Antioxidant effect of organic purple grape juice on exhaustive exercise

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Abstract

This study aimed to assess the potential protective effect of organic purple grape juice (PGJ) upon oxidative stress produced by an exhaustive exercise bout in rats. To test this hypothesis, rats were acutely treated with organic PGJ (Vitis labrusca) and were subsequently submitted to an exhaustive exercise bout. Parameters of oxidative stress, such as thiobarbituric acid reactive species (TBARS) levels, 2′,7′-dichlorofluorescein diacetate (DCFH-DA) oxidation and nonprotein sulfhydryl levels (NP-SH) in brain, skeletal muscle, and blood were evaluated. Enzyme activity of Na\(^+\), K\(^+\)-ATPase, Ca\(^{2+}\)-ATPase, and δ-aminolevulinate dehydratase (δ-ALA-D) in brain, skeletal muscle, and blood were also assayed. Statistical analysis showed that the exhaustive exercise bout increased TBARS levels and DCFH-DA oxidation, and decreased NP-SH levels in rat tissues. Ca\(^{2+}\)-ATPase activity was increased in groups exposed to both exercise and PGJ treatment. The results herein presented indicate that organic PGJ intake was able to protect against the oxidative damage caused by an exhaustive exercise bout in different rat tissues.

Keywords: Exhaustive exercise; oxidative stress; purple grape juice; polyphenols; antioxidant capacity; neuroprotection.
1. Introduction

It is well known that acute exhaustive exercise leads to reactive oxygen species (ROS) production which in turn induces lipid and protein oxidative damage (Malaguti et al. 2009). Among target tissues, brain is especially susceptible to ROS production due to its high demand for molecular oxygen, the polyunsaturated fatty acids enrichment in membrane phospholipids, and the relatively low levels of antioxidant defenses (Sun et al. 2008). Exhaustive exercise increases oxidative stress in brain by several pathways, including dopamine synthesis which may form ROS (Sutoo and Akiyama 2003). Exhaustive exercise also leads to increased serum glucocorticoid levels, which increases the toxicity of oxygen radical generators (McIntosh and Sapolsky 1996), and alters the efficiency of antioxidant activities in the brain (McIntosh et al. 1998).

In line with this, both acute exhaustive exercise and exercise training increase the consumption of various antioxidant molecules. On the other hand, antioxidants deficiency severely hampers proper functioning of the corresponding antioxidant system, exacerbating exercise-induced oxidative stress and tissue damage (Ji 1995). In fact, endogenous antioxidants can be depleted after exhaustive exercise causing an oxidative stress (Margaritis and Rousseau 2008). As a result, it has been suggested that dietary supplementation with specific antioxidants may be beneficial against oxidative stress brain damage (Ji 1995). Moreover, preventing oxidative stress by antioxidant supplement may enhance skeletal muscle function recovery which also relays on ROS levels (Margaritis and Rousseau 2008).

Epidemiological studies have indicated that a high vegetable and fruit consumption is consistently associated with low risk to oxidative stress-associated diseases, such as cancer, neurodegenerative disorders, and aging (Ames et al. 1993; Lau
et al. 2005; Prior et al. 2007). These effects are mainly attributed to the antioxidant properties of the phenolic compounds found in fruits and vegetables (Lau et al. 2005). In this line, grape juice is a rich source of polyphenols, such as flavonoids and anthocyanides, and nonflavonoids, including resveratrol (Frankel et al. 1998; Fuleki and Ricardo-Da-Silva 2003). Indeed, previous studies observed that the chronic grape juice intake, both organic and conventional, was able to reduce oxidative stress damage induced by carbon tetrachloride (CCl$_4$) in brain, liver, and plasma of rats (Dani et al. 2008a, 2008b). Nowadays there is much interest in a healthy, environmentally friendly method for fruits production (Dani et al. 2010). Organic products have shown differences in the phenolic content when compared to conventional procedures. In fact, organic leaf extract showed a resveratrol concentration 10-fold higher than the conventional extract (phenolic content of grapevine leaves) (Dani et al. 2010).

