Green juice as a protector against reactive species in rats

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Abstract

Introduction: Green juice is popularly known for introducing antioxidants, improving intestinal function and reducing weight gain.

Objectives: In the present study we determine the antioxidant effect of green juice comparing it with orange juice.

Methods: Rats were divided into three experimental groups and submitted to supplementation for 15 days: the (GJ) group received green juice, the (OJ) group received orange juice and the control group received water. We evaluated the antioxidant activity and total phenolic content of green and orange juices, as well as rat weight gain. We also investigated some oxidative stress parameters, namely thiobarbituric acid-reactive substances (TBARS), superoxide dismutase and catalase in rat cerebral cortex.

Results and discussion: Results showed that GJ had significantly less weight gain than the control group. With respect to antioxidant activity screening, the remaining percentage of DPPH at dilutions 1:10, 1:100 and 1:1000 of green juice was 22.8%, 58% and 78%, and orange juice, at the same dilutions, was 5.6%, 5.6% and 77.2%, respectively. The ability of juices to reduce the ABTS radical was 3.5 mmol trolox/L for green juice and 5.2 mmol trolox/L for orange juice. Additionally, the green juice did not present any difference in total phenolic acid content when compared to orange juice. TBARS were decreased in GJ and OJ. Besides, GJ supplementation decreased catalase activity. In conclusion, our data showed that green juice reduced weight gain, lipoperoxidation and catalase activity, suggesting that this supplementation may have a protective effect against reactive species.

DOI:10.3305/nh.2013.28.5.6505

Key words: Green juice. Oxidative stress. Antioxidant. Weight gain. Rats.

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Abbreviations

- ABTS: 2,2'-azino-bis-3-ethyl benzothiazoline-6-sulfonic acid.
- CAT: Catalase.
- DPPH: 2,2-diphenyl-1-pycrylhydrazyl.
- GJ: Green juice.
- GPx: Glutathione peroxidase.
- GSH: Glutathione.
- OJ: Orange juice.
- RS: Reactive species.
- SOD: Superoxide dismutase.
- TBARS: Thiobarbituric acid-reactive substances.
- TEAC: Trolox equivalent antioxidant capacity.

Introduction

Reactive species (RS) such as hydrogen peroxide, superoxide, hydroxyl radical can induce damage to cellular macromolecules, including lipids, proteins and DNA. It is known that cellular redox state is a consequence of the balance between the levels of oxidizing and reducing agents and endogenous antioxidants. However, during some pathological process may occur an imbalance between oxidants and antioxidants, namely as oxidative stress. RS are kept at physiological levels by antioxidant defense systems, including non-enzymatic antioxidants such as glutathione (GSH), bilirubin, uric acid, vitamins C and E, and endogenous compounds with enzymatic properties such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT).

Epidemiologic studies have demonstrated that the consumption of fruits and vegetables is inversely associated with morbidity and mortality from degenerative diseases. Thus, some research studies reported that antioxidants present in foods, such as vitamins C and E, selenium, polyphenols and β-carotene, may prevent some of the processes involved in the progression of cancer and cardiovascular disease. However, the precise contribution of these dietary components to maintain health and delay disease onset is uncertain.

Apples contain various types of polyphenols, such as phenolic acid derivatives and flavonoids, which present many physiological functions. A relationship has been reported between the consumption of apples or related products and chronic human diseases such as cancer and cardiovascular disease. Furthermore, fruit juice, particularly orange juice, is also an excellent source of antioxidant in diet. It is rich in bioactive compounds such as ascorbic acid, vitamin B, fiber, iron, β-carotene, flavonoids and others. There are many reports showing the major of orange juice in the protection of DNA against oxidative damage. Besides, vitamin C seems to be essential for antioxidant homeostasis and as a cofactor in a series of post-translational events.

Green juice contains fruits and vegetables such as apple, orange, dark green vegetables, and it is considered a great source of fibers, vitamins and minerals. This juice is popularly known for introducing antioxidants, improving intestinal function and reducing weight gain. Although there are no studies showing the effect of this juice on biological systems, it has aroused great interest among researchers because its components have different functional properties such as improving the immune system and reducing the action of RS that contribute to the progression of many diseases.

