

Effects of *Passiflora edulis* (Yellow Passion) on Serum Lipids and Oxidative Stress Status of Wistar Rats

Maricelma da Silva Soares de Souza,^{1,2} Sandra Maria Barbalho,^{3,4} Débora Cristina Damasceno,²
Marilza Vieira Cunha Rudge,² Kleber Eduardo de Campos,⁵ Ana Carolina Goyos Madi,¹
Bruno Ribeiro Coelho,¹ Rodrigo César Oliveira,¹ Rodrigo Cartaxo de Melo,¹
and Vinícius Castrequini Donda¹

Departments of ¹Pharmacology and ³Biochemistry, School of Medicine and Health Sciences,
University of Marília, Marília, São Paulo, Brazil.

²Laboratory of Experimental Research of Gynecology and Obstetrics, Department of Gynecology
and Obstetrics, Botucatu Medicine School, São Paulo State University, Botucatu, São Paulo, Brazil.

⁴Department of Food Research, Faculty of Food and Technology of Marília, Marília, São Paulo, Brazil.

⁵Institute of Sciences and Health, Mato Grasso Federal University, Sinop, Mato Grasso, Brazil.

ABSTRACT The aim of this study was to evaluate the effects of *Passiflora edulis* f. *flavicarpa* Degener (yellow passion) juice on the lipid profile and oxidative stress status of Wistar rats. Adult male Wistar rats were divided in two groups ($n = 8$ animals per group): the control group, which received water, and the treated group, which was given *P. edulis* juice (1,000 mg/kg). Both groups received by gavage treatment twice a day for 28 days. The treated group showed an increased high-density lipoprotein-cholesterol level and decreased low-density lipoprotein-cholesterol and free fatty acid levels compared with the control group. Levels of triglycerides and and very low-density lipoprotein-cholesterol, superoxide dismutase activity, and total glutathione concentration were not statistically different between the two groups, but the thiobarbituric acid–reactive substances concentration (indicating lipid peroxidation) decreased in the treated group. These findings suggests that *P. edulis* juice in the experimental conditions used showed beneficial effects on lipid profile and improved lipid peroxidation in Wistar rats.

KEY WORDS: • cholesterol • lipids • oxidative stress • *Passiflora edulis* • rat

INTRODUCTION

MANY PLANTS ARE KNOWN and widely used as drugs for the treatment of many diseases, including type 2 diabetes mellitus and high levels of serum lipids. Several studies have shown that many commonly used plants have significant beneficial effects.^{1–3} According to the World Health Organization, about 80% of the people in the world use medicinal herbs to treat and prevent diseases instead of primary medical assistance because of low prices and the ease of obtaining such medicinal plants.⁴

Literature data confirm the significant effects of alternative medicine to control hyperglycemia^{5,6} and lipid levels.^{7–9}

Hypercaloric diets are very common nowadays and increase the occurrence of hypercholesterolemia and low levels of high-density lipoprotein-cholesterol (HDL-c), resulting in higher risks of cardiovascular diseases.¹⁰ Hypercholesterolemia and low HDL-c levels may be associated

with endothelium dysfunction and inflammation, which are often followed by atherosclerosis. The endothelial dysfunction may be prevented by controlling serum lipids.¹¹ High levels of total cholesterol, low-density lipoprotein-cholesterol (LDL-c), and triglycerides associated with lower HDL-c levels may also induce insulin resistance. These parameters together may be conducive to the occurrence of the metabolic syndrome, which increases the risk for development of vascular diseases.^{12,13}

The genus *Passiflora* is largely found in Brazil and include several native plants known as *maracujá* (yellow passion), but only two of them have commercial importance: *Passiflora edulis*, usually called juice *maracujá*, and *Passiflora alata*, called sweet *maracujá*. *Passiflora quadrangularis* has been shown to have hemolytic activity like hemolysin.¹⁴ The insoluble fiber fraction derived from *P. edulis* seeds has effects on plasma and hepatic lipids from hamsters.¹⁵ *Passiflora incarnata* has anxiolytic effects, and its leaves have anti-asthmatic activity.¹⁶ Biochemical parameters and antioxidant defenses (superoxide dismutase [SOD] and glutathione peroxidase [GSH-Px] activities and total glutathione [GSH-t] and thiobarbituric acid–reactive substances [TBARS] concentrations) were affected when male Wistar rats were treated with

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Address correspondence to: Profa. Dra. Maricelma da Silva Soares de Souza, University of Marília, Rua José Bertonha, 78, Jardim Tangará, CEP 17516-010, Marília, SP, Brazil. E-mail: maricelma.soares.souza@gmail.com

P. alata.¹⁷ A dry extract of *Passiflora* has been shown to exert positive effects in the control of glycemia and lipid levels.^{18–20}

The aim of the present study was to evaluate the effects of *P. edulis* (yellow passion) juice on serum lipid levels and oxidative stress status of Wistar rats.

