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ORIGINAL RESEARCH

Effect of Chronic Treatment with Conventional and Organic Purple Grape Juices (*Vitis labrusca*) on Rats Fed with High-Fat Diet

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Abstract Serra Gaucha is described as the most important wine region of Brazil. Regarding cultivars widespread in the Serra Gaucha, about 90 % of the area is occupied by vines of *Vitis labrusca* that is the most important specie used in grape juice production. The objective of this study was to investigate the antioxidant and neuroprotective effect of chronic intake of purple grape juice (organic and conventional) from Bordo variety (*V. labrusca*) on oxidative stress in different brain regions of rats supplemented with high-fat diet (HFD) for 3 months. A total of 40 male rats were randomly divided into 4 groups. Group 1 received a standard diet and water, group 2 HFD and water, group 3 HFD and conventional grape juice (CGJ), and group 4 HFD and organic grape juice (OGJ). All groups had free access to food and drink and after 3 months of treatment the rats were euthanized by decapitation and the cerebral cortex, hippocampus and cerebellum isolated and homogenized on ice for oxidative stress analysis. We observed that the consumption of calories in HFD and control groups, were higher than the groups supplemented with HFD and grape juices and that HFD diet group gain more weight than the other animals. Our results also demonstrated that HDF enhanced lipid peroxidation (TBARS) and protein damage (carbonyl) in cerebral cortex and hippocampus, reduced the non-enzymatic antioxidants defenses (sulfhydryl) in cerebral cortex and cerebellum, reduced catalase and superoxide dismutase activities in all brain tissues and enhanced nitric oxide production in all cerebral tissues. CGJ and OGJ were able to ameliorate these oxidative alterations, being OGJ more effective in this protection. Therefore, grape juices could be useful in the treatment of some neurodegenerative diseases associated with oxidative damage.

Keywords Antioxidants · Grapes · *Vitis labrusca* · High-fat diet

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Introduction

It is well described in the literature that increases in high-fat diet (HFD) consumption and obesity has lead to an increase in the prevalence of type 2 diabetes and cardiovascular diseases (Grundy et al. 2005; Mielke et al. 2006; Fontana and Klein 2007). Furthermore, some studies proposed that a HFD influences the normal development of the central nervous system and provokes the enhancement of reactive species and free radicals formation (Kalmijn 2000; Molteni et al. 2002; Solfrizzi et al. 2003; Amin et al. 2011). In this context, deficits in brain functions due to oxidative stress may be caused in part to a decline in the endogenous antioxidant defense mechanisms and to the vulnerability of the brain to the deleterious effects of oxidative damage (Halliwell and Gutteridge 2007; Hatcher and Adibhatla 2008).

The harmful effects caused by oxidative stress could be retarded or even reversed by increasing antioxidant levels, particularly phytochemicals such as polyphenols (Castilla

et al. 2006; Burin et al. 2010). Therefore, several studies have suggested an inverse relationship between the consumption of polyphenol-rich foods and beverages and the risk of degenerative diseases, cancers, and cardiovascular diseases (Peters et al. 2001; Lau et al. 2005; Kondrashov et al. 2009; Venturini et al. 2011). Moreover, some researches have also shown that diets rich in fruits and vegetables are associated with a low risk of oxidative stress-induced diseases being the polyphenols the main compounds believed to be responsible for these beneficial effects (Zern and Fernandez 2005; Silver et al. 2011). Consequently, polyphenols have been linked to a reduction in the risk of major chronic diseases, such as Parkinson's, Alzheimer's, and other neurodegenerative diseases (Baur et al. 2006; Halliwell and Gutteridge 2007; Valko et al. 2007; Ozyurt and Olmez 2012; Li and Pu 2011).

Between foods and beverages present in the human diet, purple grape juice is considered a very rich source of polyphenols, such as flavonoids, tannins, and resveratrol (Dani et al. 2007). It has been already reported that grape juice compounds can prevent: platelet aggregation, LDL oxidation, oxidative damage to DNA, coronary diseases, atherosclerosis and brain oxidative damage caused by a convulsing drug [pentylenetetrazole (PTZ)] and carbon tetrachloride (CCl_4) (Day et al. 1997; Frankel et al. 1998; Osman et al. 1998; Dani et al. 2008, 2009; Rodrigues et al. 2012). Nowadays, in Brazil, it is possible to find two kinds of grapes juices, the organic (free of pesticides and genetic engineering) and the conventional (traditional cultivation with pesticide use and/or genetic engineering) (Dani et al. 2007; Rodrigues et al. 2012).

Considering that the consumption of foods and beverages containing phenolic compounds have been reported due to the benefits they produce on human health (Balu et al. 2005; Fernández-Fernández et al. 2012) the objective of this study was to investigate the antioxidant and neuroprotective effect of chronic consumption of purple grape juice (organic and conventional) from Bordo variety on oxidative stress in different brain regions of rats supplemented with a HFD.

