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Jaboticaba peel and jaboticaba peel aqueous extract shows *in vitro* and *in vivo* antioxidant properties in obesity model

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ABSTRACT

This study evaluated the chemical composition of freeze-dried jaboticaba peel (FJP) and jaboticaba peel aqueous extract (JE) and their antioxidant capacity *in vitro* and *in vivo* in obesity model. Phenolic compounds, total anthocyanins, flavonoids and antioxidant capacity (DPPH, FRAP, ABTS and ORAC assays) in FJP methanolic extract and JE were measured. Specific phenolics (ellagic and gallic acids) and anthocyanin (cyanidin-3-*O*-glucoside) were measured by LC-DAD-ESI/MS. In the biological assay thirty-six *Wistar* rats were divided in six groups: AIN-93 M normal control diet; HFF (obese control) feed a high-fat and fructose diet; Prevention FJP (P. FJP) and Treatment FJP (T. FJP) feed HFF diet with 2% of FJP powder, for 12 and 6 weeks respectively; Prevention JE (P. JE) and Treatment JE (T. JE) were feed with HFF diet and the water was substituted by JE, for 12 and 6 weeks, respectively. FRAP, TBARS, GSH and antioxidant enzymes (GPx, GR, CAT and SOD) were determined in the plasma and liver. The limit of significance was set at $P < 0.05$. The FJP methanolic extract showed higher levels of total phenolics and anthocyanins, ellagic acid and DPPH, FRAP and ORAC assay, but JE showed higher levels of cyanidin-3-*O*-glucoside, gallic acid and ABTS assay. Antioxidant potential of the FJP and JE were confirmed by important markers in animals, such as TBARS and GSH levels and CAT activity, but not by FRAP assay, and SOD, GR and GPx enzymes. Thus, FJP and JE showed an important antioxidant effect *in vitro* and *in vivo*.

1. Introduction

Excessive production of reactive oxygen species (ROS) is identified as one of the key mechanisms in the development of obesity and other metabolic disorders such as insulin resistance (IR) and diabetes (Ando & Fujita, 2009; Feillet-Coudray, Sutra, Fouret, et al., 2009). The excess of reactive species can damage cell lipids, proteins and DNA by oxidative action, which might result in loss of function and even cellular death (Habib & Ibrahim, 2011), which has linked the oxidative stress to some diseases (Durackova, 2010).

Studies have shown that hypercaloric/high-fat diets can induce oxidative stress and metabolic disorders in obesity induced animal model by excessive production of reactive species and decrease in the antioxidant protection (Aschbachera, Kornfeldc, Picardd, et al., 2014; Tsuchiya, Ebataa, Sakabeb, Hamaa, Kogurea, & Shiota, 2013). The combat against oxidative damage is endogenously mediated by enzymatic antioxidant system, compound for the enzymes superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx) and catalase (CAT), and non-enzymatic systems: thiol reduced (GSH), vitamins, minerals and polyphenols (Rezaie, Parker, & Abdollahi, 2007).

Nutritional alternatives to counteract the oxidative damage are necessary, in order to avoid the appearance and progression of obesity and chronic diseases. The role of phytochemicals in the oxidative damage combat it has been studied (Habib & Ibrahim, 2011; Han, Shen, & Lou, 2007). Phenolic compounds are the principal class of dietary phytochemical, naturally present in plant foods, especially fruits, and have important antioxidant potential/capacity (Prior, Wilkes, Rogers, Khanal, Wu, & Howard , 2010).

The jaboticaba (*Myrciaria Jaboticaba* (Vell.) Berg), a Brazilian typical fruit, has high nutritional value. Mainly, jaboticaba peel has a significant content of minerals, soluble and insoluble fiber, and phenolic compounds (Alezandro, Dubé, Desjardins, Lajolo, & Genovese, 2013a; Alezandro, Granato, & Genovese, 2013b; Lima, Duarte, Carvalho, Patto, & Dantas-Barros, 2008). Leite, Malta, Riccio, Eberlin, Pastore, & Marostica (2011) identified cyanidin-3-*O*-glucoside and delphinidin-3-*O*-glucoside as predominant anthocyanins in freeze-dried jaboticaba peel. In addition to anthocyanins, jaboticaba peel has high concentrations of tannins, gallic acid and ellagic acid that have important antioxidant effect (Abe, Lajolo, & Genovese, 2012; Wu, Dastmalchi, Long, & Kennelly, 2012; Wu, Long, & Kennelly, 2013).

Due to high bioactive compounds concentration, the *in vitro* and *in vivo* antioxidant potential of jaboticaba peel has been reported by several authors (Alezandro, Dubé, Desjardins, Lajolo, & Genovese,

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2013a; Alezandro, Granato, & Genovese, 2013b; Batista, Lenquiste, Cazarin, et al., 2014; Leite, Malta, Riccio, Eberlin, Pastore, & Marostica, 2011). In these studies, the jaboticaba peel showed elevated values of DPPH, FRAP, ABTS and ORAC assays, as well increases GSH and antioxidant enzymes in animal model. Thus, the objective of present study was to identify and quantify the bioactive compounds in the freeze-dried jaboticaba peel (FJP) and in the FJP aqueous extract (JE) and to evaluate their antioxidant capacity by *in vitro* assays. Furthermore, we evaluated the antioxidant potential of the FJP and JE supplementation in animals with oxidative stress induced by high fat and high fructose diet.

2. Methods

2.1. Preparation of freeze-dried jaboticaba peel (FJP)

Jaboticabas (*M. Jaboticaba* (Vell.) Berg) were acquired in Campinas Central Supply (CEASA), Brazil. Fruits were selected and cleaned. The peels were manually separated and were frozen, freeze-dried and milled to get a homogeneous powder. The powder was stored at −80 °C in dark flasks, prior to analysis and preparation for the *in vivo* assays.

2.2. Proximate centesimal composition of FJP

Analyses of humidity, total protein and ash were performed according to methods described by Association of Official Analytical Chemists (AOAC, 2002). Total lipids were determined by Bligh & Dyer (1959). Soluble and insoluble fibers were determinate according to Asp, Johansson, Hallmer, & Siljestrom (1983). Carbohydrate content was obtained by difference using the equation: 100 − (moisture + protein + lipid + ash + dietary fiber) and energy value was determined in isoperibol automatic calorimeter (PARR 1261) with oxygen pump (PARR 1108).

2.3. FJP extracts preparation

FJP MeOH extract was prepared mixing 0.5 g of FJP powder with 12.5 mL of MeOH:H2O (70:30) (v/v). After vortex-mixing, sample was sonicated for 10 min. Extracts were filtered on paper filter and re-extracted twice. Filtrates were mixed and filtered again on 0.45 μm filter. FJP aqueous extract (JE) were obtained by weighing 2.0 g FJP powder and 100 mL boiling water addition. The extract was maintained in infusion for 30 min and manually homogenized each 15 min. Afterward extract was filtered under vacuum pressure and stored at 4 °C until analysis.

