics Committee of the Centro Universitário Metodista IPA, number 298/2009. The animals were randomly assigned to one of three experimental groups ($n = 16$ per group) as follows: group 1 served as control and received saline, while groups 2 and 3 were given, by gavage, organic or conventional grape juice (10 μL/g of body weight), respectively, once a day over the course of 17 days. The doses of purple grape juice were determined by calculating the amount of juice consumed on average by a 70-kg human male, i.e., approximately 500 mL/day (Park et al., 2003).

Table 1
Main chemical characteristics of purple grape juices.

	Organic grape juice	Conventional grape juice
Carbohydrates (%)	17.50 ± 0.01	17.50 ± 0.20
Lipids (%)	1.25 ± 0.06	1.07 ± 0.04
Proteins (%)	0.38 ± 0.02	0.50 ± 0.01*
Moisture (%)	87.20 ± 0.01	85.70 ± 0.05*
Ashes (%)	0.40 ± 0.01	0.20 ± 0.01*
Total acidity (% acid solution in molar)	8.40 ± 0.01	8.20 ± 0.01
pH	3.71 ± 0.01	3.49 ± 0.01*
Ascorbic acid (mg %)	45.34 ± 1.16	26.71 ± 1.17*

The grape juice determinations were performed according to the Association of Official Analytical Chemists (2005).
* Statistically different from organic grape juice.

2.4. Open field test

In order to assess if purple grape juices intake could alter the behavioral parameters, the treated rats were evaluated through the open field test. Anxiety, locomotion and exploratory activities were evaluated in the animals following the conclusion of the treatment (day 18). Experiments were carried out between 8:00 a.m. and 13:00 p.m. in a noise-free room. Rats were placed in a wooden box in which the floor was divided by black lines into 12 equal squares. Initially, the rats were placed in the middle of the quadrant and were allowed to explore the box freely for five minutes. The latency to start locomotion, the number of black line crossing, rearing, grooming and fecal bolus during exploration were measured and recorded manually (Holzmann et al., 2011; Galani and Patel, 2010).

2.5. Pentylenetetrazole (PTZ)-induced seizures

After the open field test, half of the rats from each group ($n = 8$) received a single, intraperitoneal (i.p.) dose of PTZ (60 mg/kg of body weight) dissolved in sterile isotonic saline. This dose is between half of the effective dose to cause seizures (33 mg/kg) and the median lethal dose (75 mg/kg) (Ilhan et al., 2005). The other half of the rats (negative control) received saline solution (i.p.). The animals were observed for 30 min by a blind researcher, and the resulting seizures were classified according to the stages proposed by Racine et al. (1972). We observed the latency to seizure onset, the tonic-clonic seizure time, the total seizure time, the number of seizures and how many seizures reached the fifth stage on Racine's scale (tonic-clonic seizures).

2.6. Neuroprotective effects of grape juice in brain tissues

Following the seizure tests, all animals, with or without PTZ treatment, were killed by decapitation. The hippocampus, cerebellum and cerebral cortex were isolated and stored at −80 °C. Prior to each assay, the tissues were homogenized in phosphate buffered saline (pH 7.4) using a ground-glass-type Potter–Elvehjem homogenizer and were centrifuged for five minutes. The supernatant was used in all assays. All processes were carried out under cold conditions.

To evaluate a possible neuroprotective effect of the juices, we measured the lipid and protein oxidative damage, the nitric oxide content and the enzymatic (superoxide dismutase and catalase)