Methods

Chemicals

All chemical reagents were purchased from Sigma (St. Louis, MO, USA), except for thiobarbituric acid which was purchased from Merck (Darmstadt, Germany).

Diets

The animals received a standard diet Nuvilab® (Colombo, Paraná, PR, Brazil) or a HFD containing 59 % of kcal in fat, basically formed by saturated fatty acids, purchased from Pragsoluções Biosciences (Jau, São Paulo, SP, Brazil). The compositions of both diets are demonstrated in Table 1.

Grape Juices

The purple grape juices samples used in this study were from *Vitis labrusca* grapes, Bordo variety. Organic grape juice (OGJ) was produced with grapes cultivated without pesticides, obtained from Cooperativa Aecia (Antonio Prado, Rio Grande do Sul, RS, Brazil) and was certified by Rede de Agroecologia ECOVIDA. Conventional grape juice (CGJ), produced with grapes cultivated using traditional methods, was obtained from Vinícola Perini (Farroupilha, Rio Grande do Sul, RS, Brazil). Validity periods were observed, and the same brands were used for the entire study. Grape juices were manufactured in 2010. The juices were manufactured by heat extraction (~50 °C), with a subsequent pressing in order to separate the pulp, and then submitted to pasteurization (at 85 °C). All juices were manufactured by heat extraction, immediately followed by bottling at 80 °C.

Grape Juice Chemical Analysis and Nutritional Evaluation

All analyses were performed in duplicate. Carbohydrates (%), and humidity levels [ashes (g/L) and moisture (g/L)], as well as ascorbic acid (mg%) were determined according to AOAC International official methodologies [Association Official Agriculture Chemistry (AOAC) 1998].

Quantification of the Phenolic Compounds in the Grape Juices

The total phenolic content of CGJ and OGJ were measured using the modification of the Folin–Ciocalteau colorimetric method, as described by Singleton et al. (1999). Two

Table 1 Diets composition

	Standard diet	High-fat diet
kcal/kg	3,440	5,123
Composition (w/w)	%kcal	%kcal
Proteins	25.5	21
Lipids	11.7	59
Carbohydrates	62.8	20
Fibers	0	0
Vitamins/minerals	0	0
Total	100	100

hundred microliters of grape juice was assayed with 1,000 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, USA) with a diode array detector system. The chromatographic analyses were a modification of the methods described by Lamuela-Raventos and Waterhouse (1994). A Zorbax SB C18 (250 × 4.6 mm^2), 5 m particle size, with a flow of 0.5 mL/min, was used for the stationary phase. After filtration on a 0.2-μm 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 230 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 and (−)-epicatechin). Quantification was performed using the standards by establishing calibration curves for each identified compound. Results are shown in mg/L. 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).

Animals

Forty male Wistar rats of 21-day-old were obtained from our own breeding colony. They were maintained at 22 ± 2 °C, on a 12 h light/12 h dark cycle, with free access to food and drink. The "Principles of laboratory animal care" (NIH publication no. 80-23, revised 1996) were followed in all our experiments and the research protocol was approved by the Ethical Committee for Animal Experimentation of the Centro Universitário Metodista do IPA. All efforts were made to minimize animal suffering and to use only the minimum of animals necessary to produce reliable scientific data.

Treatment

The animals were randomly divided into four groups. Group 1: standard diet + water; Group 2: HFD + water; Group 3: HFD + CGJ; Group 4: HFD + OGJ. The animals were subjected to 12 weeks of treatment.

Evaluation of Food and Drink Consumption

The diets, water, and juices were controlled daily. The consumption of chows and juices was measured by the difference between the initial and final weight in a period of 24 h, the results were expressed weekly in total calories (kcal).

Body Composition

Animal body weight was assessed weekly on electronic balance (Crystal 200, Gibertini, Italy).

Oxidative Stress Measurements

Tissue Preparation

After 12 weeks of treatment the animals were euthanized by decapitation and the brain was quickly excised on a Petri dish, placed on ice. The cerebral cortex, hippocampus, and cerebellum were dissected and kept chilled until homogenization which was performed using a ground glass type Potter–Elvejhem homogenizer. Fresh tissue was homogenized in 1.5 % KCl. The homogenates were centrifuged at 800×*g* for 10 min at 4 °C, the pellet was discarded and the supernatants were kept at −70 °C until the determinations.

Thiobarbituric Acid Reactive Substances (TBARS) Measurement

Thiobarbituric acid reactive substances was used to determine lipid peroxidation and was measured according to the method described by Ohkawa et al. (1979). Briefly, 50 μL of 8.1 % sodium dodecyl sulfate (SDS), 375 μL of 20 % acetic acid (pH 3.5), and 375 μL of 0.8 % thiobarbituric acid (TBA) were added to 200 μL of homogenates and then incubated in boiling water bath for 60 min. After cooling, the mixture was centrifuged (1,000×*g*/10 min).