For phenolic LC-DAD-ESI/MS analysis FJP powder (1.0 g) was mixed with 15 mL of MeOH:H2O:Acetic acid (85:15:0.5) (v/v), vortex-mixed for 30 s and sonicated for 5 min. Samples were vortex-mixing for 30 s, two times, until complete 10 min. Samples were centrifuged at 3500 rpm for 10 min at 25 °C. The extraction process was repeated with 10 mL of the solution. Thereafter, extracts were combined and diluted to 25 mL with the same solvent. All samples were filtered through 0.45 μm filter. HPLC analyses used the same aqueous extract as described above. Extracts were prepared in triplicate for all analyses.

2.4. Total phenolic determination

Total phenolic content was determined using the Folin–Ciocalteau method as described by Singleton, Orthofer, and Lamuela-Raventos (1999). Samples and a gallic acid standard curve were read at 725 nm. Results were expressed as mg gallic acid equivalent g^{-1} or mL^{-1}. Total flavonoid concentration was quantified using the colorimetric method described by Herald, Gadgil, & Tilley (2012). Samples and catechin standard curve were read at 510 nm. Results were expressed as mg catechin equivalent g^{-1} or mL^{-1}. Total anthocyanin concentration was performed by the pH differential method described by Fuleki & Francis (1968), with some modifications. The samples were read at 300 and 700 nm. Results were expressed as mg 100 g or mL^{-1}. Assays were performed in triplicate for all samples.

2.5. Phenolic compounds analysis by LC-DAD-ESI/MS

Cyanidin-3-*O*-glucoside identification and quantification was performed according to general procedure for screening of phenolics in plant materials with modifications (Lin & Harnly, 2007). LC-DAD-ESI/MS instrument consisted of a Varian 250 HPLC (Varian, CA) coupled with a diode array detector (DAD) and a 500-MS IT mass spectrometer (Varian, CA). A Symetry C18 (Varian Inc., Lake Forest, CA) column (3 μm, 250 × 2 mm) was used at a flow rate of 0.4 ml min^{-1}. The column oven temperature was set at 30 °C. Mobile phase consisted of a combination of A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). Gradient was varied linearly from 10% to 26% B (v/v) in 40 min, to 65% B at 70 min, and finally to 100% B at 71 min and held at 100% B to 75 min. The DAD was set at 270 and 512 nm for real-time read-out and UV/VIS spectra, from 190 to 650 nm, were continuously collected. Mass spectra were simultaneously acquired using electrospray ionization in the positive and negative ionization modes (PI and NI) at a fragmentation voltage of 80 V for the mass range of 100–1000 amu. A drying gas pressure of 35 psi, nebulizer gas pressure of 40 psi, a drying gas temperature of 370 °C, capillary voltages of 3500 V for PI and 3500 V for NI, and spray shield voltages of 600 V were used. LC system was coupled to the MSD with a splitting of 50%. Results were expressed as μg g^{-1} or mL^{-1} of cyanidin equivalent. Determinations were performed in triplicate for all samples.

Ellagic acid and gallic acid were quantified in a HPLC (HPLC Agilent 1100 Series, Englewood, CO, USA) with manual injection. 20 μL sample loop and ternary pump, coupled to a diode array detector (DAD Agilent G13158). The oven (Agilent 1100) was operated at 25 ± 2 °C. Data was obtained and processed using the software ChemStation (Hewlett Packard, Germany). A reverse phase chromatographic column (C18 Eclips XDB (5 μm × 250 mm × 4.6 mm), Agilent, Englewood, CO, USA) was used. Mobile phase was 1% orthophosphoric acid in water (v/v) (A) and acetonitrile (B). Elution gradient started at 95:05 (A:B) at 0.7 mL min^{-1}. This condition was maintained for 5 min and then concentration of A was decreased (75:25, A:B), and at 25 min it reached 60:40 (A:B) followed by a linear increase of solvent A to 95% until 35 min. Detection was done at 210, 254, 280, 300 and 340 nm, which allowed simultaneous quantification and identification of the phenolic compounds separated by the HPLC. Content of identified compounds were calculated from the analytical curves. The results were expressed as μg g^{-1} or mL^{-1}. Determinations were performed in triplicate for all samples.

2.6. Antioxidant assay in vitro

Antioxidant capacity in FJP MeOH and FJP aqueous extract was determined by DPPH, ABTS $^{+}$, FRAP and ORAC assays. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was done according to Brand-Williams, Cuvelier, & Berset (1995), with some modifications. The decreasing in absorbance of samples and Trolox standard curve was measured after 30 min of reaction and read at 515 nm. Results were expressed as μM Trolox g^{-1} or mL^{-1}.

The $ABTS^{+}$ assay was based on the method developed by Miller, Rice-Evans, Davies, Gopinathan, & Milner (1993), with modifications according to Rufino, Alves, Brito, Pérez-Jiménez, Saura-Calixto, & Mancini-Filho (2010). The working solution was prepared by reacting 7 mM ABTS (2,2 azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammoninum salt) stock solution with 140 mM potassium persulfate after 16 h incubation in the dark at room temperature. The monitored decrease of absorbance over time (30 min with 1 min interval) was plotted and the differences between samples and control were

calculated. The samples and Trolox standard curve were read at 734 nm. Results were expressed as μM Trolox g^{-1} or mL^{-1}.

The ferric reducing antioxidant power (FRAP) of samples was determined according to Rufino, Alves, Brito, Pérez-Jiménez, Saura-Calixto, & Mancini-Filho (2010). The FRAP reagent was prepared in the dark with 300 mmol L^{-1} acetate buffer (pH 3.6), 10 mmol L^{-1} TPTZ (2,4,6-tris(2-pyridyl)-S-triazine) in a 40 mmol L^{-1} HCl solution and 20 mmol L^{-1} $FeCl_3$. The samples and Trolox standard curve were read at 595 nm. Results were expressed as μM Trolox g^{-1} or mL^{-1}.

ORAC assay (oxygen radical absorbance capacity test) was carried out adding 20 μL of samples extract or standard solutions, 120 μL of fluorescein diluted in phosphate buffer (pH 7.4), and 60 μL of AAPH (2,2′-azobis (2-methylpropionamidine) dihydrochloride) to black microplates, in the dark (Dávalos, Gómez-Cordovés, & Bartolomé, 2004). ORAC values were expressed in μM Trolox g^{-1} or mL^{-1}. Appropriate calculations were used for check the linearity between the net area under the curve and the concentration for the samples.

