

## Full Length Research Paper

## Effects of jaboticaba (*Myrciaria jaboticaba*) peel on blood glucose and cholesterol levels in healthy rats

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Received 2 July, 2014; Accepted 2 September, 2014

The jaboticaba (*Myrciaria jaboticaba* (Vell.) Berg) peel was lyophilized and the proximate composition, total anthocyanins and polyphenolic content were determined. The effect of the freeze-dried jaboticaba peels (FJP) in the plasmatic levels of glucose, lipid fractions, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in *Wistar* adult male rats was investigated. The animals were distributed in four groups G0 (control), G1, G2, G4, which received 0, 1, 2 and 4% FJP powder added to normal diet, respectively. The chemical analyses showed that FJP is a source of fiber, anthocyanins, gallic and ellagic acids. The FJP supplementation was responsible for a reduction in the plasmatic glucose levels in G2 group. Total triglycerides and cholesterol levels were reduced in the G1 animals as compared to G0, but the animals treated with 2 and 4% FJP showed increased total cholesterol level and LDL-cholesterol fraction. HDL-cholesterol and hepatic probes showed no significant changes in the experimental groups G1, G2 and G4 in comparison to G0.

**Key words:** Polyphenols, glucose and cholesterol levels, Jaboticaba, *Myrciaria jaboticaba* (Vell.) Berg.

### INTRODUCTION

The consumption of exotic fruits and their byproducts has been strongly associated with reduced risk for developing chronic diseases such as obesity, cardiovascular diseases, type 2 diabetes, insulin resistance, neurodegenerative diseases, cancer and others (Dragano et al., 2013; Lenquiste et al., 2012; Leite-Legatti et al., 2012; Papandreou et al., 2009). There are strong evidences that these properties are related to phytochemicals that may be able to combat the formation

of free radicals and the increased oxidative stress. Moreover, these compounds present in seeds, peels and pulps of these fruits have anti-inflammatory, hypoglycemic and hypolipidemic effects (Dragano et al., 2013; dos Santos et al., 2010; Esteves et al., 2011).

The berries are highlighted in this context, because they are rich in polyphenols and have significant amounts of fiber (Kim et al., 2010). These compounds purified or within the whole food, seem to promote beneficial effects

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**Abbreviations:** AIN-93M, American Institute of Nutrition 93- adult maintenance diet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BHT, 2,6-di-tert-butyl-4-methylphenol; FJP, freeze-dried jaboticaba peels; HDL, high-density lipoprotein; HPLC-DAD, high performance liquid chromatography - diode array detector; LDL, low-density lipoprotein; ORAC, oxygen radical absorbance capacity; TEAC, trolox equivalents antioxidant capacity.

to the body, both in the treatment and prevention of chronic diseases (Tsuda et al., 2003; Guo et al., 2012).

Studies have shown that diets rich in polyphenols can attenuate the development of type 2 diabetes, since they could protect the pancreas against oxidative stress, preserve  $\beta$  pancreatic cells, increase the excretion of insulin by these and inhibit the glucose absorption from intestine (Lenquist et al., 2012; Jayaprakasam et al., 2006). Besides phenolic compounds, the dietary fibers also exhibit an important role in reducing blood glucose (Esteves et al., 2011; Kim et al., 2010).

The bioactive compounds from products of blueberries are related to the reduction of cholesterol and bile acids hepatic synthesis, increasing fecal excretion of lipids and thereby reducing plasma cholesterol levels (Kim et al., 2010). Pigs fed with a diet containing cereals/grains supplemented with fiber source and 1.5% blueberries resulted in reduced total cholesterol (Kalt et al., 2008). Thus, it is possible that consumption of foods containing a combination of phenolic compounds and fibers provide improvements of glycemic and lipids control *in vivo*.

Jaboticaba is a tropical wild berry from Southeastern Brazil. *Myrciaria jaboticaba* (Vell.) Berg. and *Myrciaria caulifolia* (DC) Berg are the varieties more suitable for 'in natura' consumption, as well as for food industry applications (Pinto et al., 2011). The berry diameter is about 3-4 cm; it has one to four seeds inside, and the peel is very purple. The pulp is white and sweet. Normally, the peel is not consumed and discarded. However, recent researches have been performed with the purpose of adding value to the use of this byproduct by industry (Pinto et al., 2011).