The supernatant was removed and absorbance was read at 535 nm on a spectrophotometer (T80 UV/VIS Spectrometer, PG Instruments). Commercially available malondialdehyde was used as a standard. Results were expressed as nmol/mg protein.

Carbonyl Assay

Carbonyl assay was used to determine oxidative damage to proteins. Homogenates were incubated with 2,4 dinitrophenylhydrazine (DNPH 10 mmol/L) in 2.5 mol/L HCl solution for 1 h at room temperature, in the dark. Samples were vortexed every 15 min. Then 20 % TCA (w/v) solution was added in tube samples, left in ice for 10 min and centrifuged for 5 min at 1,000×*g*, to collect protein precipitates. Another wash was performed with 10 % TCA. The pellet was washed three times with ethanol:ethyl acetate (1:1) (v/v). The final precipitates were dissolved in 6 mol/L guanidine hydrochloride solution, left for 10 min at 37 °C, and read at 360 nm (T80 UV/VIS Spectrometer, PG Instruments) (Reznick and Packer 1994). The results were expressed as nmol/mg protein.

Sulfhydryl Assay

This assay is based on the reduction of 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) by thiols, generating a yellow derivative (TNB) whose absorption is measured spectrophotometrically at 412 nm (Aksenov and Markesberry 2001). Briefly, 0.1 mM DTNB was added to 120 µL of the samples. This was followed by a 30-min incubation at room temperature in a dark room. Absorption was measured at 412 nm (T80 UV/VIS Spectrometer, PG Instruments). The sulfhydryl content is inversely correlated to oxidative damage to proteins. Results were reported as nmol/mg protein.

Determination of Antioxidant Enzyme Activities

Superoxide dismutase (SOD) activity, expressed as USOD/mg protein was based on the inhibition of the ratio of autocatalytic adrenochrome formation at 480 nm (T80 UV/VIS Spectrometer, PG Instruments) (Bannister and Calabrese 1987). Catalase (CAT) activity was determined by following the decrease in 240 nm absorption of hydrogen peroxide (H_2O_2) (T80 UV/VIS Spectrometer, PG Instruments). It was expressed as UCAT/mg protein (Aebi 1984).

Nitric Oxide Production

Nitric oxide (NO) was determined by measuring the stable product nitrite through the colorimetric assay described by Hevel and Marletta (1994). In brief, the Griess reagent was prepared by mixing equal volumes of 1 % sulfanilamide in 0.5 N HCl and 0.1 % *N*-(1-naphthyl)ethylenediamine in deionized water. The reagent was added directly to the homogenates and incubated under reduced light at room temperature for 30 min. Samples were analyzed at 550 nm on a microplate spectrophotometer. Controls and blanks were run simultaneously. Nitrite concentrations were calculated using a standard curve prepared with sodium nitrite (0–80 mM). Results were expressed as nmol/mg protein.

Protein Determination

Protein concentrations were determined by the method of Lowry et al. (1951) using bovine serum albumin as standard.

Statistical Analysis

Grape juices composition was analyzed by student *t* test. Changes on body weight, and food and drink consumption were analyzed by one-way ANOVA for repeated measures and data from all other experiments were analyzed statistically by one-way ANOVA followed by the Tukey test. Values of $P < 0.05$ were considered to be significant. All analyses were carried out using the Statistical Package for Social Sciences (SPSS) software (version 17.0).

Results

Grape Juices Composition

Conventional grape juice presented a higher carbohydrates content and ashes compared to OGJ (Table 2). Whereas OGJ demonstrated higher ascorbic acid, total phenolic content, resveratrol, catechin, epicatechin, as compared to CGJ (Table 2).

Effect of HFD and Grape Juices Treatment on the Pattern of Food and Drink Consumption

We evaluated diets and grape juices consumption in kcal per week on a period of 12 weeks (Fig. 1). We observed that as regards to food consumption control and HFD groups showed a higher consumption of calories as compared to the groups treated with HFD and grape juices, specially between the second and sixth weeks ($P < 0.05$) (Fig. 1a). We also evaluated the calories of grape juices in kcal consumed during the treatment and verified that the rats had the same pattern of consumption of both conventional and OGJ (Fig. 1b). The animals maintained the same consumption on the first 3 weeks, but after the fourth week they started to drink more grape juice until the end of the