To date exercise-induced oxidative stress brings out the question of optimal conditions for antioxidant adaptations to stress stimuli, and antioxidant nutrient requirements have been arousing growing interest during the last years (Margaritis and Rousseau 2008). The understanding of mechanisms that underlie dietary supplementation, oxidative stress protection, and exercise performance is of key importance to sports outcomes. Therefore, the present study was designed to analyze the possible protective effect of organic PGJ (Vitis labrusca) intake on the oxidative stress induced by an exhaustive exercise bout in rats.

2. Materials and Methods

2.1. Chemicals

Thiobarbituric acid, aminolevulinic acid, and DL-dithiothreitol (DTT) were obtained from Sigma Aldrich (St. Louis, MO, USA). 2',7'-Dichlorofluorescin diacetate
(DCFH-DA) was purchased from Molecular Probes (Eugene, OR, USA). HgCl$_2$, NaCl, K$_2$HPO$_4$, KH$_2$PO$_4$, trichloroacetic acid (TCA), para-dimethylaminobenzaldehyde, and glacial acetic acid were purchased from Reagen (Rio de Janeiro, RJ, Brazil). All other chemicals were purchased from Merck (Darmstadt, Germany).

Ecologically produced (organic) PGJ was obtained from grapes of the bordo variety (*Vitis labrusca*) that were cultivated in 2007, and the juice was prepared in the same year. The organic PGJ was donated by Econatura Produtos Ecológicos e Naturais LTDA (Garibaldi, RS, Brazil), and the concentration (mg/L) of the main phenolic compounds in the PGJ was earlier determined to be the following: resveratrol 3.95 ± 0.01, quercetin 8.95 ± 0.09, rutin 3.75 ± 0.03, gallic acid 81.07 ± 2.03 and caffeic acid 30.28 ± 2.00 as previously described (Machado et al. 2011).

2.2. Animals

Male Wistar rats weighing 270–320 g and with aging 3–3.5 months from our own breeding colony were kept in cages with four animals each. Animals were kept at room temperature (22 ± 3 °C) with a 12 h light/dark cycle with lights on at 7:00 a.m. with food and water *ad libitum*. All experiments were conducted in accordance with national and international legislation (Brazilian College of Animal Experimentation [COBEA], and the Canadian Council of Animal Care [CCAC]) and with previous approval of the Ethics Committee for Animal Research of the Universidade Federal de Santa Maria (UFSM) under protocol number of 35/2008.

2.3. Treatment and exhaustive exercise bout

Animals were firstly divided into two groups (n=16 each): non-exercised (NE) and exercised (EX). Further NE and EX were divided into two subgroups (n=8 each): control (C), and grape juice (GJ). The GJ rats received organic PGJ treatment in the drinking water (diluted 1:1 in water) *ad libitum* 24 h before the exhaustive exercise.
bout, whereas C rats had only water access. The amount of the PGJ drunk by each rat was estimated to be around 25 mL. Swimming was performed in a cylindrical tank that is 80 cm in length, 50 cm in width, and 90 cm in depth, containing water at 32 °C. Exhaustive exercise consisted on 15 sets of 20 s swimming bouts with an overload of 15 % of body weight. A 10 s recovery period between bouts was allowed (Terada et al. 2001). Rats were sacrificed by decapitation immediately after the exhaustive exercise bout. Swimming bouts were monitored by the same person, and were always performed between 9:00 and 11:00 am.

2.4. Tissue preparation

The brain was immediately removed after decapitation, placed on ice, and dissected into four specific regions: cerebellum, cortex, hippocampus, and striatum. Each section was weighted and homogenized in 10 volumes of 10 mM Tris-HCl. The gastrocnemius muscle was also dissected and homogenized at 5 volumes of 10 mM Tris-HCl. Both the homogenates were centrifuged at 4000 x g for 10 min to yield a low-speed supernatant fraction (S1) which was used for the biochemical and enzymatic assays. Blood samples were collected in heparinized tubes and fractioned to biochemical analyses further described.

2.5. Lipid peroxidation assay

Thiobarbituric acid reactive species (TBARS) were determined as described by Ohkawa et al. (1979). Briefly, samples were incubated at 100 °C for 1 h in a medium containing 8.1 % sodium dodecyl sulfate, 1.4 M acetic acid, pH 3.4, and 0.6 % thiobarbituric acid. The pink chromogen produced in the reaction was measured spectrophotometrically at 532 nm. Results are expressed as percent of controls.