The absorbances or fluorescences of these assays were read in a microplate reader SynergyHT, Biotek (Winooski, USA); with Gen5™2.0 data analysis software spectrophotometer.

2.7. Antioxidant assay in vivo

2.7.1. Animals and diets

Thirty-six male *Wistar* rats, recently weaned, were obtained from the Multidisciplinary Center for Biological Research at Unicamp (CEMIB). The experiment was approved by the Ethics Committee on Animal Experiments (CEUA/UNICAMP), protocol number 3272-1, and followed all the ethical requirements of the Brazilian College of Animal Experimentation (COBEA). Animals were maintained in growth period for 4 weeks, feed by commercial pelleted diet. Afterward, animals were randomly distributed into six groups (n = 6) and remained at individual cages with food and water under the system of free access, controlled temperature and humidity, with a range of 22 ± 1 °C and 60–70% respectively, and light/dark cycle of 12 h, throughout the experimental period.

Two control diets were given during the experiment: a normal control diet, prepared in accordance with the American Institute of Nutrition (Reeves, Nielsen, & Fahey, 1993), AIN-93 M, with protein concentration of 12%, lipid concentration of 4% and 70% approximately of total carbohydrates and a high-fat fructose control diet, AIN-93 M-modified with 12% protein and 35% of fat, 4% vegetable oil (soybean) and 31% of animal origin (lard) and 20% of fructose (HFF) (Marineli, Moura, Moraes, et al., 2015). The HFF-FJP experimental diet was formulated from high-fat fructose diet adding 2% of FJP powder. Diets composition is showed in Table 1. The prevention groups (P. FJP and P. JE) consumed the supplemented diet or JE for 12 weeks and the treatment groups (T. FJP and T. JE) consumed HFF diet for 6 weeks to induce obesity and started the supplementation as from the sixth week. Groups P. JE and T. JE had the substitution of water by FJP aqueous extract (JE). The experimental design is presented in Fig. 1.

Table 1
Composition of modified AIN-93 M diets fed to rats.

INGREDIENTS	AIN-93 M (g Kg^{-1})	HFF (g Kg^{-1})	HFF–FJP (g Kg^{-1})
Casein (78% prot.)	143.89	143.89	143.89
Corn starch	461.69	133.18	133.18
Maltodextrin	155.00	44.67	44.67
Sucrose	100.00	28.82	28.82
Soybean oil	40.00	40.00	40.00
Cellulose	50.00	50.00	50.00
Fructose	–	200.00	200.00
Mineral mix	35.00	35.00	35.00
Vitamin mix	10.00	10.00	10.00
L-cystine	1.80	1.80	1.80
Choline bitartarate	2.50	2.50	2.50
Tert-butyl hydroquinone	0.008	0.008	0.008
Lard	–	310.00	310.00
FJP powder	–	–	20.00
Energy value (Kcal g^{-1})	4.17	5.45	5.51

In the HFF diet, 31% of lard was added and, consequently, starch, sucrose and maltodextrin contents were reduced. HFF–FJP diet was added of 2% of freeze-dried jaboticaba peel powder. Value expressed in Kcal g^{-1} diet, obtained by calorimetry.

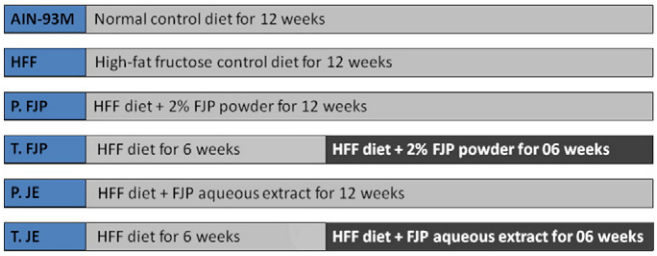

Fig. 1. Experimental design. The animals were maintained on a commercial pelleted diet for 4 weeks for growth. After 4 weeks, they were divided in six groups (n = 6): AIN-93 M (normal control) standard diet; HFF (obese control) received a high-fat and fructose diet containing 4% (w/w) soybeanoil, 31% (w/w) lard and 20% fructose (w/w); P. FJP and T. FJP received the HFF diet with 2% of FJP powder (w/w), for 12 and 6 weeks respectively; P. JE and T. JE received the HFF and had the water substituted by FJP aqueous extract, for 12 and 6 weeks respectively. All diets were based on the AIN-93 M diet.

Diet intake was monitored every 2 days and weight gain once a week. The FJP aqueous extract (JE) was prepared daily and its consumption was monitored every day: JE was measured (25 mL) and placed in animal's bottles, in the next day the JE leftover was measured and the two volumes were recorded. The water consumption of HFF group was monitored to be used as control for the P. JE and T. JE groups.

At 16 experimental weeks, animals were euthanized by decapitation preceded by 12 h fasting. Blood was collected in tubes with anticoagulant EDTA to obtain plasma. After exsanguination, liver was removed, cleaned with saline solution, weighed, frozen in liquid nitrogen and stored in a freezer at −80 °C. Frozen liver was divided in aliquots of the 100 mg approximately and the remaining tissue was freeze-dried. The freeze-dried liver was stored in freezer at −20 °C for further analysis.

2.7.2. Lipid peroxidation by thiobarbituric acid reactive substances (TBARS) assay

TBARS determinations were done in liver and plasma according to Ohkawa, Ohishi, & Yagi (1979), with adaptations. For liver 10 mg of the freeze-dried tissue were mixed with 1 mL of acetate buffer pH 3.6 and sonicated for 30 min in ice bath. The plasma (100 μL) was directly pipetted in the tubes by reaction. The 8.1% sodium dodecyl sulfate (SDS) and working reagent (2-thiobarbituric acid — TBA, 5% acetic acid and 20% sodium hydroxide) were added in the samples. After heating at 95 °C for 60 min, samples remained in ice bath for 10 min and centrifuged at 14.000 rpm for 10 min. Supernatant was read at 532 nm, using a clear 96-well microplate. Standard curve was obtained using malondialdehyde standard (MDA). Results were expressed in nmol MDA mg tissue $^{-1}$ or nmol MDA mL $^{-1}$ plasma.

2.7.3. Antioxidant potential in liver and plasma by FRAP assay

FRAP assay was used for determination of the antioxidant capacity in the tissues. Plasma was treated with ethanol, ultrapure water and 0.75 mol L $^{-1}$ metaphosphoric acid (Leite, Malta, Riccio, Eberlin, Pastore, & Marostica, 2011). Liver homogenate in phosphate buffer (PB) was centrifuged and supernatant was used in FRAP assays (Rufino, Alves, Brito, Pérez-Jiménez, Saura-Calixto, & Mancini-Filho, 2010) as described above.