Table 2 Grape juices composition

Parameters	Conventional grape juice	Organic grape juice
Carbohydrates (%)	12.60 ± 0.141	11.67 ± 0.106*
Moisture (g/L)	153.37 ± 0.212	143.30 ± 0.141
Ashes (g/L)	3.30 ± 0.282	2.46 ± 0.615*
Ascorbic acid (mg%)	26.71 ± 1.17	45.34 ± 1.16*
Total phenolic compounds (mg catechin/mL)	72.30 ± 0.141	101.19 ± 0.021*
Resveratrol (mg/L)	0.210 ± 0.028	0.850 ± 0.014*
Catechin (mg/L)	8.09 ± 0.01	24.76 ± 0.01*
Epicatechin (mg/L)	5.87 ± 0.01	8.20 ± 0.001*

* $P < 0.05$, by student's t test

study (Fig. 1b). Regards to total calories consumption we observed that the animals treated with normal chow and HFD had increased kcal consumption as compared to the animals treated with HFD and CGJ or OGJ ($P < 0.05$) (Fig. 1c).

We also studied the animal's body weight during the 12 weeks of treatment (Fig. 2). We verified that all four groups showed significant changes during the 12 weeks treatment. The animals of all groups gained weight during all treatment period. After 30 days we observed that the HFD group gained more weight compared to the other groups ($P < 0.05$). Moreover, we also verified that the animals that consumed HDF + grape juice groups (CGJ and OGJ) showed less gain weight compared to HDF from 30 days until the end of the treatment ($P < 0.05$).

Effect of HFD and Grape Juices Treatment on Oxidative Stress Parameters

First, we demonstrated that the HFD treatment was able to induce oxidative damage in a different pattern according to the parameter analyzed and the brain area in rats (Figs. 3, 4, 5, 6). The HFD enhanced lipid peroxidation and protein oxidation (carbonyl) in cerebral cortex and hippocampus, while cerebellum was not affected by this treatment (Fig. 3). We also verified that only the OGJ was able to reduce the TBARS levels in cerebral cortex and cerebellum (Fig. 3a) and that both grape juices (conventional and organic) were able to decrease protein oxidation in these tissues (Fig. 3b).

Next, we observed the effect of the HFD on the non-enzymatic antioxidant defenses by measuring protein sulfhydryl groups. Figure 4 shows that sulfhydryl groups were reduced by this treatment in the cerebral cortex and cerebellum and that OGJ was able to prevent this inhibition in both tissues whereas in the cerebral cortex CGJ also could prevent this effect. The hippocampus was not affected by the treatment.

Moreover, we investigated the effect of HFD on the enzymatic antioxidant defenses by measuring CAT and SOD activities. Figure 5 shows that CAT and SOD activities were reduced by the HFD in cerebral cortex, hippocampus, and cerebellum. OGJ and CGJ were able to prevent CAT inhibition in all tissues studied (Fig. 5a). Both juices enhanced SOD activity in cerebral cortex and hippocampus while none of the grape juices were able to improve the activity of SOD in cerebellum (Fig. 5b).

Figure 6 demonstrates that HFD increased NO production in all cerebral tissues studied and that both grape juices were able to prevent this effect in the cerebellum whereas in cerebral cortex and hippocampus only OGJ was able to reduce NO levels.

Discussion

Grapes have been widely studied because of its antioxidant properties (Park et al. 2003; Zern and Fernandez 2005; Dani et al. 2007; Robb et al. 2008; Abeywardena and Leifert 2008). It is well described in the literature the neuroprotective activity of this fruit, especially in neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, as well as in metabolic diseases (e.g., diabetes and hypertension) (Sinha et al. 2002; Han et al. 2004; Raval et al. 2006; Vingtdeux et al. 2008; Xia et al. 2010; Venturini et al. 2011). In addition, the pathogenesis of these diseases are also linked by the raised of reactive species, failure of oxidative defenses, and elevated consumption of HFD (Moses et al. 2006; Puglielli 2007). Therefore in the present study we evaluated the antioxidant and neuroprotective effect of chronic use of organic and conventional purple grape juices on oxidative stress in different brain regions of rats supplemented with a HFD.

In the present study we observed that the HFD was able to induce oxidative stress in the brain of rats, and that the grape juices were able to ameliorate this effect. This result could be explained by the composition of the grape juices. Our results showed that both grape juices are rich in polyphenols, being the OGJ richer than the CGJ in all phenolic compounds. This different composition of the juices could be due to the fact that the organic products are cultivated without protection and for this reason the plant produced more phenolic compounds to protect itself against the aggressive agents, such as fungal, and weather (Carbonaro et al. 2002). This is line with other studies that also showed a raise in phenolic compounds in organic cultivation (Durak et al. 1999; Carbonaro et al. 2002; Fuleki and Ricardo-da-Silva 2003; Dani et al. 2007; Gollücke et al. 2008; Rodrigues et al. 2012). Moreover, it is important to consider the fact that the grape juice is a