2.6. Estimation of ROS production
The ROS production was estimated as previously described (Ali et al. 1992). An aliquot of 50 µL of brain regions and skeletal muscle S1 or 20 µL of total blood was added to 2.45 mL of Tris-HCl 10 mM and incubated in the presence of 5 µM DCFH-DA for 1 h at room temperature. Fluorescent signals were recorded at the end of the incubation at 488 nm excitation and 525 nm emission wavelengths. Results are expressed as percent of controls.

2.7. Nonprotein sulfhydryl (NP-SH) levels

To determine NP-SH levels, 500 µl of 10% TCA were added to 500 µl of the samples and further centrifuged at 4000 x g at 4°C for 10 min. The protein pellet was discarded and free -SH groups were determined in the clear supernatant, which was neutralized with 0.1 M NaOH using the method of Ellman (1959). Results are expressed as percent of controls.

2.8. Enzyme activity assays

2.8.1. Na\(^+\), K\(^+\)-ATPase

The measurement of Na\(^+\), K\(^+\)-ATPase activity was performed according to Wyse et al. (2000). The assay medium consisted on 30 mM Tris-HCl buffer (pH 7.4), 0.1 mM EDTA, 50 mM NaCl, 5 mM KCl, 6 mM MgCl\(_2\) and 50 µg of protein in the presence or absence of 1 mM ouabain in a final volume of 350 µL. The reaction was started by the addition of adenosine triphosphate (ATP) to a final concentration of 5 mM. After 30 min at 37 °C, the reaction was stopped by the addition of 70 µL of 50% (w/v) TCA. Saturating substrate concentrations were used, and the reaction was linear with protein and time. Appropriate controls were included in the assays for non-enzymatic ATP hydrolysis. The amount of inorganic phosphate (Pi) released was quantified using KH\(_2\)PO\(_4\) as reference standard. Specific Na\(^+\), K\(^+\)-ATPase activity was calculated by
subtracting the ouabain-insensitive activity from the overall activity in the absence of ouabain and expressed in nmol Pi/mg protein/min.

2.8.2. Ca\(^{2+}\)-ATPase

The Ca\(^{2+}\)-ATPase enzyme activity was determined in skeletal muscle S1 samples according to Zaidi and Michaelis (1999) with few modifications. The S1 skeletal muscle aliquots (20 µL) were added to a reaction medium containing 1 mM MgCl\(_2\), 50 mM KCl, 0.2 mM EGTA, and 25 mM Tris-HCl buffer (pH 7.4), with or without 150 µM CaCl\(_2\) to ensure a final concentration of 1 µM of Ca\(^{2+}\) ions in the medium. The experimental procedures were similar to those used for the Na\(^{+}\), K\(^{+}\)-ATPase activity determination previously described.

2.8.3. δ-aminolevulinate dehydratase (δ-ALA-D)

The δ-ALA-D activity was assayed according to the method of Sassa (1982) by measuring the rate of product formation (porphobilinogen/PBG). The reaction product was determined using a modified Ehrlich’s reagent at 555 nm with a 6.1 x 10\(^4\) M\(^{-1}\) molar absorption coefficient for the Ehrlich-PBG salt. The incubation medium contained an aliquot of the total blood and 0.084 M potassium phosphate buffer (pH 6.8). The reaction was initiated by the addition of 2.4 mM δ-ALA, and the incubations were carried out during 90 min at 39 °C. Next, the reaction was stopped by the addition of 10% TCA containing 0.01 M HgCl\(_2\). The δ-ALA-D activity was expressed as nmol of PBG/mg of protein/h. A set of tubes was assayed in parallel using the same protocol, except that 2 mM DTT was added to obtain the reactivation index. DTT is a –SH reducing agent that has been used \textit{in vitro} to prevent and/or revert δ-ALA-D inhibition by oxidizing agents. This index indicates the extent of the δ-ALA-D activity reactivation, and was calculated as follows:
(δ-ALA-D activity with DTT - δ-ALA-D activity without DTT) x 100%

δ-ALA-D activity with DTT

2.9. Protein measurement

Protein was assayed by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

2.10. Statistical analysis

Data were analyzed statistically by one-way ANOVA, followed by Duncan’s post-hoc tests. The results were considered statistically significant when p<0.05. Normality assumption was tested with Kolmogorov–Smirnov test.