and non-enzymatic (sulfhydryl protein) antioxidant defenses. We used the formation of thiobarbituric acid-reactive species (TBARS) during an acid-heating reaction as an index of lipid peroxidation, as previously described by Wills (1996). The results were expressed as nmol of malondialdehyde (MDA)/mg protein. The oxidative damage to proteins was assessed by the formation of carbonyl groups based on the reaction with dinitrophenylhydrazine, as previously described by Levine et al. (1990). The results were expressed as nmol/mg of protein. Nitric oxide production was determined based on the Griess reaction (Green et al., 1981). Nitrite concentration was determined from a standard nitrite curve generated using sodium nitroprusside. The results were expressed as mg/mL of sodium nitroprusside/mg protein. Superoxide dismutase (SOD) activity was assayed by measuring the inhibition of adrenaline auto-oxidation, as previously described (Bannister and Calabarese, 1987), and the results were expressed as U SOD (units of enzyme activity)/mg of protein. One unit was defined as the amount of enzyme that inhibits the rate of adrenochrome formation in 50%. Catalase (CAT) activity was assayed by measuring the rate of decrease in hydrogen peroxide (H_2O_2) absorbance at 240 nm, as previously described (Aebi, 1984), and the results were expressed as mmol H_2O_2/min/ mg of protein. The protein sulfhydryl content was evaluated by the 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) method (Aksenov and Markesbery, 2001), and the results are expressed as nmol DTNB/mg of protein. Protein concentration was measured by the Bradford method Bradford (1976) using bovine serum albumin as a standard.

2.7. Quantification of the phenolic compounds in the grape juices

The total phenolic content of the organic and conventional grape juices were measured using the modification of the Folin–Ciocalteau colorimetric method, as described by Singleton et al. (1999). Two hundred microliters of grape juice was assayed with 1000 μL of Folin–Ciocalteu reagent and 800 μL of sodium carbonate (7.5%, w/v). After 30 min, the absorbance was measured at 765 nm, and the results were expressed as mg/L catechin equivalent. High-performance liquid chromatography (HPLC) analysis was used to quantify the presence of individual phenolic compounds. Prior to the HPLC analysis, 1.5 mL of each sample was filtered through a cellulose membrane (diameter 0.2 μm). The equipment used in the analysis consisted of an LC-DAD Series 1100 liquid chromatographic system (Hewlett–Packard, Palo Alto, CA) with a diode array detector system.

The chromatographic analyzes were a modification of the methods described by Lamuela-Raventós and Waterhouse (1994). A Zorbax SB C18 (250 × 4.6 mm), 5 m particle size, with a flow of 0.5 mL/min, was used for the stationary phase. After filtration on a 0.2 m Millipore membrane, five microliters of grape juice was injected into the HPLC system. The solvents used for the separation were as follows: solvent A (50 mM dihydrogen ammonium phosphate adjusted to pH 2.6 with orthophosphoric acid), solvent B (20% of solvent A with 80% acetonitrile) and solvent C (0.2 M orthophosphoric acid adjusted with ammonia to pH 1.5). The gradient conditions were as follows: solvent A 100% (0–5 min), solvents A 96% and B 4% (5–15 min), solvents A 92% and B 8% (15–25 min), solvents B 8% and C 92% (25–45 min), solvents B 30% and C 70% (45–50 min), solvents B 40% and C 60% (50–55 min), solvents B 80% and C 20% (55–60 min) and solvent A 100% (60–65 min). Chromatograms were monitored at 204 nm, and identification was based on the retention time relative to authentic standards ((+)-catechin, (−)-epicatechin, procyanidin B1, B2 and gallic acid). Quantification was performed using the standards by establishing calibration curves for each identified compound. Results are shown in mg/L. To determine cyanidin-3-glucoside, delphinidin-3-glucoside, peonidin-3-glucoside, malvidin-3-diglucoside and malvidin-3-glucoside, we used a mobile phase with solvents A (ultrapure water, formic acid, and acetonitrile) and B (ultrapure water, formic acid, and acetonitrile) in a constant flow of 0.8 mL/minute with a controlled temperature of 40 °C. The gradient conditions were as follows: solvents A 94% and B 6% (0 min), solvents A 70% and B 30% (0–15 min), solvents A 50% and B 50% (15–30 min), solvents A 40% and B 60% (30–35 min), solvents A 94% and B 6% (35–41 min). The peak was detected at 518 nm, and the amount of sample injected was 50 μL (OENO, 2003). To quantify the resveratrol compound, we used a mobile phase of ultrapure water and acetonitrile (75:25 vol/vol) (pH 3.0) with a constant flow of 1.0 mL/min for 20 min with a controlled temperature of 25 °C. The gradient conditions were as follows: solvents A 10% and B 90% (0 min), solvents A 85% and B 15% (0–23 min), solvents A 95% and B 5% (23–30 min), solvents A 10% and B 90% (30–35 min). The peak was detected at 385 nm, and the amount of sample injected was 20 μL (McMurtrey et al., 1994).