2.7.4. Enzymatic and non-enzymatic endogenous antioxidant system

2.7.4.1. Thiol group content (GSH). GSH levels were determined in the PB homogenates of liver and plasma using Ellman's reagent (DTNB) (Ellman, 1959), with modifications. GSH solution (2.5–500 nmol GSH mL^{-1}) was used as standard and absorbance was read at 412 nm. Reduced thiol contents were expressed in nmol GSH mg $protein^{-1}$.

2.7.4.2. Glutathione peroxidase activity (GPx). GPx activity was quantified in plasma and PB homogenate of liver. The oxidation of 10 mmol reduced glutathione by glutathione peroxidase coupled to the oxidation of 4 mmol NADPH by 1 U enzymatic activity of GR in the presence of 0.25 mmol H_2O_2 is measured in this assay. The rate of NADPH oxidation was monitored by the decrease in absorbance at 365 nm (Flohe & Gunzler, 1984). Results were expressed in nmol NADPH consumed min^{-1} mg $protein^{-1}$.

2.7.4.3. Glutathione reductase activity (GR). GR activity was measured in plasma and in liver PB homogenates, following the decrease in absorbance at 340 nm induced by 1 mmol oxidized glutathione in the presence of 0.1 mmol NADPH in phosphate buffer (Carlberg & Mannervik, 1985). Results were expressed in nmol NADPH consumed min^{-1} mg $protein^{-1}$.

2.7.4.4. Superoxide dismutase activity. SOD activity was analyzed in liver and plasma. Samples (100 μL) were added in 96-well microplate and 150 μL of the working solution (0.1 mmol hypoxanthine, 0.07 U xanthine oxidase, and 0.6 mmol NTB in PB in 1:1:1 proportions) and the kinetic reaction was monitored at 560 nm (Winterbourn, Hawkins, Brian, & Carrell, 1975). Area under the curve (AUC) was calculated and the SOD activity was expressed as U mg $protein^{-1}$.

2.7.4.5. Catalase activity. Catalase assay is based on reaction of the enzyme with methanol in optimum concentrations of H_2O_2. The produced formaldehyde is measured colorimetrically with Purpald (chromophore) (Johansson & Borg, 1988; Wheeler, Salzman, Elsayed, et al., 1990). Liver homogenate and plasma were pipetted (20 μL) in 96-well microplate with 100 μL assay buffer, 30 μL methanol and 20 μL H_2O_2. Reaction occurred for 20 min in shaker of the dark. Thirty μL of potassium hydroxide stop the reaction. Purpald was added (30 μL) and the plate was read at 540 nm. The formaldehyde standard curve was done and used for calculate catalase activity. The results were expressed as nmol min^{-1} mL^{-1} or g $protein^{-1}$.

For FRAP assay, enzymatic and non-enzymatic endogenous antioxidant system analyze in liver, the protein concentration of tissue homogenates was done by Bradford method (Bradford, 1976).

2.8. Statistical analysis

Phenolics compounds and antioxidant capacity between the FJP MeOH extract and FJP aqueous extract were analyzed by Student's *t-test* and the limit of significance was set at $p < 0.05$. For biological assays, difference in averages between AIN-93 M and HFF groups was analyzed by Student's *t-test* with limit of significance of 0.05. Difference between HFF and supplemented groups was analyzed by Analysis of Variance (ANOVA; $\alpha = 5\%$) with post hoc Tukey's range test with significant differences between means ($p < 0.05$). Data analyses were carried out with GraphPad Prism 5.0 (GraphPad Software, Inc. La Jolla, CA, USA) software.

3. Results and discussion

3.1. Chemical composition

The jaboticaba peel is composed mainly of carbohydrates, including soluble and insoluble fiber, and water (Table 2). Possibly, the high content of carbohydrate corresponds the large amount of simple and complex sugars which are the major constituents of fruit peel (Damodaran, Parkin, & Fennema, 2010). The content of insoluble and soluble fibers, protein and lipids has higher in the present study than in similar studies (Alezandro, Dubé, Desjardins, Lajolo, & Genovese, 2013a; Alezandro, Granato, & Genovese, 2013b; Leite, Malta, Riccio, Eberlin, Pastore, & Marostica, 2011; Lenquiste, Batista, Marineli, et al., 2012). These results in the approximate composition can be justified by harvest time and ripeness of used jaboticaba across studies.

Table 2
Proximate composition of freeze-dried jaboticaba peel.

NUTRIENTS	g 100 g^{-1}
Protein	7.31 ± 0.17
Lipids	2.71 ± 0.17
Moisture	13.88 ± 0.08
Total solids	86.12 ± 0.08
Ashes	3.84 ± 0.02
Soluble fibers	24.95 ± 0.15
Insoluble fibers	7.88 ± 0.22
Carbohydrates	33.96
Energy value (Kcal g^{-1})	2.55 ± 0.06

Data are presented as means ± standard deviation. The values are expressed as percentage. The carbohydrates were calculated by difference.

3.2. Antioxidant assay in vitro

3.2.1. Phenolic compounds and antioxidant capacity in FJP extracts

Jabuticaba peel has a great potential as functional food and as an additive in processed foods (Dessimoni-Pinto, Moreira, Cardoso, & Pantoja, 2011). On the other hand, jabuticaba peel can be consumed as tea such as formulated in the present study. This proposal becomes practicable once tea is the second most popular beverage in the world. Furthermore, plants infusion contains several flavonoids, substances that are among the polyphenolic compounds, which can have positive biological effects (El-Beshbishy, 2005).

Phenolics compounds and antioxidant capacity in freeze-dried jaboticaba peel (FJP) MeOH extract and FJP aqueous extract are showed in Table 3. Total phenolic compounds, anthocyanins and flavonoids were higher in FJP MeOH extract than in FJP aqueous extract. Total phenolics and anthocyanins in the FJP MeOH extract were similar to those obtained by Alezandro, Dubé, Desjardins, Lajolo, & Genovese (2013a) and Alezandro, Granato, & Genovese (2013b), but lower than those obtained by Batista, Lenquiste, Cazarin, et al. (2014).