3. Results

3.1. Lipid peroxidation levels

Lipid peroxidation was evaluated in the brain (cortex, hippocampus, striatum, and cerebellum), skeletal muscle, and blood across TBARS production. Exhaustive exercise caused an increase in TBARS levels in the cortex and cerebellum of exercised rats (Figures 1A, and 1D, respectively; p<0.05). PGJ intake protected against the exercise-induced TBARS production in these brain regions (p<0.05). Specifically, PGJ intake decreased lipid peroxidation in hippocampus, striatum, and skeletal muscle of rats submitted to the exhaustive exercise bout (Figures 1B, 1C, and 1E, respectively; p<0.05). TBARS levels in blood were not affected by exercise or either PGJ intake as seen in Figure 1F.

3.2. Estimation of ROS production

ROS production was estimated by assessing DCFH oxidation. PGJ intake protected against the exercise increased ROS production in different brain regions.
(Figures 2A, 2B, and 2D; p<0.05), excepting the striatum (Figure 2C). Similarly, the exhaustive exercise bout increased the DCFH oxidation in skeletal muscles of exercised rats (Figure 2E; p<0.05). PGJ intake was able to decrease the ROS production both in skeletal muscle and blood (Figures 2E, and 2F, respectively; p<0.05).

3.3. NP-SH levels

The exhaustive exercise decreased NP-SH levels in the striatum (Figure 3C) and cerebellum, skeletal muscle, and blood when compared to the other groups (Figures 3D, 3E, and 3F, respectively; p<0.05). Furthermore, EX-GJ group showed higher NP-SH levels when compared to EX-C for all tissues (Figure 3; p<0.05), indicating a clear PGJ protection for this parameter.

3.4. Enzyme activity assays

3.4.1. Na\(^+\), K\(^+\)-ATPase

The exhaustive exercise did not affect the Na\(^+\), K\(^+\)-ATPase activity in the brain regions analyzed as seen on Figure 4. Nevertheless, NE-GJ group presented higher activity when compared to EX-C (Figure 4D; p<0.05).

3.4.2. Ca\(^{2+}\)-ATPase

Ca\(^{2+}\)-ATPase activity in skeletal muscle increased 125% in EX-C compared to the NE-C group (Figure 5; p<0.05). The NE-GJ group increased approximately 50% the Ca\(^{2+}\)-ATPase activity when compared to the NE-C (Figure 5; p<0.05). For EX-GJ group Ca\(^{2+}\)-ATPase activity remained at the same level as the NE-GJ, and significantly different from the EX-C group (Figure 5; p<0.05).

3.4.3. δ-ALA-D activity

Blood δ-ALA-D activity was augmented in the EX-GJ group when compared to the others (Figure 6A, p<0.05). DTT was added and the enzyme activity increased in all
groups (Figure 6B; p<0.05). On the other hand, no differences were observed in the reactivation index of the enzyme among the groups (Figure 6C).

4. Discussion

In the present study organic PGJ intake proved to be effective in protecting brain, skeletal muscle and, to a minor extent, blood from oxidative stress induced by an exhaustive exercise bout. Results herein found pointed out to decreased TBARS level, DCFH-DA oxidation, and higher NP-SH levels and enzyme activities after an exhaustive exercise bout with previous organic PGJ intake. These data point out to an acute antioxidant effect of organic PGJ in a well-recognized ROS inducing model. Furthermore, PGJ-protected NP-SH groups from the oxidation induced by exhaustive exercise can be associated to an increase in the antioxidant status by the polyphenols present in the juice. These results are corroborated by other studies that show the recovering of GSH levels (Andrade et al. 2011) and the increase in serum antioxidant status by purple grape juice (Rowe et al. 2011).

It is well known that unaccustomed or exhaustive exercise induces pro-oxidant unbalance and subsequent oxidative stress and tissue damage (Ji 1995; Liu et al. 2000). There have been several reports which detected ROS production in rat muscle and liver after exercise (Davies et al. 1982; Somani and Arroyo 1995). Results from such studies include increased oxidative damage biomarkers, such as TBARS (Sen et al. 1997); effect on mitochondrial function (Ravalec et al. 1996; Willis and Jackman 1994); and decreased antioxidants levels and enzymatic activities in several tissues (Liu et al. 2000).