2.8. Statistical analyzes

All measurements were performed in duplicate, at least. All values were presented as mean and standard error. The main chemical characteristics and phenolic compounds of organic and conventional grape juices were subjected to Student's t-test ($p \leqslant 0.05$). Other results were subjected to an analysis of variance (ANOVA) with Tukey's post hoc test. The SPSS 17.0 software package (SPSS Inc., Chicago, IL) was used for all statistical analyzes.

3. Results

The results of the open field test showed that neither organic nor conventional grape juice altered the behavior parameters (latency for locomotion, total crossings, total rearings, grooming and fecal bolus) for any of the animals evaluated (Fig. 1). Furthermore, neither juice type was able to prevent the convulsant effects induced by PTZ (latency of seizure time, tonic-clonic seizure time, total seizure time, number of seizures and number of seizures reaching stage five on Racine's scale) (Fig. 2).

When compared to the saline group, organic and conventional grape juice treatments did not induce lipid or protein damage, nor did they increase nitric oxide content in the hippocampus, cerebellum or cerebral cortex. In addition, neither of the juices induced a decrease in the antioxidant enzymes SOD or CAT or in the sulfhydryl protein content of any of the tissues compared to the saline group (Table 3–5).

When compared to the saline group, pentylenetetrazole treatment induced an increase in lipid peroxidation (TBARS), protein damage (carbonyl protein content) and nitric oxide levels in all brain tissues. In addition, SOD and CAT activities and sulfhydryl protein were all reduced in the PTZ group for all of the tissues assayed (Tables 3–5).

Treatment with organic or conventional grape juices attenuated the PTZ-induced oxidative damage to lipids and proteins and the increase in nitric oxide concentration in the hippocampus, cerebellum and cortex. In all tissues, the organic juice also inhibited the decrease in SOD and CAT activity induced by PTZ. Both juices prevented the reduction in sulfhydryl protein concentration that is typically induced by PTZ (Tables 3–5).

Organic grape juice has a higher phenolic content compared to conventional juice (Table 2). Additionally, organic juice also shows higher concentrations of catechin, cyanidin, epicatechin, malvidin diglycoside, procyanidin B1 and resveratrol compared to conventional juice. The gallic acid and procianidin B2 concentrations were higher in conventional grape juice (Table 2).