MATERIALS AND METHODS

Extraction of plant material

P. edulis seeds were authenticated by experts from the Botanical Department at our institution (São Paulo State University, Botucatu, SP, Brazil) where a voucher specimen has been deposited. The material was prepared by grinding the pulp, similar to the folk-medicine preparation method. The material was filtered through several layers of gauze, and the extract was divided into aliquots and stored and maintained in a freezer until use. A sample was evaporated to determine the total extract concentration (150 mg/mL).

Animals

Wistar rats were obtained from the University of Marília, Marília, SP, Brazil. After a 10-day acclimatization, virgin male Wistar rats, weighing 200 ± 20 g each, were maintained under standard conditions of temperature ($21 \pm 4^\circ\text{C}$) and a 12-hour light/dark cycle, with access to water and food *ad libitum*. The animals were cared for according to the guidelines of the U.S. National Institutes of Health for the care and use of experimental animals.

Experimental procedure

The rats received daily treatment by gavage, twice a day (in the morning and in the afternoon), for 28 days. The rats were randomly divided into two experimental groups: the control group ($n=8$) received vehicle (water), which was also used to dilute the plant extract, and the treated group ($n=8$) was given a dose of *P. edulis* juice at 1,000 mg/kg of body weight.

Assay for blood samples

On day 28 of treatment, the rats were anesthetized with sodium pentobarbital (Penta-Hypnol® [3%], Agrovot Market S.A., Lima, Peru), and blood was then collected for biochemical analysis. Blood samples were divided in two. One portion of blood put into anticoagulant-free test tubes, kept at low temperature for 30 minutes, and then centrifuged at 1,300 g for 10 minutes at 4°C . The supernatant was collected as serum and stored at -80°C for determination of the lipid profile. The other portion of blood put into anti-coagulant tubes was centrifuged at 90 g for 10 minutes at room temperature for assay of oxidative stress status.

Assays for oxidative stress status

The biomarkers estimated for oxidative stress were SOD, GSH-t, GSH-Px, and TBARS as an index of lipid peroxidation.

Lipid peroxides were estimated in the washed erythrocytes using thiobarbituric acid. In brief, 1.0 mL of washed

erythrocytes was added to a test tube containing 1.0 mL of 3.0% sulfosalicylic acid, agitated for 10 seconds, centrifuged at 18,000 g for 3 minutes, and allowed to rest for 15 minutes. The sample was diluted to 500 μL of 0.67% thiobarbituric acid solution. The mixture was heated to 80°C for 30 minutes, and the absorbance was measured at a wavelength of 535 nm. The results were expressed as nmol of TBARS per gram of hemoglobin (nmol/g Hb).²¹

SOD activity was determined from its ability to inhibit the autooxidation of pyrogallol. The reaction mixture (1.0 mL) consisted of 5.0 mM Tris (pH 8.0), 1.0 mM EDTA, double distilled water, and 20 μL of sample. The reaction was initiated by the addition of pyrogallol (final concentration, 0.2 mM), and the absorbance was measured by a spectrophotometer with a wavelength of 420 nm (25°C) for 5 minutes. The enzymatic activity unit was defined as the amount of SOD units able to produce 50% of pyrogallol oxidation inhibition. All data were expressed in units of SOD per milligram of hemoglobin.²¹

The content of GSH-t, which consists of reduced and oxidized glutathiones, was enzymatically determined using 5,5'-dithiobis(2-nitrobenzoic acid) and glutathione reductase in the presence of a reduced form of NADPH, forming 2-nitro-5-thiobenzoic acid. A mixture consisting of 1,290 μL of distilled water, 200 μL of Tris-HCl buffer (1 M [pH 8.0] with 5 mM EDTA), 200 μL of 10 IU/mL glutathione reductase (Sigma, St. Louis, MO, USA), 200 μL of 2 mM NADPH (Sigma), and 100 μL of 12 mM 5,5'-dithiobis(2-nitrobenzoic acid) (Sigma) was added to 10 μL of the sample. Activity was measured at 412 nm with a spectrophotometer. One unit of activity was equal to 1 μmol of substrate reduced per gram of hemoglobin.²¹