The polyphenols from *M. jaboticaba* were not extensively explored. However, some bioactive compounds were already described: anthocyanins, mainly cyanidin and delphinidin 3-glucoside; ellagitannins, ellagic acid, vitamin C, limonene, terpenes, dietary fibers and others (Leite-Legatti et al., 2012; Abe et al., 2012; Lima et al., 2011; Alezandro et al., 2013). In our studies, 1 and 2% of FJP in diets increased systemic antioxidant status. Antioxidant assays performed in the plasma of animals increased 70 and 30% of antioxidant activity by ORAC and TEAC (Leite et al., 2011).

We hypothesized that the antioxidant capacity of plasma from rats fed FJP could contribute to the improvement of glycemia and lipid profile control. The aim of this study was to investigate the chemical composition and the effects of the addition of a jaboticaba byproduct to the diet of adult Wistar rats concerning biochemical parameters.

## MATERIALS AND METHODS

### Preparation and chemical composition of the freeze-fried jaboticaba peels

Jaboticaba fruits (*M. jaboticaba* (Vell.) Berg) were bought on a local market in Campinas in September, 2008. The fruits were washed,

manually peeled and the peels were frozen at  $-18^{\circ}\text{C}$ . The peels were then lyophilized in a freeze-dryer (Liobras, Brazil) at  $30^{\circ}\text{C}$ , 300  $\mu\text{m Hg}$  for 95 h. The freeze-dried product (FJP powder) was stored at  $-80^{\circ}\text{C}$ .

### Proximate composition

The contents of protein (Kjeldahl method), moisture, ash, lipids, fiber (acid hydrolysis) and total sugars of freeze-dried jaboticaba peels were determined (AOAC, 1995; Bligh and Dyer, 1959; Lane and Eynon, 1923; IAL, 1985).

### Extraction of bioactive compounds

The FJP powder was weighed into a centrifuge tube (250 mg) and extracted with 15 mL of ethanol/water (60:40, v v<sup>-1</sup>). The sample was shaken in a vortex for 30 s and then allowed in water bath at  $70^{\circ}\text{C}$  for 1 h, under agitation for each 15 min. The extract was filtered and allowed in an amber bottle. A second extraction was performed in the residue with 10 mL ethanol/water using the same procedure, and the supernatants combined and used to the following analysis (Colomeu et al., 2014). Each analysis was performed in triplicate.

### Folin-Ciocalteu reagent reducing substances (FCRRS)

The Folin-Ciocalteu method was used to determine total polyphenols contents and was defined as FCRRS, since it is known to be affected by several interfering substances (Silva et al., 2013; O'Brien, et al., 2013). The total phenolic content was determined by using the adapted Folin-Ciocalteu method (Swain and Hillis, 1959). Water, Folin-Ciocalteu reagent and sodium carbonate were added to the extract or standard solutions, and after 2 h in the dark at room temperature the absorbance of samples and standard curve was read at 725 nm. The measurements were done using 96-well microplate and a microplate reader (Sinergy HT, Biotek, Winooski - USA). The results were expressed as gallic acid equivalents (GAE  $\text{mg g}^{-1}$  FJP).

### Total anthocyanins analysis

The total anthocyanins were also quantified in the ethanol extract according to the pH-differential method described by Wrolstad (Wrolstad, 1993).

### Oxygen radical absorbance capacity (ORAC) assay

The ORAC test (Davalos et al., 2004) was carried out adding 20  $\mu\text{L}$  of samples extract or standard solutions, 120  $\mu\text{L}$  of fluorescein diluted in potassium phosphate buffer (pH 7.4), and 60  $\mu\text{L}$  of AAPH (2,2'-azobis(2-methylpropionamide) dihydrochloride) to black microplates, in the dark. Trolox (( $\pm$ )-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was used as standard and the microplate reader (Synergy HT, Biotek, Winooski, USA) was set using fluorescent filters: excitation wavelength, 485 nm; emission wavelength, 520 nm. ORAC values were expressed in  $\mu\text{mol trolox equivalent} (\mu\text{mol TE})$  per gram of FJP by using the standard curves (2.5 - 80.0  $\mu\text{mol TE L}^{-1}$ ) for each assay. The fluorescence readings were used to the appropriate calculations.