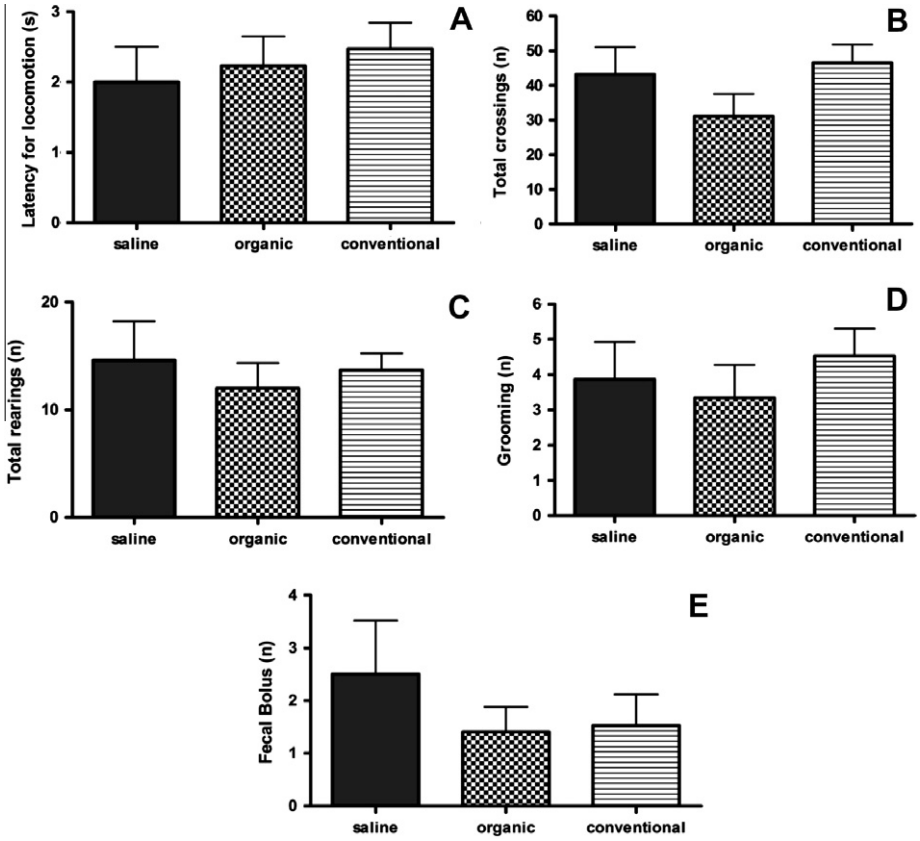

Fig. 1. Effects of treatment on the behavior of Wistar rats with or without organic or conventional grape juices on (A) the latency to start locomotion, (B) the total crossings, (C) rearings, (D) grooming, (E) fecal bolus.

4. Discussion

In the central nervous system (CNS), the disruption of the naturally existing balance between the concentrations of inhibitory and excitatory neurotransmitters is thought to be the main cause of convulsive episodes. GABA deficiency (inhibitory neurotransmission) and the stimulation and modification of either the density or sensitivity of different glutamate receptor subtypes (excitatory neurotransmission) are associated with epilepsy. In contrast, the stimulation of GABA receptors or an increase in positive modulators produces anxiolysis, sedation, anesthesia, myorelaxation and anticonvulsant actions (Silva et al., 2009). The activation of excitatory amino-acid receptors by glutamate or N-methyl-D-aspartic acid has been known to accompany the generation of ROS and reactive nitrogen species, such as superoxide anion radicals, hydrogen peroxide, nitric oxide and peroxide anions, that lead to neuronal damage (Mori et al., 2004). Studies have shown that polyphenols, such as 6-methylflavanone (Hall et al., 2005), (−)-epigallocatechin gallate (Vignes et al., 2006), flavan-3-ol derivatives (Fernandez et al., 2008) and resveratrol (Li et al., 2010), are positive modulators of GABA receptors. Grape juices are rich in polyphenols, which have important antioxidant effects (Dani et al., 2007). In this study, we evaluated the neuroprotective and anticonvulsant effects of organic and conventional grape juices in an experimental model in which epilepsy was induced in Wistar rats by PTZ. Furthermore, we also evaluated possible behavioral changes and the phenolic profiles of rats treated with the juices.

Although both grape juices contain flavan-3-ol derivatives and resveratrol, neither were able to inhibit the seizures induced by PTZ (as measured by tonic-clonic seizure time, total seizure time, number of seizure and number of seizures reaching stage five on Racine's scale) (Fig. 2). This result could be explained by the fact that the amounts of polyphenols present in grape juices are lower than those reported to be effective in binding to GABA receptors (Fernandez et al., 2008; Li et al., 2010).