Brain is a target to oxidative damage induced by exhaustive exercise due to its high metabolic rate and elevated polyunsaturated fatty acids content (Ji 1995). All the
brain regions in this study were susceptible to the oxidative stress induced by the exhaustive exercise bout. Concurrent with the ROS generation, exercise was found to increase lipid peroxidation and decrease NP-SH levels in the brain. In line with this, it is known that thiols levels are involved in the antioxidant system and therefore are important to brain protection from oxidative stress (Cechetti et al. 2012).

The brain regions showed differential responses to the treatments, where cerebellum was the most susceptible to oxidative stress among the brain regions, followed by cortex, hippocampus, and striatum. However, organic PGJ was able to counteract oxidative stress in all these brain regions. Dani et al. (2008b) have described the antioxidant role of PGJ in substantia nigra and striatum of rats exposed to carbon tetrachloride-induced oxidative stress (Dani et al. 2008b). The same report also found that PGJ intake increases catalase and superoxide dismutase activities in rat brain (Dani et al. 2008b). Presumably, PGJ antioxidant properties played a major role on oxidative stress refrain, although other redox status related mechanisms may not be ruled out. In this sense, the effect of an exhaustive exercise bout on Na\(^+\), K\(^+\)-ATPase activity was also evaluated, considering that this enzyme is a known ROS target and may be involved in glutamate uptake inhibition (Hexum and Fried 1979). However, Na\(^+\), K\(^+\)-ATPase activity was not affected by exercise and/or PGJ in brain regions in this study.

The exhaustive exercise bout provoked an increase in lipid peroxidation, ROS production, and decreased NP-SH levels in skeletal muscle. Nevertheless, PGJ intake was able to prevent these alterations, confirming the antioxidant effect of the organic PGJ to a ROS inducing model. Previous studies with antioxidant supplementation have also pointed out to muscle tissue protection from ROS damage after an exhaustive exercise bout (Malaguti et al. 2009). Therefore, the effect of an exhaustive exercise bout on Ca\(^{2+}\)-ATPase activity of skeletal muscle was also assayed, considering that this
enzyme plays a prominent role in muscle and calcium homeostasis during the excitation/contraction coupling (Inesi et al. 2008; Traaseth et al. 2008). Exhaustive exercise caused approximately a two-fold increase in Ca\(^{2+}\)-ATPase activity in skeletal muscle of rats submitted to the exhaustive exercise bout. In this case, PGJ alone or combined with exercise also caused an increase in skeletal muscle Ca\(^{2+}\)-ATPase activity, but to a minor extent than exercise alone. The results of Bonner et al. (1976) showed that endurance-trained rats presented a higher sarcoplasmic reticulum Ca\(^{2+}\)-ATPase activity at exhaustion than untrained rats, which substantiates our results. However, to the best of our knowledge this is the first evidence of Ca\(^{2+}\)-ATPase activity increase in exercised rats supplement with organic PGJ.

Organic PGJ intake also protected blood from exercise-induced oxidative stress through decreased DCFH oxidation and increased NP-SH levels. Here, lipid peroxidation (TBARS) and ROS production (DCFH oxidation) were not significantly affected by exercise in blood. These results are in agreement with a previous study in humans which has shown that the oxidative stress and exercise-induced pathogenesis might be prevented via antiinflammatory and antioxidant defense mechanisms induced during exercise in the circulation (Suzuki et al. 2003). Of note, organic PGJ intake enhanced δ-ALA-D activity in exercised rats in the experiments performed in DTT presence or absence, whereas exercise and PGJ intake alone did not alter δ-ALA-D activity. The reactivation index for δ-ALA-D was not modified by exercise or PGJ intake, indicating that treatments did not affect -SH groups of the enzyme. The δ-ALA-D is a SH-containing enzyme highly susceptible to oxidizing agents and is inhibited in different pro-oxidant situations (Folmer et al. 2003). However, in this study, the oxidative stress induced by a bout of exhaustive exercise did not affect δ-ALA-D activity.
We would like to call attention to the fact that the techniques used in this study do not reveal the exact mechanism of action of the organic PGJ. In this way, the use of more precise methods such as PCR, Western blot or immunohistochemical analysis would be important in order to confirm the findings of this study and clarify the mechanisms underlying the organic PGJ effects. Also, the TBARS assay used in this study has some limitations since thiobarbituric acid (TBA) is nonspecific for malondialdehyde (MDA) and can react with nonlipid-related materials and fatty peroxide-derived decomposition products other than MDA (Janero 1990). In this manner, other indices of fatty peroxide formation and decomposition should be considered in future studies in order to provide more accurate data.