The gallic acid and cyanidin-3-*O*-glucoside content were higher in FJP aqueous extract. However, ellagic acid was higher in the FJP MeOH extract. Gallic acid values showed in FJP MeOH extract was 18% lower than found by Wu, Dastmalchi, Long, & Kennelly (2012), which evaluated the phenolic compounds by HPLC in the jaboticaba peel and jaboticaba juice. Nevertheless, in the present study results are in agreement with those demonstrate by Batista, Lenquiste, Cazarin, et al. (2014). Ellagic acid content in FJP MeOH extract was similar to those reported by Abe, Lajolo, & Genovese (2012) – 22.50 g Kg^{-1} – and higher than the 348.08 mg 100 g^{-1} obtained by Batista, Lenquiste, Cazarin, et al. (2014). In Alezandro, Dubé, Desjardins, Lajolo, & Genovese (2013a), Alezandro, Granato, & Genovese (2013b), and Wu, Dastmalchi, Long, & Kennelly (2012) studies ellagic acid levels were lower than those obtained in this study. Anthocyanin cyanidin-3-*O*-glucoside in FJP MeOH was higher than those showed by Leite, Malta, Riccio, Eberlin, Pastore, & Marostica (2011) and Wu, Dastmalchi, Long, & Kennelly (2012), 1964.00 mg 100 g^{-1} and 29.80 mg 10 g^{-1}, respectively.

Anthocyanins are more soluble in water and methanol. However, it is known that heating may enhance the extraction of certain

Table 3
Phenolics compounds and antioxidant capacity in freeze-dried jaboticaba peel (FJP) MeOH extract (g), FJP aqueous extract (g) and FJP aqueous extract (mL).

	FJP MeOH extract (g)	FJP aqueous extract (g)	FJP aqueous extract (mL)
Phenolic compounds			
Total phenolics	$48.61^{a} \pm 1.18$	$36.12^{b} \pm 3.05$	0.72 ± 0.06
Anthocyanins	$630.46^{a} \pm 21.76$	$404.56^{b} \pm 35.85$	8.09 ± 0.72
Flavonoids	$7.59^{a} \pm 0.12$	$7.22^{b} \pm 0.02$	0.15 ± 0.01
Specific Phenolics			
Gallic acid	$36.92^{b} \pm 2.28$	$177.76^{a} \pm 2.26$	3.55 ± 0.05
Ellagic acid	$3045.44^{a} \pm 90.97$	$1581.61^{b} \pm 135.50$	31.63 ± 2.71
Cyanidin-3-*O*-glucoside	$32{,}945.24^{b} \pm 347.80$	$34{,}242.88^{a} \pm 594.70$	685.20 ± 11.90
Antioxidant capacity			
DPPH	$346.77^{a} \pm 30.23$	$320.85^{b} \pm 29.90$	6.42 ± 0.60
FRAP	$449.68^{a} \pm 18.72$	$410.65^{a} \pm 10.60$	8.21 ± 0.21
ABTS	$194.95^{b} \pm 4.66$	$223.10^{a} \pm 2.28$	4.46 ± 0.05
ORAC	$317.98^{a} \pm 9.03$	$213.16^{b} \pm 15.06$	4.26 ± 0.31

Data are presented as means ± standard deviation. Total phenolics are expressed as mg gallic acid equivalent g or mL^{-1}. Total flavonoids are expressed as mg catechin equivalent g or mL^{-1}. Total anthocyanins are expressed as mg 100 g or mL^{-1}. Specific flavonoids are expressed as μg g or mL^{-1}. Cyanidin-3-*O*-glucoside is expressed as μg g or mL^{-1} of cyanidin equivalent. Antioxidant activities are expressed as μM Trolox g or mL^{-1}. Statistical differences between FJP MeOH 70% and FJP aqueous extract are represented by different letters according Student's *t-test* ($p < 0.05$). The FJP aqueous extract (mL) column was not statistically compared to other columns.

compounds, by breaking hydrogen bonds. Therefore, the concentration of anthocyanins in aqueous extract can be improved by increasing solubility of these compounds through boiling water infusion (Azmir, Zaidul, Rahman, et al., 2013). Daneshfar, Ghaziaskar, & Homayoun (2008) evaluated the solubility of gallic acid in different solvents such as methanol, ethanol and water and its relationship with temperature. These authors observed that heating increases gallic acid solubility of in water. This may explain the higher concentration of gallic acid in the aqueous extract since it was heated to 95 °C.

Antioxidant capacity of jaboticaba peel extracts was measured by DPPH, FRAP, ABTS and ORAC assays (Table 3). In DPPH and ORAC assays, methanol extract was more efficient than the aqueous extract. The results of FRAP assay were statistically similar for both extracts and the ABTS assay showed higher values in the aqueous extract.

Leite-Legatti, Batista, Dragano, et al. (2012) and Abe, Lajolo, & Genovese (2012) evaluated the antioxidant capacity by DPPH assay in jaboticaba peel and showed lower values, 45.38 μg mL^{-1} and 62.60 mmol TE kg^{-1} fresh weight, respectively, than those presented in this study. Otherwise, Alezandro, Dubé, Desjardins, Lajolo, & Genovese (2013a) and Alezandro, Granato, & Genovese (2013b) showed higher DPPH value (600.00 TE 100 g^{-1}). FRAP assay carried out by Alezandro, Dubé, Desjardins, Lajolo, & Genovese (2013a), Alezandro, Granato, & Genovese (2013b), and ABST by Leite-Legatti et al. (2012) showed 68% and 51% lower values than those showed in this study. However, ORAC values obtained by Leite-Legatti, Batista, Dragano, et al. (2012) and Batista, Lenquiste, & Cazarin, et al. (2014) were higher when compared to the present results — 25,514.24 μM TE g^{-1} and 519.11 μM TE g^{-1}, respectively.

The bioactive compounds in plants, as well as their antioxidant capacity and biological effects, could be an arduous work due to the large content and composition variation that depends of geography, photoperiod and climate. These differences have been reported as influence factors on secondary metabolites biosynthesis such as many flavonoids. Thus, it can affect antioxidant potential range (Jaakola & Hohtola, 2010; Kumazawa, Hamasaka, & Nakayama, 2004).

3.3. Antioxidant capacity in vivo

3.3.1. Weight gain and intake parameters

Weight gain, food intake, energy intake and FJP aqueous extract (JE) intake was showed in Table 4. Daily and cumulative weight gains were higher in the HFF group compared to AIN-93 M. Supplemented groups had lower weight gain than the HFF group. Thus, the HFF diet was able to induce obesity in animals when compared to the AIN-93 M group. Furthermore, FJP and JE supplemented groups were able to counteract this process. Food intake was higher in the AIN-93 M groups than in the HFF group, however energy intake did not differ among groups. There was no difference in JE consumption in P. JE and T. JE groups when compared to water consumption of HFF group.

Studies have been evaluated the effects of purified anthocyanins and fruits rich in these compounds supplementation in high-fat diet on the development of obesity. Jayaprakasam, Olson, Schutzki, Tai, & Nair

Table 4
Weight gain, food and tea intake.