### HPLC-DAD analysis

For chromatographic analysis, 1 g of FJP was extracted in duplicate with 25 mL of 80% methanol at  $37^{\circ}\text{C}$  for 3 h in a shaking water bath

bath. The analysis of the phenolic compounds from the methanol extracts was carried out in a high performance liquid chromatography (HPLC) (Agilent 1100), with manual injection (three injections each extract), 20  $\mu\text{L}$  sample loop and ternary pump, coupled to a diode array detector (DAD) (Agilent G1315B), at room temperature. The data was obtained and processed using the software ChemStation (Hewlett Packard, Germany). A reverse phase chromatographic column (C18 Eclips ODS (250 mm  $\times$  4.5 mm, Agilent) was used. The mobile phase was 1% orthophosphoric acid in water (v.v<sup>-1</sup>) (A) and acetonitrile (B). The elution gradient started at 95: 05 (A: B) at 0.7 mL min<sup>-1</sup>. This condition was maintained for 5 min and the concentration of A was decreased so that at 25 min it reached 60:40 (A:B) followed by a linear increase of solvent A to 95% until 35 min. The detection was done at 210, 254, 280, 300 and 340 nm, which allowed the simultaneous quantification and the tentative identification of the phenolic compounds separated by the HPLC. The comparison parameters were elution time, spectra of absorption and sample fortification. The identification was carried out using the chromatograms obtained at each injection and compared with the absorption spectra and retention time of the standards. Co-chromatography was performed to confirm the identity of the compounds. The concentrations of the identified compounds were calculated from the analytical curves obtained using commercial products (ellagic acid, quercetin and gallic acid from Sigma, St Louis, MO, USA) under identical chromatographic conditions (Port's et al., 2013).

### ***In vivo* experiment**

The *in vivo* assay was performed with 32 Wistar adult rats (individual weight of approximately 250 g), from Unicamp Biotherium Center (CEMIB), divided into four groups with eight animals each. The animals were kept in individual cages and received AIN-93M diet (Reeves et al., 1993) added with FJP.

The amount of proteins was reduced to 12% and the amount of sucrose in the diets supplemented with FJP was modified to make them isocaloric. The ingredients used and distributed equally among the diets were: starch (46.57%); dextrin (15.5%); casein (14.2%); cellulose (5.0%); soy oil (4.0%); mineral mix (3.5%); vitamins mix (1.0%); L-cystine (0.18%); choline substrate (0.25%); and BHT (0.0008%). The groups denominated G0, G1, G2 and G3 received diets added by 0, 1, 2, and 4% FJP powder (described above) in diet, respectively and the amount of sucrose was adjusted to 10, 9.31, 8.62 and 7.24% for the same groups.

The *in vivo* experiment was carried during 28 days. The animals were kept under controlled temperature (22  $\pm$  2°C) during the whole experiment, with alternated periods of 12 h in light and dark. All animals were weighed at every two days and the diet consumed was monitored. At the end of the feeding period, the animals were fasted (12 h), killed by decapitation, the blood was collected in heparin-coated tubes and the plasma was obtained by centrifugation. The livers were carefully removed, rinsed with saline solution and weighted. The plasma samples were stored at -80°C.

The experiment was performed according to ethical principles for experiments with animals adopted by the Brazilian College for Animal Experimentation (COBEA) and it was previously approved by the Committee for Ethics in Animal Research - CEUA/ Unicamp, University of Campinas, Brazil (#1627-1).