Various dietary antioxidant supplements have been commercialized and used by athletes as a means of counteracting oxidative stress related to exercise outcomes (Urso and Clarkson 2003). In this study, we demonstrated that an exhaustive exercise bout increases the production of ROS in the brain, skeletal muscle, and blood of rats, but pre-treatment with organic PGJ intake was able to counteract the oxidative damage in these tissues. This protection is possibly linked to the polyphenols antioxidant activity present in the PGJ. These results are important in the way that organic PGJ could be a potential healthy environmental friendly supplement to high-performance athletes, since its ingestion before exercise could have several health benefits, including preventing and counteracting ROS generation thus preventing disorders associated with oxidative stress. Nevertheless, further studies are of need to clarify the precise mechanisms underlying the organic PGJ protection from oxidative stress produced during exhaustive exercise in different rat tissues and confirm its benefits to sports outcomes.

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**Conflict of interest statement**

The authors declare that there are no conflicts of interest.
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Legends for Figures

Figure 1. Effect of PGJ and acute, exhaustive exercise on lipid peroxidation levels in cortex (A), hippocampus (B), striatum (C), cerebellum (D), skeletal muscle (E), and blood (F) of rats. Data are expressed as the mean ± S.E.M., n=8. Experiments were performed in duplicate. Letters denote significant (p<0.05) differences among the treatments by one-way ANOVA followed by Duncan’s multiple range test. The TBARS levels in the control groups were 0.12 ± 0.06, 0.12 ± 0.04, 0.11 ± 0.03, 0.11 ± 0.03, 0.18 ± 0.06, and 0.55 ± 0.12 nmol TBARS/mg protein for cortex, hippocampus, striatum, cerebellum, skeletal muscle and blood, respectively. NE: non-exercised; EX: exercised; C: control; GJ: grape juice.

Figure 2. Effect of PGJ and acute, exhaustive exercise on ROS production in the cortex (A), hippocampus (B), striatum (C), cerebellum (D), skeletal muscle (E), and blood (F) of rats. Data are expressed as the mean ± S.E.M., n=8. Experiments were performed in duplicate. Letters denote significant (p<0.05) differences among the treatments by one-way ANOVA followed by Duncan’s multiple range test. NE: non-exercised; EX: exercised; C: control; GJ: grape juice.

Figure 3. Effect of PGJ and acute, exhaustive exercise on NP-SH levels in the cortex (A), hippocampus (B), striatum (C), cerebellum (D), skeletal muscle (E), and blood (F) of rats. Data are expressed as the mean ± S.E.M., n=8. Experiments were performed in duplicate. Letters denote significant (p<0.05) differences among the treatments by one-way ANOVA followed by Duncan’s multiple range test. The NP-SH levels in control groups were 5.6 ± 0.8, 3.9 ± 0.7, 6.1 ± 0.5, 6.0 ± 0.5, 3.6 ± 0.3, 41.6 ± 6.5 nmol SH/mg protein for cortex, hippocampus, striatum, cerebellum, skeletal muscle and blood, respectively. NE: non-exercised; EX: exercised; C: control; GJ: grape juice.
Figure 4. Effect of PGJ and acute, exhaustive exercise on Na⁺, K⁺-ATPase activity in the cortex (A), hippocampus (B), striatum (C), and cerebellum (D) of rats. Data are expressed as the mean ± S.E.M., n=8. Experiments were performed in duplicate. Letters denote significant (p<0.05) differences among the treatments by one-way ANOVA followed by Duncan’s multiple range test. NE: non-exercised; EX: exercised; C: control; GJ: grape juice.