	AIN-93 M	HFF	P. FJP	T. FJP	P. JE	T. JE
Daily weight gain (g rat day^{-1})	$2.65^{B} \pm 0.16$	$3.51^{Aa} \pm 0.40$	$3.05^{b} \pm 0.51$	$3.09^{b} \pm 0.16$	$2.77^{b} \pm 0.27$	$2.65^{b} \pm 0.37$
Cumulative weight gain (g rat^{-1})	$226.30^{B} \pm 15.94$	$307.90^{Aa} \pm 25.81$	$250.41^{b} \pm 40.38$	$259.42^{b} \pm 13.68$	$233.30^{b} \pm 23.07$	$235.40^{b} \pm 31.83$
Food intake (g rat day^{-1})	$23.81^{A} \pm 1.28$	$17.49^{Ba} \pm 1.22$	$17.54^{a} \pm 1.12$	$17.63^{a} \pm 1.49$	$16.60^{a} \pm 1.35$	$16.14^{a} \pm 1.10$
Energy intake (Kcal rat day^{-1})	$91.94^{B} \pm 4.31$	$95.71^{Aa} \pm 4.02$	$97.20^{a} \pm 8.18$	$96.10^{a} \pm 5.56$	$91.15^{a} \pm 7.39$	$89.98^{a} \pm 4.71$
Water or JE intake (mL rat day^{-1})	–	$18.18^{a} \pm 2.90$	–	–	$17.98^{a} \pm 2.68$	$15.97^{a} \pm 1.38$

Data presented as means ± standard deviation. Statistical differences between AIN-93 M and HFF groups are represented by different uppercase letters and statistical differences between HFF and supplemented groups are represented by different lowercase letters. HFF group had the water consumption measured to order to serve as a control for P. JE and T. JE groups. AIN-93 M (normal control) standard diet; HFF (obese control) received a high-fat and fructose diet containing 4% (w/w) soybeanoil, 31% (w/w) lard and 20% fructose (w/w); P. FJP and T. FJP received the HFF diet with 2% of FJP powder (w/w), for 12 and 6 weeks respectivelly; P. JE and T. JE received the HFF and had the water substituted by FJP aqueous extract, for 12 and 6 weeks respectivelly.

(2006) and Prior, Wu, Gu, Hager, Hager, & Howard (2008) showed that the supplementation of different sources of cyanidin-3-*O*-glucoside (C3G) extracted in a high-fat diets reduced mice weight gain. However, the consumption of freeze-dried foods as a source of anthocyanins added to high-fat diet has not shown effects on weight gain neither in body fat in animal models (Batista, Lenquiste, Cazarin, et al., 2014; DeFuria, Bennett, Strissel, et al., 2009; Dragano, Marques, Cintra, et al., 2013; Lenquiste, Batista, Marineli, et al., 2012; Prior, Wilkes, Rogers, Khanal, Wu, & Howard, 2010).

The effects of phenolic compounds on obesity have been studied and its effect on weight gain and body mass are still divergent. Nevertheless, acceptable mechanisms for antiobesogenic potential of these compounds have been proposed. Alezandro, Granato, & Genovese (2013b) demonstrated significant inhibitory activity of α-amylase and α-glucosidase enzymes in induced diabetic rats fed with jaboticaba peel. Sergent, Vanderstraeten, Winand, Beguin, & Schneider (2012) showed that phenolic compounds of green tea inhibited pancreatic lipase activity which delayed or decreased *in vivo* fatty acid absorption by enterocytes. Thus, the enzymatic inhibition can be a mechanism in the obesity regulation. In recent study, Dragano, Marques, Cintra, et al. (2013) reported that despite the consumption of FJP added to the high-fat did not effected on mice weight gain, insulin resistance and modulated proteins associated with obesity, inflammation and diabetes were improved.

3.3.2. Antioxidant potential and lipid peroxidation

Bioactive compounds have been studied due to their effects on preventing damage caused by ROS and free radicals which are widely produced during metabolic processes. In order to reduce oxidative stress, the organisms have developed mechanisms. These mechanisms include the non-enzymatic and enzymatic antioxidant defenses produced in endogenously and others provided by diet (exogenous) (Han, Shen, & Lou, 2007).

There were no difference in plasma and liver FRAP assay (Fig. 2A, B). Leite, Malta, Riccio, Eberlin, Pastore, & Marostica (2011) evaluated plasma antioxidant capacity by ABTS assay after FJP diet supplementation. They reported that the addition of 1 and 2% de FJP powder in a normolipidic diet increased the antioxidant potential. However at 4% FJP powder diet it showed an inverse effect. Additionally, Batista, Lenquiste, Cazarin, et al. (2014) evaluated the antioxidant potential in several organs of rats which consumed HF diet supplemented with FJP at 1, 2 and 4%. These authors observed that in plasma samples TEAC assay showed higher values in animals fed with 2 and 4% of FJP. Nevertheless, ORAC and FRAP assay did not differ among groups. In liver samples, they showed that ORAC assay was higher in the FJP supplemented groups compared to HF group, but TEAC and FRAP assay did not differ among groups. FRAP assay was used by Alezandro, Granato, & Genovese (2013b) to evaluated oxidative stress in streptozotocin-mediated diabetic rats. In this study, plasma antioxidant capacity of diabetic rats was increased (2 to 2.5 times) after both jaboticaba doses (1.0 and 2.0 g dry weight kg^{-1} body weight) supplementation for 40 days.

Lipid peroxidation in plasma and liver was measured by TBARS assay. HFF group showed higher MDA level in plasma and liver than AIN-93 M, showing increased lipid peroxidation. This damaging process was reversed by FJP and JE supplementation (Fig. 2C, D). Wu, Ma, Li, Deng, Yin, & Huang (2015) evaluated serum MDA levels in mice fed with normal or three types of flavonoids extracts in supplemented diets. They observed that the extracts groups had lower MDA level than the control groups. Several studies has also shown positive effects of foods rich in phenolic compounds on lipid peroxidation (Alezandro, Granato, & Genovese, 2013b; Batista, Lenquiste, Cazarin, et al., 2014; Silva, Souza, Thomazini, et al., 2014). Thus, MDA levels are an important marker of lipid peroxidation and can be modulated by phenolic compounds intake.