PTZ may trigger a variety of biochemical processes, including the activation of membrane phospholipases, proteases and nucleases, causing the degradation of membrane phospholipid metabolism and proteolysis and protein phosphorylation; thus, PTZ could lead to a release of lipid peroxides and free radicals (Naziroglu et al., 2009; Obay et al., 2008; Silva et al., 2009). The present study shows that PTZ induces an increase in oxidative damage through lipid and protein oxidation in the hippocampus, cerebellum and cortical tissues assayed. The rats treated with organic and conventional grape juices showed an attenuation in the PTZ-induced increase in lipid and protein oxidation in all brain tissues (Tables 3–5). Similar results were found with α-tocopheryl-L-ascorbate-2-O-phosphate diester (Yamamoto et al., 2002), lipoic acid (Militão et al., 2010), erdostein (Ilhan et al., 2005) and isopulegol (Silva et al., 2009) in different experimental models of induced epilepsy in rats.

The inactivation of ROS can be accomplished by antioxidant enzymes. The enzyme SOD plays a key role in detoxifying the superoxide anions from hydrogen peroxide and oxygen (Fridovich, 1998). The hydrogen peroxide that is formed may be decomposed by CAT in water and oxygen (Naziroglu et al., 2009). We observed that PTZ decreased SOD and CAT activity in the hippocampus (Table 3), cerebellum (Table 4) and cortex (Table 5). This effect may be due to a depletion of enzymes, as previously described by Obay et al. (2008) and Silva et al. (2009), in brain tissues treated with PTZ. Organic grape juice attenuates this decrease in the activities of SOD and CAT, as previously shown for erdosteine (Ilhan et al.,

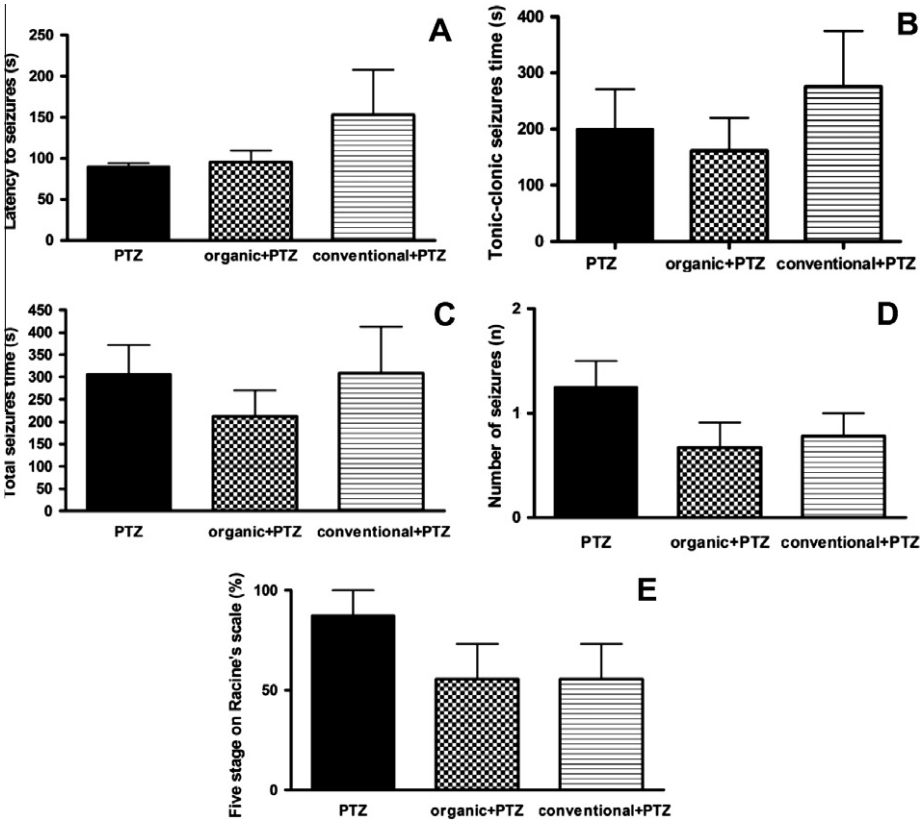

Fig. 2. Effects of pentylenetetrazole treatment of Wistar rats with or without organic or conventional grape juice on (A) the latency to seizure, (B) tonic-clonic seizure time, (C) total seizure time, (D) number of seizures, (E) percentage of rats that reached stage five on Racine's scale.