### **Blood analysis**

Plasma lipids and glycemia were determined by using colorimetric enzymatic methods that employed the commercial kits Laborlab for quantifying blood glucose (CAS #02200), triglycerides (CAS #02700), total cholesterol (CAS #01400), HDL-cholesterol (CAS

#08900), ALT (#00200) and AST (#00300), and Wienerlab (São Paulo, Brazil) for LDL-cholesterol (CAS #1220104) were determined in a spectrophotometer (Beckman®, DU640, Corona, USA).

### **Statistics**

Shapiro Wilk was used as normality test. ANOVA and Tukey test were carried out. A 5% significance level was used and GraphPad Prism 5.0 (GraphPad Software, Inc. La Jolla, CA, USA) was used as software.

## **RESULTS**

The FJP powder is constituted mostly of sugars, fibers, polyphenols and anthocyanins with significant antioxidant activity (Table 1). A linear trend line showed a correlation between the total antioxidant activity and the anthocyanins values ( $r^2 = 0.9744$ ;  $r = -0.9871$ ;  $P = 0.1023$ ;  $y = 906.03 - 0.1789x$ ). The chromatographic analysis showed that FJP possessed four identified compounds: ellagic and gallic acid, cyanidin 3-glucoside and quercetin (Table 1, Figure 1).

There was no statistical difference in food intake among G0, G1, G2, and G3 rats (data ranging from 21.2  $\pm$  1.4 to 22.8  $\pm$  1.1 g per day). The intake of jaboticaba peel by the experimental groups showed a dependent-dose response (214, 456 and 884 mg day<sup>-1</sup> or 98.43, 130.29 and 252.57 mg 100g<sup>-1</sup> day<sup>-1</sup> for G1, G2 and G3, respectively). As expected, the results showed no differences in the weight gain among animals from groups G0 and G1, G2 and G3. The final body weight was similar among the groups with means ranging from 356.9  $\pm$  27.74 to 356.9  $\pm$  22.96. The weight of livers from G1 rats (2.81  $\pm$  0.12 g%) were smaller than the G0 group (3.31  $\pm$  0.25 g%).

The reduction of the blood glucose in group G2 was significant when compared to the control (G0). According to these results (Figure 2A), the addition of 2% FJP to the diet of rats decreased 19.8% in plasma glucose in comparison to G0 (117.4 mg dL<sup>-1</sup> in G2 and 94.15 mg dL<sup>-1</sup> in G0).

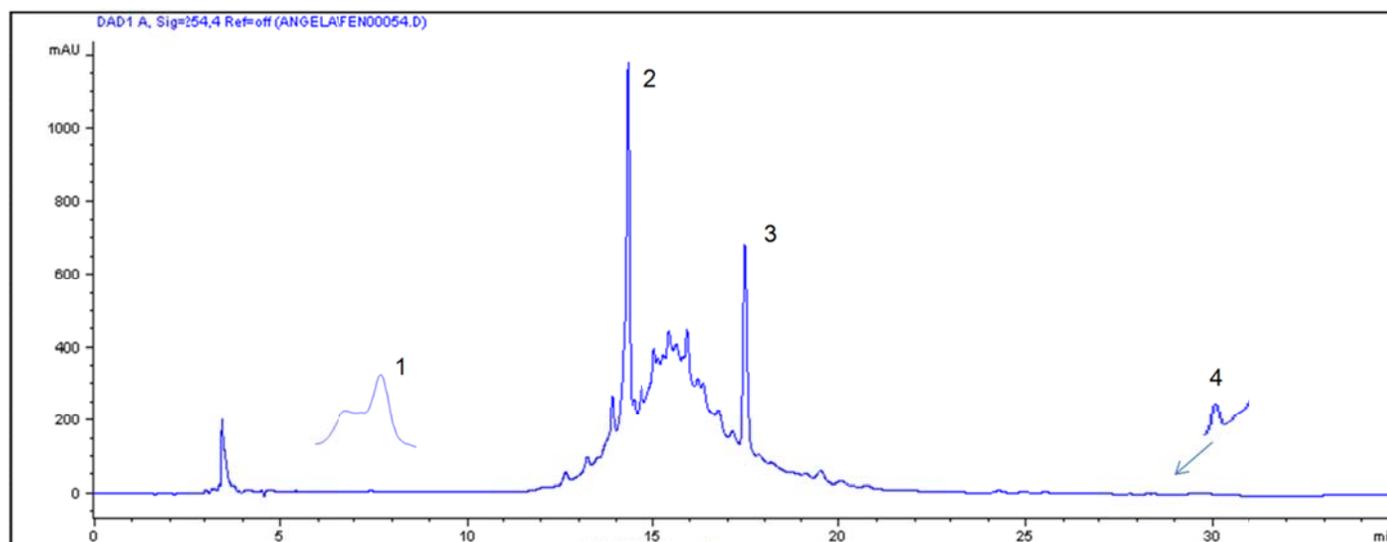
The G1 group showed a reduction in total cholesterol and triglycerides levels (Figure 2B and C). The intake of 1% FJP caused a significant decrease (70.75%) in the total cholesterol levels of animals in comparison to G0. However, the trend was not linear: cholesterol level increased 61.94% in G3 compared to control group, reaching 47.37  $\pm$  6.28 mg dL<sup>-1</sup>, which was an unexpected result, and could indicate that jaboticaba peels, when consumed in excess, may generate undesirable effects. A similar effect was showed in the LDL-cholesterol analysis (Figure 2D). Although, no change was observed in the levels of HDL of the experimental groups (Figure 2E).