3.3.3. Thiol group content and antioxidant enzymes

The increased oxidative stress is related to an overproduction of free radicals or deficiency in the antioxidant defense system (Habib & Ibrahim, 2011). Plasma GPx and GR did not differ among groups (Fig. 3A, B). However, plasma GSH level was significantly lower in HFF group compared to the AIN-93 M group. FJP and JE supplemented

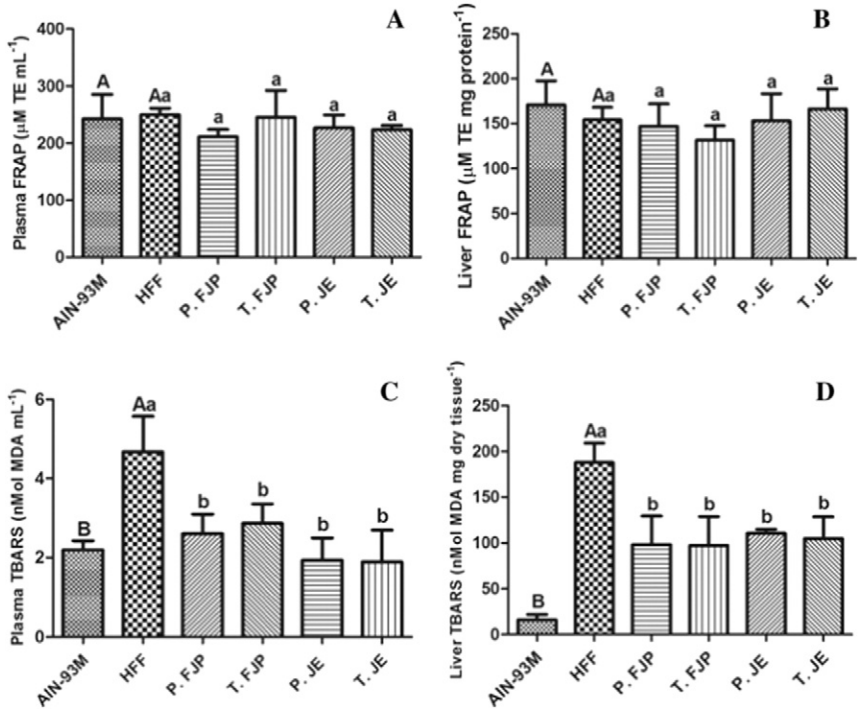

Fig. 2. FRAP and TBARS levels in plasma and liver of experimental animals. A — plasma FRAP; B — liver FRAP; C — plasma TBARS; D — liver TBARS. Data presented as means ± standard deviation. Statistical differences between AIN-93 M and HFF groups are represented by different uppercase letters and statistical differences between HFF and supplemented groups are represented by different lowercase letters.

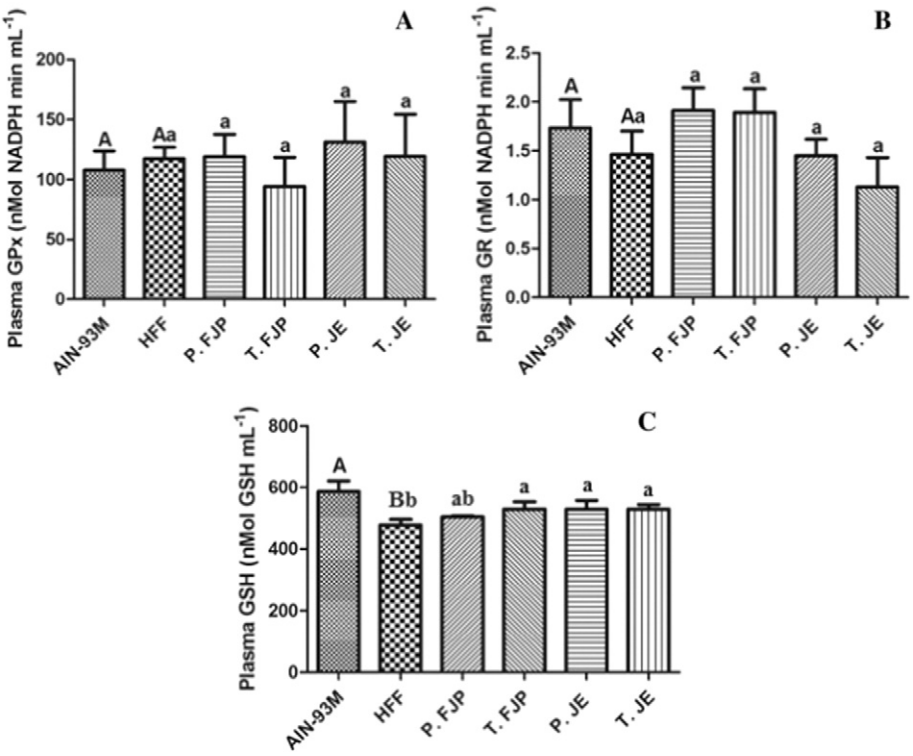

Fig. 3. Glutathione peroxidase (GPx) and glutathione reductase (GR) enzymes and reduced glutathione (GSH) in plasma of experimental animals. A — GPx enzyme; B — GR enzyme; C — GSH. Data presented as means ± standard deviation. Statistical differences between AIN-93 M and HFF groups are represented by different uppercase letters and statistical differences between HFF and supplemented groups are represented by different lowercase letters.

groups showed an increase in this biomarker compared to HFF group (Fig. 3C). In liver, GPx level was higher in AIN-93 M group compared to HFF group and there was no difference among HFF and supplemented groups (Fig. 4A). Liver GR level was lower in AIN-93 M group compared to HFF group. Supplements FJP and JE did not reduce the GR level compared to HFF group (Fig. 4B). Liver GSH level was lower in HFF group