Figure 5. Effect of PGJ and acute, exhaustive exercise on Ca²⁺-ATPase activity in skeletal muscle of rats. Data are expressed as the mean ± S.E.M., n=8. Experiments were performed in duplicate. Letters denote significant (p<0.05) differences among the treatments by one-way ANOVA followed by Duncan’s multiple range test. NE: non-exercised; EX: exercised; C: control; GJ: grape juice.

Figure 6. The effect of PGJ and acute exhaustive exercise on the δ-ALA-D activity in blood of rats. Enzyme activity determined without DTT (A), in the presence of 2 mM DTT (B), and the enzyme reactivation index (C). Data are expressed as the mean ± S.E.M., n=8. Experiments were performed in duplicate. Letters denote significant (p<0.05) differences among the treatments by one-way ANOVA followed by Duncan’s multiple range test. NE: non-exercised; EX: exercised; C: control; GJ: grape juice.
Figure 1. Effect of PGJ and acute, exhaustive exercise on lipid peroxidation levels in cortex (A), hippocampus (B), striatum (C), cerebellum (D), skeletal muscle (E), and blood (F) of rats. Data are expressed as the mean ± S.E.M., n=8. Experiments were performed in duplicate. Letters denote significant (p<0.05) differences among the treatments by one-way ANOVA followed by Duncan’s multiple range test. The TBARS levels in the control groups were 0.12 ± 0.06, 0.12 ± 0.04, 0.11 ± 0.03, 0.11 ± 0.03, 0.18 ± 0.06, and 0.55 ± 0.12 nmol TBARS/mg protein for cortex, hippocampus, striatum, cerebellum, skeletal muscle and blood, respectively. NE: non-exercised; EX: exercised; C: control; GJ: grape juice.
Figure 2. Effect of PGJ and acute, exhaustive exercise on ROS production in the cortex (A), hippocampus (B), striatum (C), cerebellum (D), skeletal muscle (E), and blood (F) of rats. Data are expressed as the mean ± S.E.M., n=8. Experiments were performed in duplicate. Letters denote significant (p<0.05) differences among the treatments by one-way ANOVA followed by Duncan’s multiple range test. NE: non-exercised; EX: exercised; C: control; GJ: grape juice.
Figure 3. Effect of PGJ and acute, exhaustive exercise on NP-SH levels in the cortex (A), hippocampus (B), striatum (C), cerebellum (D), skeletal muscle (E), and blood (F) of rats. Data are expressed as the mean ± S.E.M., n=8. Experiments were performed in duplicate. Letters denote significant (p<0.05) differences among the treatments by one-way ANOVA followed by Duncan’s multiple range test. The NP-SH levels in control groups were 5.6 ± 0.8, 3.9 ± 0.7, 6.1 ± 0.5, 6.0 ± 0.5, 3.6 ± 0.3, 41.6 ± 6.5 nmol GSH/mg protein for cortex, hippocampus, striatum, cerebellum, skeletal muscle and blood, respectively. NE: non-exercised; EX: exercised; C: control; GJ: grape juice.
Figure 4. Effect of PGJ and acute, exhaustive exercise on Na+, K+-ATPase activity in the cortex (A), hippocampus (B), striatum (C), and cerebellum (D) of rats. Data are expressed as the mean ± S.E.M., n=8. Experiments were performed in duplicate. Letters denote significant (p<0.05) differences among the treatments by one-way ANOVA followed by Duncan’s multiple range test. NE: non-exercised; EX: exercised; C: control; GJ: grape juice.
Figure 5. Effect of PGJ and acute, exhaustive exercise on Ca2+-ATPase activity in skeletal muscle of rats. Data are expressed as the mean ± S.E.M., n=8. Experiments were performed in duplicate. Letters denote significant (p<0.05) differences among the treatments by one-way ANOVA followed by Duncan’s multiple range test. NE: non-exercised; EX: exercised; C: control; GJ: grape juice.
Figure 6. The effect of PGJ and acute exhaustive exercise on the δ-ALA-D activity in blood of rats. Enzyme activity determined without DTT (A), in the presence of 2 mM DTT (B), and the enzyme reactivation index (C). Data are expressed as the mean ± S.E.M., n=8. Experiments were performed in duplicate. Letters denote significant (p<0.05) differences among the treatments by one-way ANOVA followed by Duncan’s multiple range test. NE: non-exercised; EX: exercised; C: control; GJ: grape juice.