There was no statistical difference in the ALT activity among the groups (Figure 2F). The AST enzyme

**Table 1.** Proximate composition and polyphenol compounds of the freeze-dried jaboticaba peel.

| Component                      | Content (%) | SD <sup>a</sup> |
|--------------------------------|-------------|-----------------|
| Moisture                       | 22.72       | 0.23            |
| Lipids                         | 1.27        | 0.07            |
| Ash                            | 3.01        | 0.07            |
| Crude protein                  | 3.90        | 0.12            |
| Crude fiber                    | 6.45        | 0.28            |
| Total sugars <sup>b</sup>      | 59.04       | 0.99            |
| Reducing sugars                | 40.95       | 0.57            |
| Sucrose                        | 18.09       | 0.01            |
| Gallic acid (mg/ 100 g)        | 3.87        | 0.15            |
| Ellagic acid (mg/ 100 g)       | 362.43      | 3.67            |
| Quercetin (mg/ 100 g)          | 5.17        | 0.05            |
| Total anthocyanins (mg/ 100 g) | 926.78      | 16.55           |
| FCRRS (mg GAE/g)               | 86.67       | 1.05            |
| ORAC (μmol TE/g)               | 807.00      | 4.83            |

<sup>a</sup>Standard deviation. <sup>b</sup>Total sugars were calculated by the sum of reducing sugars and sucrose.