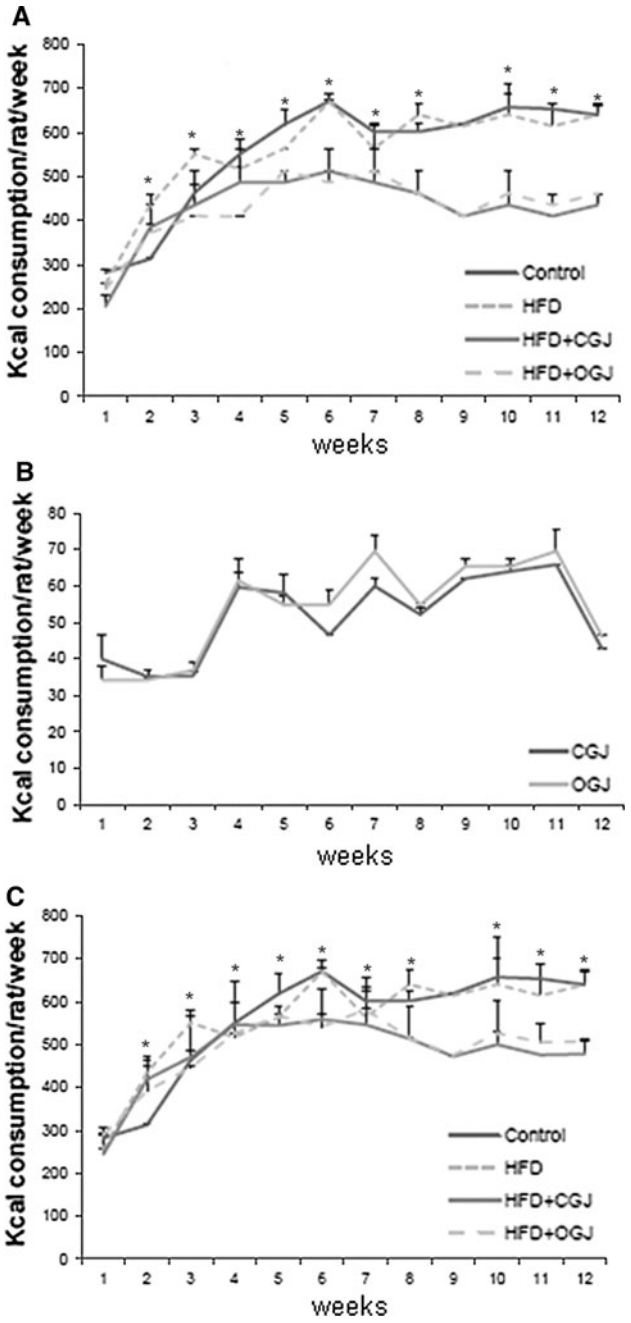

Fig. 1 Consumption of kcal of diets (**a**); purple grape juices (**b**); and diets + purple juices (**c**) during the 12 weeks of treatment. Data are reported as mean ± SD for ten animals per group. One-way ANOVA for repeated measures, followed by Tukey test: $^{*}P < 0.05$, from control, HFD + CGJ and HFD + OGJ; $^{\#}P < 0.05$, from HFD + CGJ and HFD + OGJ. *HFD* high-fat diet, *CGJ* conventional grape juice, *OGJ* organic grape juice

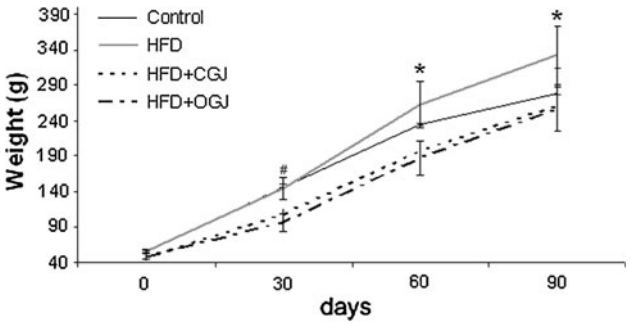

Fig. 2 Effect of chronic treatment with high-fat diet and purple grape juices on body weight of rats. Data are reported as weight variation (g) ± SD of ten animals per group. One-way ANOVA for repeated measures, followed by Tukey test: $^{*}P < 0.05$, from HFD + CGJ and HFD + OGJ. *HFD* high-fat diet, *CGJ* conventional grape juice, *OGJ* organic grape juice