2005), ghrelin (Obay et al., 2008) and isopulegol (Silva et al., 2009) treatments in rats. In contrast, conventional juice was not able to block the modulation of enzymes induced by PTZ. While other studies are needed, it is possible that this effect could be due the reduced polyphenol (Table 2) and ascorbic acid (Table 1) content of the conventional grape juice. Organic juice also showed higher concentrations of catechin, cyanidin, epicatechin, malvidin diglycoside, procyanidin B1 and resveratrol compared to conventional juice (Table 2). Phenolic compounds are secondary metabolites that are produced and accumulated in plant tissues. Organic farming is currently practiced worldwide and does not use pesticides or synthetic fertilizers. As pesticides are not used, plants are more susceptible to the actions of phytopathogens, and this susceptibility causes the plant to produce higher amounts of polyphenols as a means of defending itself (Dani et al., 2007; Soleas et al., 1997).

It has been demonstrated that seizures induced by PTZ produce chances in nitric oxide metabolism (Naziroglu et al., 2009). The generation of nitric oxide results in lipid peroxidation, which may also induce epileptic activity by the direct inactivation of glutamine synthase, thereby permitting an abnormal buildup of the major excitatory neurotransmitter glutamate (Dilliogluglil et al., 2010; Militão et al., 2010; Tomé et al., 2010). In all tissues, both organic and conventional grape juices were able to attenuate the increase in nitric oxide content induced by PTZ. Similar results were observed for rats treated with lipoic acid (Militão et al., 2010) and α-tocopherol (Tomé et al., 2010) in a pilocarpine model of epilepsy.

Nitric oxide could react with superoxide, generating the potent tissue-damaging moiety peroxynitrite, which has a high affinity for sulfhydryl groups and thus inactivates several key sulfhydryl-bearing enzymes (Katzung, 2004). This effect is probably the reason that sulfhydryl proteins are reduced in the PTZ group. In contrast, in all tissues assayed, the treatment with either organic or conventional grape juice protected sulfhydryl groups from the oxidation induced by PTZ (Tables 3–5).

We did not observe differences in the results obtained from the different tissues assayed. The hippocampus is part of the limbic system, and it is important for learning and memory (Hansen and Koeppen, 2002). In addition, the hippocampus is a structure that is involved in the expression and propagation of seizures (Bear and Lothman, 1993). The cortex represents the highest center for sensory and motor processing, and González-Ramírez et al. (2010) suggest that this tissue also participates in the expression and propagation of seizures. The cerebellum coordinates smooth motor activities and processes muscle position (Hansen and Koeppen, 2002). More studies are needed to evaluate the association of these tissues with epileptic seizures.

Table 2
Total phenolic content (mg catechin/%) and major phenolic compounds (mg/L) of organic and conventional purple grape juice.

	Organic grape juice	Conventional grape juice
Total phenolic compounds	146.32 ± 1.01	125.76 ± 1.71*
Catechin	25.51 ± 0.01	8.11 ± 0.01*
Cyanidin	33.61 ± 0.06	25.44 ± 0.03*
Delphinidin	0.00 ± 0.00	0.00 ± 0.00
Epicatechin	8.17 ± 0.01	5.95 ± 0.01*
Gallic acid	5.30 ± 0.01	8.27 ± 0.01*
Malvidin	0.00 ± 0.00	0.00 ± 0.00
Malvidin diglycoside	316.15 ± 0.41	229.59 ± 0.32*
Peonidin	0.00 ± 0.00	0.00 ± 0.00
Procyanidin B1	7.53 ± 0.18	6.78 ± 0.16*
Procyanidin B2	6.94 ± 0.13	8.43 ± 0.11*
Resveratrol	0.224 ± 0.41	0.145 ± 0.01*

* Statistically different from organic grape juice.