GSH-Px activity was assayed by monitoring NADPH oxidation. The mixture consisted in the addition of 1,300 μL of distilled water, 200 μL of Tris-HCl buffer (1 M [pH 8.0] with 5 mM EDTA), 200 μL of 10 IU/mL glutathione reductase, 200 μL of NADPH (2 mM), and 40 μL of reduced glutathione (0.1 M) to 40 μL of hemolysate. The mixture was agitated in a vortex-mixer for 10 seconds. Next, 20 μL of *tert*-butylhydroperoxide (7 mM) was added and allowed to sit at 37°C for 10 minutes. Absorbance was determined with a spectrophotometer at a wavelength of 340 nm. GSH-Px activity was expressed in enzymatic activity units (IU) per gram of hemoglobin.²¹

Assay for lipid profile

Triglycerides, total cholesterol, and HDL-c concentrations were determined with a Wiener Laboratorios S.A.I.C. (Rosario Santa Fé, Argentina) assay kit.²²

Very LDL-c (VLDL-c) and LDL-c concentrations were determined by the method of Friedewald *et al.*²³ Free fatty acids (FFAs) were determined by the Reogun method.²⁴

Statistical analysis

Results are reported as mean \pm SD values. All data were statistically analyzed using analysis of variance followed by

TABLE 1. DETERMINATION OF TRIGLYCERIDES, CHOLESTEROLS, AND FREE FATTY ACIDS IN BLOOD SAMPLED FROM RATS TREATED WITH *P. EDULIS* JUICE RELATIVE TO CONTROL GROUP LEVELS

	Control group	Treated group
Triglycerides (mg/dL)	195.54 ± 113.53	180.69 ± 99.48
Cholesterol (mg/dL)	145.19 ± 29.35	109.13 ± 14.66*
LDL-c (mg/dL)	79.00 ± 43.40	22.71 ± 16.69*
HDL-c (mg/dL)	27.07 ± 7.04	50.28 ± 13.70*
VLDL-c (mg/dL)	39.11 ± 15.75	36.14 ± 10.14
FFAs (mEq/L)	1.18 ± 0.45	0.56 ± 0.41*

Data are mean ± SD values.

*Value significantly different from the control group (Student's *t* test using $P \leq .05$).

FFAs, free fatty acids; HDL-c, high-density lipoprotein-cholesterol; LDL-c, low-density lipoprotein-cholesterol; VLDL, very low-density lipoprotein-cholesterol.

Student's *t* test. Statistical significance was considered as $P \leq .05$.²⁵

RESULTS

Table 1 presents data of assay for triglycerides, total cholesterol, HDL-c, VLDL-c, LDL-c, and FFAs. Rats treated with *P. edulis* juice presented significantly increased HDL-c levels (50.28 ± 13.70 mg/dL, $P \leq .05$) compared with the control group (27.07 ± 7.04 mg/dL). The treatment with *P. edulis* juice produced decreased cholesterol (109.13 ± 14.66 mg/dL, $P \leq .05$), LDL-c (22.71 ± 16.69 mg/dL, $P \leq .05$), and FFAs (0.56 ± 0.41 mEq/L, $P \leq .05$) relative to the control group (145.19 ± 29.35 mg/dL, 79.00 ± 43.40 mg/dL, and 1.18 ± 0.45 mEq/L, respectively). Treatment with *P. edulis* juice did not significantly alter triglyceride and VLDL-c levels ($P > .05$).

Table 2 shows results of oxidative stress status. Rats treated with *P. edulis* juice presented no alteration of SOD activity, GSH-Px activity, and GSH-t concentration ($P > .05$). TBARS levels of the group given *P. edulis* juice were decreased compared with the control group (58.71 ± 14.69 vs. 75.61 ± 21.97 nM/g of hemoglobin, respectively; $P \leq .05$).

TABLE 2. DETERMINATION OF SUPEROXIDE DISMUTASE, TOTAL GLUTATHIONE, GLUTATHIONE PEROXIDASE, AND LIPID PEROXIDATION IN BLOOD SAMPLED FROM RATS TREATED WITH *P. EDULIS* JUICE RELATIVE TO CONTROL GROUP LEVELS

	Control group	Treated group
SOD (IU/g of Hb)	1,482.98 ± 370.19	1,281.90 ± 345.84
GSH-t (μmol/g of Hb)	0.44 ± 0.08	0.44 ± 0.09
GSH-Px (IU/g of Hb)	25.49 ± 7.61	31.62 ± 10.40
TBARS (nmol/g of Hb)	75.61 ± 21.97	58.71 ± 14.69*

Data are mean ± SD values.