Oxidative stress is involved in the pathogenesis of several disorders. However, in this study we evaluated the antioxidant effect of green juice to identify new dietary sources that can prevent oxidative damage.

Methods and materials

Animals

Sixty-day old male Wistar rats were obtained from the Central Animal House of Universidade Federal de Pelotas, RS, Brazil. The animals were maintained on 12 h light/12 h dark cycles at a constant room temperature (20-24°C) with free access to water and food.

Treatment

The experiments were performed after approval by the Ethics Committee on Animal Experiments (CEEA Nº 6303) of Universidade Federal de Pelotas and all efforts were made to minimize animal suffering. The animals were randomly divided into three experimental groups and submitted to supplementation for 15 days: group (GJ) received green juice, group (OJ) received orange juice and the control group received water. The rats were weighed at the beginning and the end of the experiment to determine the weight gain. All animals were handled for 15 days and were given 5 mL/kg orally. Animals were sacrificed 24 h after the last administration by decapitation without anesthesia; cerebral cortex was collected and was kept frozen until the biochemical analyzes.

Preparation of juice

Green juice was prepared using the following components: orange (Citrus sinensis), apple (Malus sp.), lettuce (Lactuca sativa), cabbage (Brassica oleracea) and cucumber (Cucumis sativus). Orange juice was prepared with orange (Citrus sinensis). All components were obtained commercially in Pelotas, RS, Brazil. Green juice preparation consisted of processing fruits and vegetables with water and then
straining to separate the solids. Orange juice was also strained after preparation. Both green and orange juices were prepared daily.

**Tissue preparation**

Cerebral cortex was homogenized in sodium phosphate buffer pH 7.4 containing KCl (1:10, w/v). The homogenates were centrifuged at 3,500 rpm for 10 min at 4°C. Immediately the supernatant was separated and used for biochemical determinations.

**Determination of antioxidant activity**

The antioxidant activity of green juice and orange juice was measured by 2,2-diphenyl-1-pycrylhydrazyl (DPPH) radical scavenging assay according to Brand-Williams with some modifications. Different dilutions of the juices were prepared: A (1:10), B (1:100) e C (1:1000), which subsequently react with DPPH (60 µM). Ascorbic acid (D) was used as standard (30 µM). The decrease in absorbance was measured and the ability to scavenge free radicals was calculated based on decreased absorbance. The antioxidant activity was expressed as the percentage of DPPH remaining.

The 2,2'-azino-bis-3-ethyl benzothiazoline-6-sulfonic acid (ABTS) method is based on the ability of antioxidant molecules to quench the long-lived ABTS+, blue-green chromophore with characteristic absorption at 734 nm, compared to trolox, a water-soluble vitamin E analog. Results were expressed as TEAC (trolox equivalent antioxidant capacity) in mmol of trolox per L of sample.

**Determination of phenolic acids**

The concentration of phenolic acids was determined using the method described by Singleton and Rossi, with modifications. The diluted sample (1:10) was added to a 10 mL volumetric with diluted Folin-Ciocalteu (1:10) CaCO3 20%. Next it was left in a water bath at 50°C for 5 min. Then, the readings were taken at 765 nm. The procedure was the same as the blank and with the standard solutions of gallic acid. The total phenolic content was expressed as gallic acid equivalents in milligrams per liter.

**Thiobarbituric acid-reactive substances (TBARS)**

TBARS, a measure of lipid peroxidation, was determined according to the method described by Ohkawa et al. Briefly, tissue supernatant was mixed with 20% trichloroacetic acid and 0.8% thiobarbituric acid and heated in a boiling water bath for 60 min. TBARS were determined by the absorbance at 535 nm and reported as nmol TBARS/mg protein.