Table 3
Determination of thiobarbituric acid reacting substances (TBARS), carbonyl protein, nitric oxide content, superoxide dismutase and catalase activities and sulfhydryl protein content in the hippocampus of Wistar rats following pentylenetetrazole-induced seizures with or without organic or conventional grape juices.

Groups	TBARS (nmol MDA/ mg of protein)	Carbonyl protein (nmol/mg of protein)	Nitric oxide content (mg/mL of sodium nitroprusside/mg protein)	Superoxide dismutase (U SOD/mg of protein)	Catalase (mmol H_2O_2/min/mg of protein)	Sulfhydryl protein content (nmol DTNB/mg of protein)
Control (saline)	$0.37 \pm 0.01^{a,c}$	1.64 ± 0.24^{a}	$1.44 \pm 0.03^{a,c}$	$56.06 \pm 3.81^{a,c}$	$10.90 \pm 1.09^{a,c}$	$0.24 \pm 0.01^{a,c}$
PTZ (60 mg/kg)	1.88 ± 0.15^{b}	3.03 ± 0.24^{b}	4.28 ± 0.20^{b}	32.50 ± 0.46^{b}	4.81 ± 0.49^{b}	0.05 ± 0.01^{b}
Organic grape juice (10 μL/g)	$0.36 \pm 0.04^{a,c}$	1.48 ± 0.06^{a}	$1.45 \pm 0.11^{a,c}$	54.50 ± 7.04^{a}	15.30 ± 1.71^{c}	0.21 ± 0.01^{c}
Organic grape juice + PTZ	$0.43 \pm 0.03^{a,c}$	1.45 ± 0.33^{a}	1.99 ± 0.09^{c}	$55.10 \pm 2.64^{a,c}$	14.42 ± 1.64^{c}	0.11 ± 0.01^{d}
Conventional grape juice (10 μL/g)	0.56 ± 0.02^{c}	1.34 ± 0.31^{a}	0.87 ± 0.06^{a}	68.91 ± 6.00^{c}	14.07 ± 1.25^{c}	0.28 ± 0.03^{a}
Conventional grape juice + PTZ	0.26 ± 0.01^{a}	1.48 ± 0.16^{a}	0.80 ± 0.03^{a}	$45.20 \pm 7.29^{a,b}$	$7.13 \pm 0.43^{a,b}$	0.14 ± 0.01^{d}

Different letters indicate a statistically significant difference for each parameter evaluated.

Table 4
Determination of thiobarbituric acid reacting substances (TBARS), carbonyl protein, nitric oxide content, superoxide dismutase and catalase activities and sulfhydryl protein content in the cerebellum of Wistar rats following pentylenetetrazole-induced seizures with or without organic or conventional grape juices.

Groups	TBARS (nmol MDA/ mg of protein)	Carbonyl protein (nmol/mg of protein)	Nitric oxide content (mg/mL of sodium nitroprusside/mg protein)	Superoxide dismutase (U SOD/mg of protein)	Catalase (mmol H_2O_2/ min/ mg of protein)	Sulfhydryl protein (nmol DTNB/mg of protein)
Control (saline)	0.30 ± 0.02^{a}	2.23 ± 0.30^{a}	1.64 ± 0.27^{a}	$8.02 \pm 0.51^{a,c}$	$6.20 \pm 0.99^{a,c}$	0.32 ± 0.03^{a}
PTZ (60 mg/kg)	0.96 ± 0.05^{b}	3.32 ± 0.24^{b}	6.33 ± 0.88^{b}	5.37 ± 0.72^{b}	3.51 ± 0.27^{b}	0.12 ± 0.01^{b}
Organic grape juice (10 μL/g)	0.17 ± 0.02^{c}	1.32 ± 0.16^{c}	1.53 ± 0.03^{a}	11.99 ± 1.95^{d}	10.30 ± 0.86^{e}	0.55 ± 0.04^{c}
Organic grape juice + PTZ	0.29 ± 0.01^{a}	2.01 ± 0.24^{a}	1.58 ± 0.10^{a}	$9.76 \pm 0.21^{c,d}$	$8.07 \pm 0.68^{c,d}$	0.32 ± 0.01^{a}
Conventional grape juice (10 μL/g)	$0.23 \pm 0.04^{a,c}$	1.06 ± 0.14^{c}	1.72 ± 0.14^{a}	11.00 ± 0.03^{d}	$9.61 \pm 0.56^{d,e}$	0.35 ± 0.03^{a}
Conventional grape juice + PTZ	$0.22 \pm 0.01^{a,c}$	1.11 ± 0.24^{c}	1.77 ± 0.15^{a}	$6.36 \pm 0.04^{a,b}$	$4.10 \pm 0.39^{a,b}$	0.30 ± 0.02^{a}