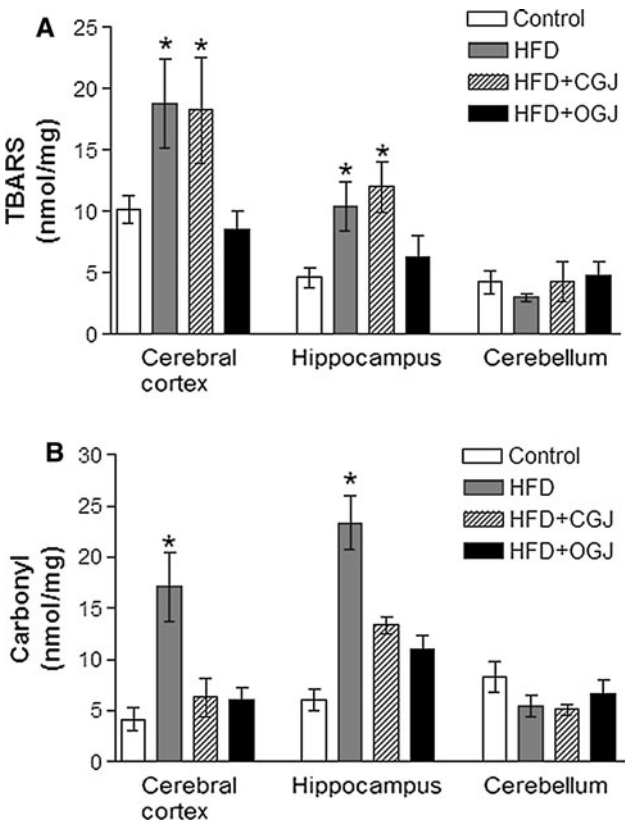

Fig. 3 Effect of chronic treatment with high-fat diet and purple grape juices on thiobarbituric acid reactive substances (TBARS) (**a**) and carbonyl formation (**b**) in the cerebral cortex, the hippocampus and the cerebellum of rats. Values are mean ± SD for 8–10 samples in each group expressed as nmol/mg. Statistically significant differences were determined by ANOVA followed by Tukey test: $^{*}P < 0.05$, from other groups. *HFD* high-fat diet, *CGJ* conventional grape juice, *OGJ* organic grape juice

complex mixture formed by other bioactive compounds such as vitamins and minerals (Dani et al. 2012), which could act in synergism to prevent the damage generated by reactive species and free radicals.

Regarding consumption of food per week the four groups had a different kcal pattern of intake. We observed that the control and HFD groups showed a higher consumption of calories at the begging of the treatment. Our data corroborate with some studies that evaluated the effect of HFD on the feeding behavior of rats, where throughout

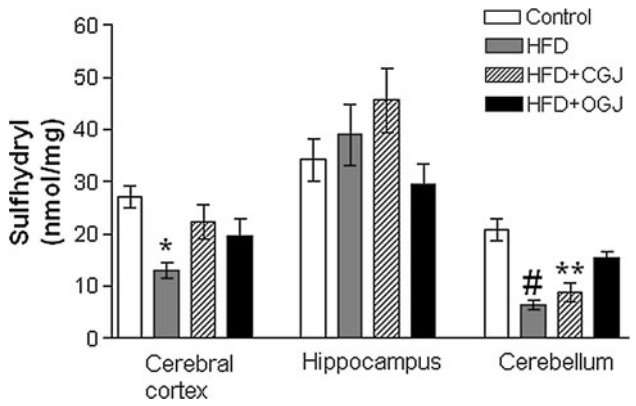

Fig. 4 Effect of chronic treatment with high-fat diet and purple grape juices on protein sulfhydryl groups in the cerebral cortex, the hippocampus, and the cerebellum of rats. Values are mean ± SD for 8–10 samples in each group expressed as nmol/mg. Statistically significant differences were determined by ANOVA followed by Tukey test: $^{*}P < 0.05$, from other groups; $^{**}P < 0.05$, from control; $^{\#}P < 0.05$, from control and HFD + OGJ. *HFD* high-fat diet, *CGJ* conventional grape juice, *OGJ* organic grape juice

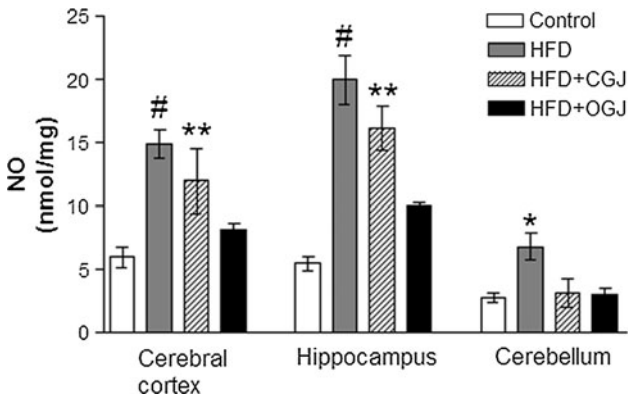

Fig. 6 Effect of chronic treatment with high-fat diet and purple grape juices on nitric oxide levels (NO) in the cerebral cortex, the hippocampus, and the cerebellum of rats. Values are mean ± SD for 8–10 samples in each group expressed as nmol/mg. Statistically significant differences were determined by ANOVA followed by Tukey test: $^{*}P < 0.05$, from other groups; $^{**}P < 0.05$, from control; $^{\#}P < 0.05$, from control and HFD + OGJ. *HFD* high-fat diet, *CGJ* conventional grape juice, *OGJ* organic grape juice