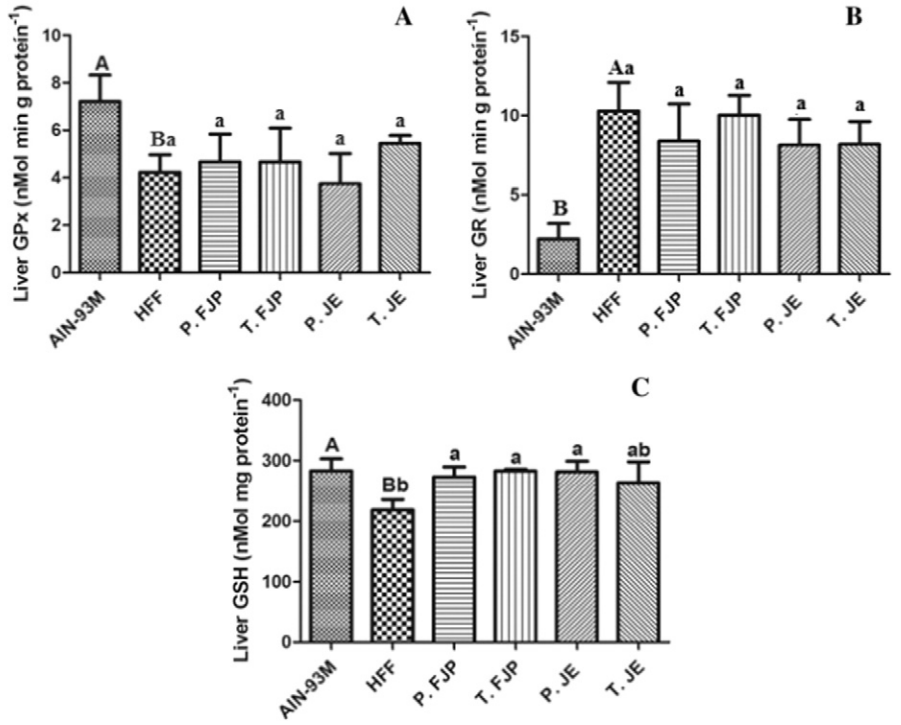

Fig. 4. Glutathione peroxidase (GPx) and glutathione reductase (GR) enzymes and reduced glutathione (GSH) in liver of experimental animals. A — GPx enzyme; B — GR enzyme; C — GSH. Data presented as means ± standard deviation. Statistical differences between AIN-93 M and HFF groups are represented by different uppercase letters and statistical differences between HFF and supplemented groups are represented by different lowercase letters.

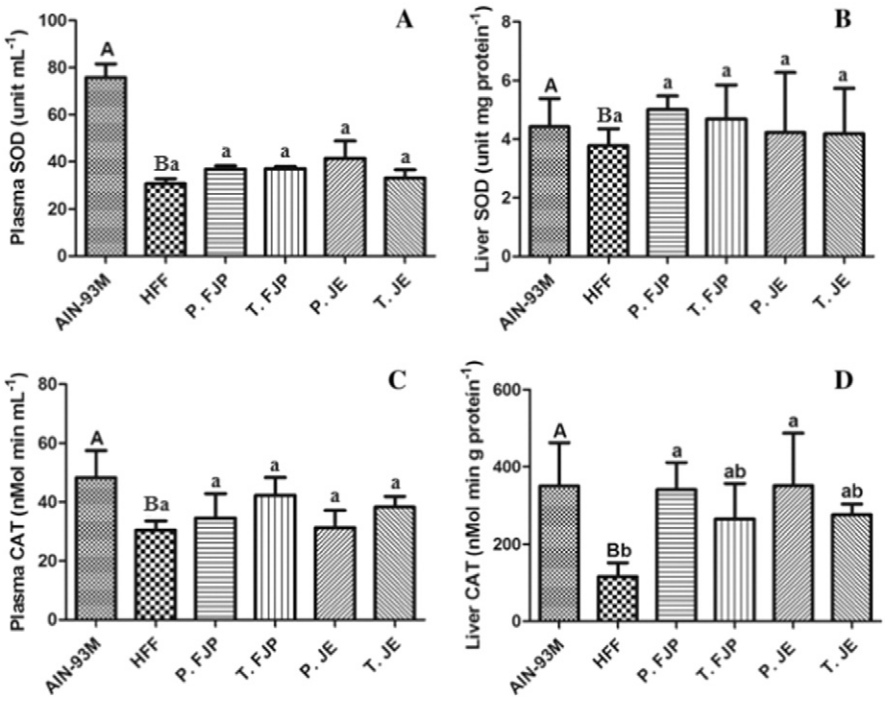

Fig. 5. Superoxide dismutase (SOD) and Catalase (CAT) activities in plasma and liver of experimental animals. A — plasma SOD; B — liver SOD; C — plasma CAT; D — liver CAT. Data presented as means ± standard deviation. Statistical differences between AIN-93 M and HFF groups are represented by different uppercase letters and statistical differences between HFF and supplemented groups are represented by different lowercase letters.

compared to AIN-93 M. FJP and JE groups showed an increase in this biomarker compared to HFF group (Fig. 4C). Thus, only plasma and liver GSH levels were positively modulated by FJP and JE intake.

Plasma and liver SOD activity was significantly lower in HFF group compared to AIN-93 M group. There was no significant difference in plasma and liver SOD activities among HFF and supplemented groups (Fig. 5A, B). There was no plasma significant difference in CAT activity among groups (Fig. 5C). Liver CAT level was lower in HFF group than in AIN-93 M group. The P. FJP and P. JE groups showed higher CAT activity than HFF group, whereas T. FJP and T. JE groups showed an increase in CAT activity. However, these values were not statistically different compared to HFF group (Fig. 5D). Thus, FJP and JE supplementation for 12 weeks increased CAT levels in animal's liver.

Glutathione is the main source of reducing power. It is maintained in reduced form of GR, which acts with NADPH. Therefore, increasing or maintaining of total GSH level added up an improvement of GR level could be an important indicator of antioxidant system enhancement (Skrzydlewska, Ostrowska, Farbiszewski, & Michalak, 2002). Wu, Ma, Li, Deng, Yin, & Huang (2015) showed an increasing in GPx and SOD activity in mice plasma supplemented with extract rich in flavonoids. Antioxidant enzymes were measured in plasma and liver of diet-induced obese rats after FJP supplementation. It was shown that the FJP promoted an increase in plasma SOD and CAT, as well as liver SOD, CAT and GPx levels (Batista, Lenquiste, Cazarin, et al., 2014). Similar results were obtained by Alezandro, Granato, & Genovese (2013b). They showed an increase of antioxidant enzymes in plasma, kidneys, brain and liver in diabetic rats supplemented with FJP extract by gavage.

In vivo studies have been evaluated the supplementation effects of foods rich in phenolics compounds on antioxidant enzymes activity (Han, Matsumoto, Shimada, Sekikawa, & Fukushima, 2007; Han, Shen, & Lou, 2007; Suwannaphet, Meeprom, Yibchok-Anun, & Adisakwattana, 2010; Tedesco, Luigi, Nazzaro, Russo, & Palumbo, 2001). Scientific evidence has shown that these compounds, especially anthocyanins, can have a powerful antioxidant effect, *in vitro* and *in vivo* studies (Alezandro, Granato, & Genovese, 2013b; Batista, Lenquiste, Cazarin, et al., 2014; Habib & Ibrahim, 2011). However, the results are still inconsistent and studies focused in possible mechanisms of action are needed.

4. Conclusion

Jaboticaba peel is a good source of bioactive compounds. FJP could have an excellent application as a food additive. Furthermore, FJP aqueous extract can be consumed as a tea which could provide an alternative bioactive compound source for human consumption. FJP and FJP aqueous extract had a higher antioxidant potential *in vitro*. The *in vivo* antioxidant potential of FJP and JE was confirmed by remarkable biomarkers, such as TBARS and GSH levels and CAT activity, but did not by FRAP assay, and SOD, GR and GPx enzymes. Thus, further studies are necessary in order to confirm the *in vivo* effects of the FJP and JE.

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