*Value significantly different from the control group (Student's *t* test using $P \leq .05$).

GSH-Px, glutathione peroxidase; GSH-t, total glutathione; Hb, hemoglobin; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances.

DISCUSSION

Changes in serum lipids are important factors in the genesis of vascular disease. These alterations can be associated with dietary patterns and related to insulin resistance, diabetes mellitus, arterial hypertension, stroke, and several types of cancer that are responsible for the increase of premature death.²⁶ The role of lipids in the pathogenesis of insulin resistance (related to obesity and/or diabetes mellitus) is one of the most important pathogenic determinants.²⁷

There are studies that have used plants for treatment or prevention of hyperlipidemias: soy,²⁸ green tea,²⁹ *Malpighia emarginata*,³⁰ and *Ananas comosus*.² Tao *et al.*³¹ showed significant reduction in glycemia, cholesterol, and triglycerides by using a traditional antidiabetic formula prepared with many plants. Adeneye and Adeyemi³² found a decrease in lipids and glycemia after using *Hunteria umbellata*.

The present study demonstrated that *P. edulis* juice increased HDL-c levels and decreased total cholesterol, LDL-c, and FFA levels (Table 1). Ramos *et al.*¹⁹ used a flour of passion fruit and also found beneficial effects on the lipid profile; the *P. edulis* juice presented relevant changes in lipid profile. The beneficial effect in HDL-c levels may be attributed to the presence of flavonoids in this species.¹⁸ Investigation using *P. alata* showed altered HDL-c levels in rats, but it did not show changes in LDL-c levels.¹⁸ This explanation might be extended to the decreased FFA and cholesterol levels in the animals treated with plant juice (Table 1). Nevertheless, treatment with *P. edulis* did not significantly modify the concentrations of triglycerides and VLDL-c. Other authors have suggested that the insoluble fiber-rich fraction prepared from *P. edulis* could potentially be hypocholesterolemic.^{15,33}

Many population studies have established a link between dietary intake of antioxidants and a reduced risk of chronic diseases. Antioxidant potential is very important because oxidative stress may be increased in many pathologies.³⁴ The increased oxidative stress is related to a hyperproduction of reactive oxygen species such as O_2^- , OH^- , and H_2O_2 or deficiency in the antioxidant defense system consisting of enzymatic (SOD, catalase, and GSH-Px/glutathione reductase systems) and nonenzymatic scavenger components. Mechanisms that contribute to the formation of free radicals may include metabolic stress resulting from changes in energy metabolism, the levels of inflammatory mediators, and the status of antioxidant defense.^{35,36} Our results did not show significant alteration in SOD and GSH-Px activities and GSH-t concentration after treatment with *P. edulis* juice (Table 2). There is increasing evidence that in certain pathological conditions the increased production and/or ineffective scavenging of such reactive species may play a crucial role in determining tissue injury by lipid peroxidation, especially in atherosclerotic and cardiovascular diseases, even in the presence of hypertension, hyperlipidemias, and smoking.^{37,38} In the present study, the TBARS concentration from rats treated with *P. edulis* juice was decreased (Table 2). TBARS are considered a lipid

peroxidation indicator.³⁹ In spite of no alteration in SOD and GSH-Px activities, the decreased TBARS concentration might be the result of action of the nonenzymatic antioxidants present in the plant juice. *Passiflora* species contain a great amount of lycopene; thus passionflower fruit is considered an alternative source of antioxidant products.⁴⁰ In this way, *P. edulis* juice may be effective in reducing lipid peroxidation and may improve protection to cellular membranes.

In conclusion, *P. edulis* juice showed beneficial effects for the lipid profile because it increased the HDL-c level and decreased levels of LDL-c, cholesterol, and FFAs. In addition, the plant juice decreased the TBARS concentration. These results show that *P. edulis* improved lipid metabolism and oxidative stress status. This study is very important because hyperlipidemia represents a major risk factor to the development of insulin resistance, the metabolic syndrome, and other diseases. However, the demonstration of the active component present in *P. edulis* that is responsible for these beneficial actions needs further investigation.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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