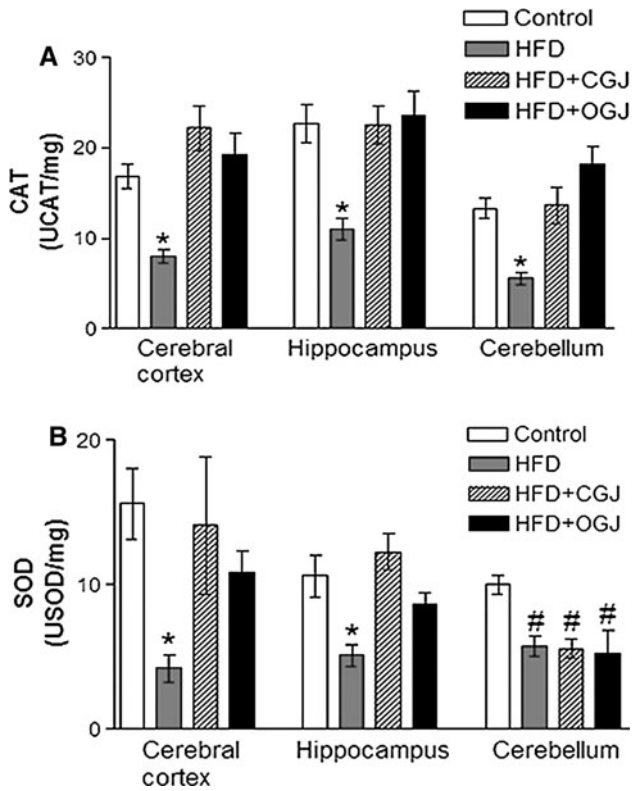

Fig. 5 Effect of chronic treatment with high-fat diet and purple grape juices on the activities of the antioxidant enzymes catalase (**a**) and superoxide dismutase (**b**) in the cerebral cortex, the hippocampus and the cerebellum of rats. Values are mean ± SD for 8–10 samples in each group. Statistically significant differences were determined by ANOVA followed by Tukey test: $^{*}P < 0.01$, from other groups; $^{\#}P < 0.05$, from control. *HFD* high-fat diet, *CGJ* conventional grape juice, *OGJ* organic grape juice

the study, animals fed with HFD had a peak of consumption of calories during the initial period and after had a slight decrease of kcal consumption maintaining an average daily food intake until the end of the study (Estadella et al. 2004). We also observed that concerning to juice consumption the animals of the four groups showed no significant difference in consumption and in the preference for the CGJ or the OGJ. An interesting finding observed in our study was that the animals supplemented with HFD and CGJ or OGJ had a lower kcal consumption compared to control and HFD groups. This is in agreement with Park et al. (2012) that observed that grape skin extract significantly lowered body weight, body fat mass, and adipocyte size in mice supplemented with HFD.

In this context, we also studied the weight of the animals during the treatment. We verified that all groups gained weight during the study. At the beginning, the animals of the control group had the most increase in body weight compared to the other groups. At the end of the experiment we observed a less intense weight gain in the animals. This relationship between first the animals gain more weight and grow and after they do not gain more weight is in line with previous studies from other researchers (Soulis et al. 2005; Banas et al. 2009; Ghalami et al. 2011). The animals treated with grape juices and HDF had a less intense gain compared to the HFD group. This fact may be explained by the composition of the grape juices that are rich in polyphenols. In this context, Emiliano et al. (2011) and Park et al. (2012) also reported that grape extracts protected against body weight gain and adiposity of adult animals fed with HFD. Moreover, our results are in line with Hollis et al. (2009), that showed that intake of concord grape juice

during 12 weeks in adults provoked a less gain weight compared to polyphenol-free grape-flavored drink. This effect could be attributed to the fact that flavonoids could increase thermogenesis and fat oxidation (Dulloo et al. 1999; Venables et al. 2008; Rumpler et al. 2001), and also might affect energy balance by reducing glucose and fat absorption via inhibition of gastrointestinal enzymes involved in nutrient digestion (Zhong et al. 2006; Hsu et al. 2006). These mechanisms may underlie the benefits on body weight and/or body composition found in some studies with polyphenols (Kataoka et al. 2004; Hollis et al. 2009; Boqué et al. 2012).