Different letters indicate a statistically significant difference for each parameter evaluated.

Table 5
Determination of thiobarbituric acid reacting substances (TBARS), carbonyl protein, nitric oxide content, superoxide dismutase and catalase activities and sulfhydryl protein content in the cerebral cortex of Wistar rats following pentylenetetrazole-induced seizures with or without organic or conventional grape juices.

Groups	TBARS(nmol MDA/mg of protein)	Carbonyl protein(nmol/mg of protein)	Nitric oxide content (mg/mL of sodium nitroprusside/mg protein)	Superoxide dismutase (U SOD/mg of protein)	Catalase (mmol H_2O_2/min/mg of protein)	Sulfhydryl protein (nmol DTNB/mg of protein)
Control (saline)	0.82 ± 0.04^{a}	2.73 ± 0.42^{a}	1.63 ± 0.15^{a}	$41.27 \pm 1.27^{a,c}$	10.88 ± 1.11^{a}	0.21 ± 0.03^{a}
PTZ (60 mg/kg)	1.56 ± 0.11^{b}	7.87 ± 0.81^{b}	6.17 ± 0.52^{b}	22.71 ± 3.98^{b}	6.15 ± 0.15^{b}	0.15 ± 0.01^{b}
Organic grape juice (10 μL/g)	0.81 ± 0.06^{a}	2.75 ± 0.23^{a}	1.58 ± 0.09^{a}	49.84 ± 4.46^{c}	11.77 ± 0.87^{a}	0.25 ± 0.01^{a}
Organic grape juice + PTZ	1.06 ± 0.12^{a}	2.00 ± 0.30^{a}	1.44 ± 0.14^{a}	48.65 ± 7.88^{c}	11.49 ± 0.53^{a}	0.24 ± 0.01^{a}
Conventional grape juice (10 μL/g)	0.24 ± 0.01^{c}	1.91 ± 0.24^{a}	1.62 ± 0.07^{a}	45.88 ± 5.43^{c}	10.25 ± 1.13^{a}	0.23 ± 0.01^{a}
Conventional grape juice + PTZ	1.09 ± 0.12^{a}	1.66 ± 0.29^{a}	1.26 ± 0.11^{a}	$27.71 \pm 0.16^{a,b}$	$9.14 \pm 0.43^{a,b}$	0.22 ± 0.01^{a}

Different letters indicate a statistically significant difference for each parameter evaluated.

The results of the present study demonstrate that both organic and conventional grape juices show important neuroprotective effects against PTZ-induced oxidative damage in rats. This effect could be important in reducing neuronal damage and, therefore, allow for a better quality of life for epileptic patients. Additionally, the open field test (Fig. 1) shows that neither grape juice affects the behavior (locomotor and exploratory activities) of animals. Still, organic grape juice shows a tendency to decrease the anxiety of the rats. These findings indicate that grape juices will provide further insights into natural neuroprotective compounds and may lead to the development of therapeutic strategies for epileptic patients in pharmaceutical or nutraceutical areas.

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