**Catalase (CAT) assay**

CAT activity was assayed in brain cortex according to Aebi based on the decomposition of H2O2 monitored at 240 nm at ambient temperature. One CAT unit is defined as one µmol of hydrogen peroxide consumed per minute and the specific activity is reported as units/mg protein.

**Superoxide dismutase (SOD) assay**

SOD activity was assayed in cerebral cortex using the SD125-RANSOD kit of Randox. The results were expressed as units of activity of the SOD/mg of protein.

**Protein determination**

Protein was determined by the method of Lowry using bovine serum albumin as standard.

**Statistical analysis**

Data were expressed as mean ± standard deviation. The comparisons of means were analyzed by Student’s t test or by analysis of variance (ANOVA) followed by one-way Tukey test when the F value was significant. A value of P < 0.05 was considered to be significant. Analyses were performed using the Statistical Package for Social Sciences (SPSS).

**Results**

The percentage of DPPH remaining at dilutions A (1:10), B (1:100) and C (1:1000) of green juice was 22.8%, 58% and 78%, and orange juice, at the same dilutions were 5.6%, 5.6% and 77.2%, respectively. The remaining percentage of DPPH in the presence of ascorbic acid was 0.4% (fig. 1). The ability of juices to reduce ABTS radical was 3.5 mmol trolox/L for green juice and 5.2 mmol trolox/L for orange juice.

![Fig. 1.—The percentage of DPPH remaining in the presence of green juice, orange juice and ascorbic acid.](image-url)
There was no significant difference in total phenolic acid content in the green juice compared to the orange juice (Table I).

The TBARS, which reflects the contents of malondialdehyde, a biomarker of lipid peroxidation, was lower in the cerebral cortex of rats supplemented with green juice when compared to the control and OJ groups \([F(2,27) = 38.745, P < 0.001]\) (fig. 2).

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\text{CAT activity was significantly lower in the group supplemented with green juice when compared to control \([F(2,27) = 12.634, P < 0.001]\) (fig. 3). No significant difference was observed in SOD activity between the supplemented groups and controls, however the OJ group presented lower SOD activity than the GJ group \([F(2,27) = 7.90, P < 0.05]\) (fig. 4).}
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With respect to weight gain, it was observed that the GJ group \((42.2 \pm 5.14 \text{ g})\) had significantly less weight gain than the control group \((52.3 \pm 6.13 \text{ g})\) \([F(2,27) = 8.446, P < 0.001]\).

Discussion

It is known that fruits, vegetables and teas provide health benefits,\textsuperscript{7,23,24,25} and that some of these effects can be assigned to antioxidant compounds.\textsuperscript{24,26} Different substances in foods, especially fruits and vegetables, such as vitamins C and E, \(\beta\)-carotene and phenolic compounds are excellent antioxidants that are able to scavenge reactive species. However, the exact health-promoting effect of these dietary components is still unclear. Therefore, the main objective of this study was to evaluate the antioxidant effect of green juice which combines fruits and vegetables.

Phenolic compounds, ubiquitous in fruits and vegetables, represent an important group of natural antioxidants. Phenolic compounds include flavonoids and phenolic acids considered desirable bioactive foods because of their antioxidant activity.\textsuperscript{24,27} In our study, no difference was observed between the phenolic acids content of orange and green juice. A higher concentration of phenolic acids in green juice was expected because it contains apple, lettuce and other vegetables;\textsuperscript{28} however, this was not observed. It is likely that other natural antioxidants are present in both juices, thus it could be useful to identify others compounds which may contribute to antioxidant activity in these beverages.

Our results also showed that orange juice presented a better DPPH and ABTS radicals scavenging capacity than green juice. The DPPH assay is based on the ability of hydrogen-donating antioxidants to scavenge the DPPH radical.\textsuperscript{17} Antioxidants may react in different manner to distinct radical or oxidant sources, sugges-
Green juice protect against reactive species


Acknowledgements

This work was supported in part by grants from “Fundação de Amparo à Pesquisa do Rio Grande do Sul” (FAPERGS), “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (CAPES) and “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq), Brazil.

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