The oxidative effects of reactive species are controlled by non-enzymatic antioxidants, such as ascorbic acid and glutathione, and also by enzymatic antioxidants (SOD and CAT). Under some conditions, the increase in oxidants and the decrease in antioxidants cannot be prevented, and the oxidative/antioxidative balance shifts toward the oxidative status (Halliwell 2001, 2006). Consequently, oxidative stress is associated with the onset and pathogenesis of several prominent central nervous system disorders, such as Parkinson's disease and Alzheimer's disease, as well as in epileptic seizures and demyelination (Bogdanov et al. 2001; Behl and Moosmann 2002; Berg and Youdim 2006; Lehtinen and Bonni 2006; Kann and Kovacs 2007).

Here we observed that HFD increased TBARS and carbonyl levels, leading to lipid and protein oxidation in the hippocampus and cerebral cortex. In this context, some studies associated HFD and oxidative stress. Fachinetto et al. (2005) and Ribeiro et al. (2009) described that HFD ingestion was associated with an increase in TBARS levels in the brain of rats fed with HFD. Moreover, Du et al. (2012) reported that HFD significantly increased the generation of reactive oxygen species, the expressions of NADPH oxidase and the uncoupling proteins, and mitochondrial changes in rats suggesting that there was mitochondrial damage in response to the excessive fat intake.

Furthermore, our results showed that the OGJ was able to ameliorate lipid and protein damages, however, the CGJ reduced only carbonyl levels. These results are in line with previous studies from Rodrigues et al. (2012) that also showed that purple grape juices (organic and conventional) were able to prevent the enhance of TBARS and carbonyl provoked by PTZ in the brain of rats. Dani et al. (2008) also showed that purple grape juices prevent the damage caused by CCl_4 in *substantia nigra* of rats. These studies attributed this prevention to the rich polyphenol content of grapes.

In our study we showed that the HFD provoked a reduction in non-enzymatic defenses, represented by sulfhydryl content (cerebral cortex and cerebellum) and a reduction in SOD and CAT activities (all brain tissues). The reduced in the antioxidant defenses was also showed by others toxic agents in the brain of rats, such as PTZ and xenobiotics (Funchal et al. 2010; Medeiros et al. 2012; Rodrigues et al. 2012). Moreover, we also demonstrated that both grape juices were able to prevent the reduced of sulfhydryl content in cerebral cortex, whereas in cerebellum only the OGJ was able to ameliorate this reduction. As regards to the enzymatic antioxidant defenses both grape juices were able to prevent the inhibition of CAT activity in all brain areas. However, SOD activity was enhanced by the chronic use of OGJ and CGJ only in cerebral cortex and hippocampus. These results are in line previous studies in brain tissues, with green tea (Mandel et al. 2008), blueberry extract (Lau et al. 2005), and grape products (Scola et al. 2010; Rodrigues et al. 2012).

Moreover in this study, we observed that the HFD provoked an increase in NO production, this fact was also showed by the use of other toxic agents, e.g., PTZ and xenobiotics (Funchal et al. 2010; Rodrigues et al. 2012).This enhance was prevented by the OGJ in all tissues and the CGJ reduced the NO levels only in cerebellum. This is in agreement with Rodrigues et al. (2012) that observed the both grape juices were capable to prevent the NO enhanced production caused by PTZ in the brain of rats.

Considering that the HFD markedly increased NO levels and reduced SOD activity in our experimental model NO could react with $O_2^{-\cdot}$ to generate nitrogen reactive species, including peroxynitrite, which is a very reactive molecule that can modify biomolecules, including DNA, lipids, and proteins (Ischiropoulos et al. 1992; Yamakura et al. 2005). Furthermore, the decrease of sulfhydryl groups observed by HFD consumption is related to the decrease of the non-enzymatic antioxidant defenses in the brain of the rats, therefore we presumed that GSH levels could be reduced intracellularly because of the excess of reactive species formation, including NO or its derivative peroxynitrite forming nitrosoglutathione or by regenerating the nitrosyl groups and, thus, limiting NO deleterious effects (Stamler and Toone 2002; Rodriguez et al. 2012).

Taken together, the HFD induced lipid peroxidation, protein damage, significantly compromised the non-enzymatic and the enzymatic antioxidant defenses and increased the levels of reactive species in the brain of rats. As a result, there was an unbalance between prooxidants and antioxidants, a situation defined as oxidative stress (Sies 1991; Halliwell and Gutteridge 2007). Grape juices, which are rich in polyphenol content, were capable to ameliorate this condition. Moreover, considering that it is well described in the literature, the association between oxidative stress and neurodegenerative diseases, we could speculate that regular intake of grape products could be considered as an adjuvant in the therapy of patients with neurodegenerative diseases, such as Parkinson's and Alzheimer's.

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Conflict of interest The authors declare no conflicts of interest.

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