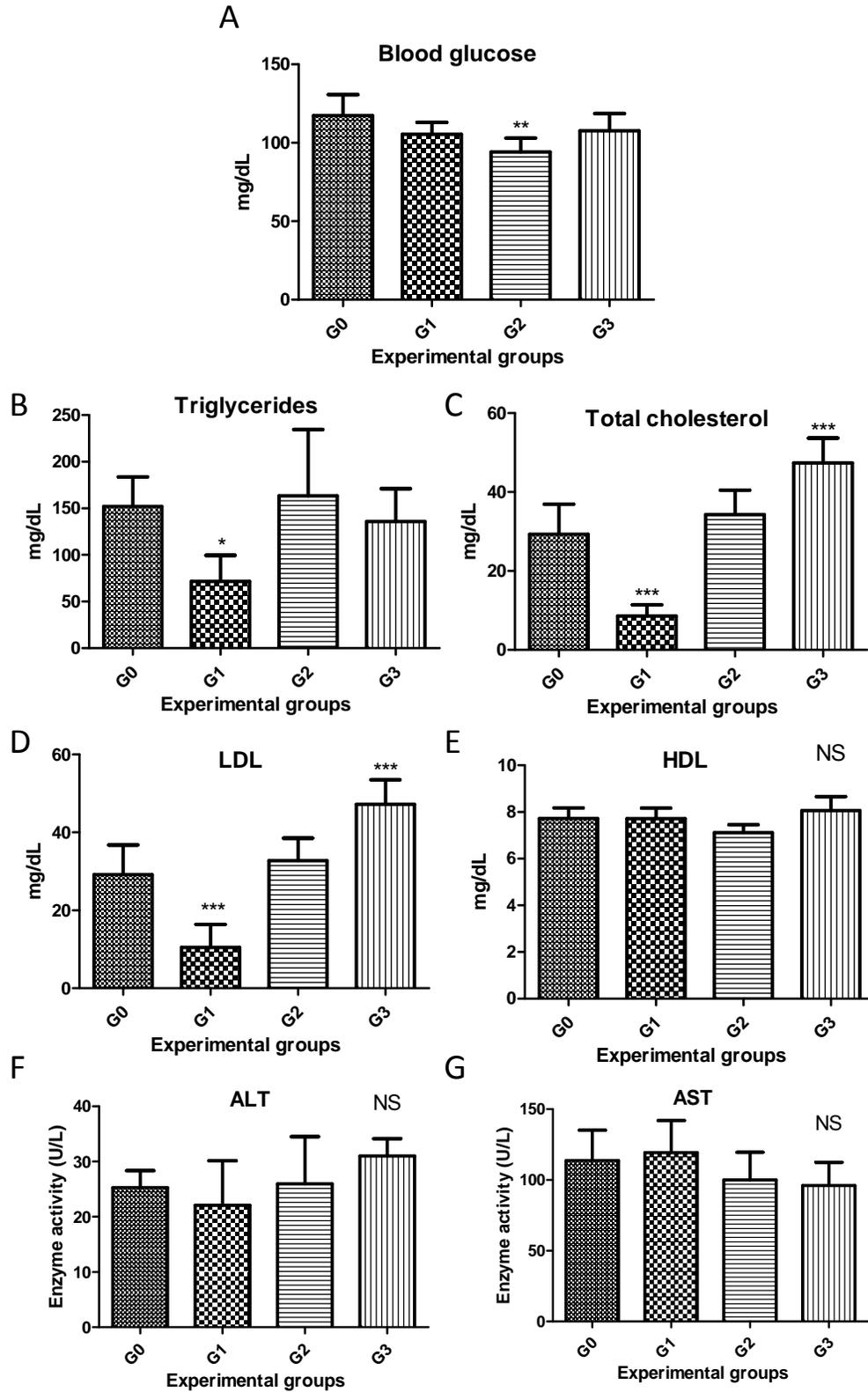
**Figure 1.** Typical chromatogram of 20 mg mL<sup>-1</sup> methanolic extract of freeze-dried jaboticaba peel by HPLC-DAD (254 nm). Compounds: 1) gallic acid; 2) cyanidin 3-glucoside; 3) ellagic acid; 4) quercetin.

presented a slight tendency for activity reduction as FJP level increases, with no significance ( $P > 0.05$ ) (Figure 2G).

## DISCUSSION

Jaboticaba peels have not been largely used by the food industry in commercial products. Although, the interest in this byproduct have increased since several bioactive

compounds were described by some authors (Pinto et al., 2011; Wu et al., 2013). The freeze-dried jaboticaba peel is a rich source of polyphenols, mainly flavonoids, anthocyanins and dietary fibers (Leite et al., 2011). Corroborating this study, ellagic acid, gallic acid, tannins, quercetin derivatives and anthocyanins were also identified by other authors (Abe et al., 2012; Alezandro et al., 2013). The polyphenols of FJP might be responsible for its antioxidant, antimutagenic, anti-inflammatory and antitumoral activities (Dragano et al., 2013; Leite-Legatti



**Figure 2.** Plasmatic glucose levels (A), total triglycerides (B), cholesterol (C), LDL-cholesterol (D), HDL-cholesterol (E) and plasmatic ALT (F) and AST enzymes (G) of freeze-dried jaboticaba peeled *Wistar* rats. The groups G0, G1, G2 and G4 received 0.00, 0.01, 0.02 and 0.04 g jaboticaba peel  $\text{kg}^{-1}$  in diet, respectively. Data were expressed as the mean  $\pm$  SD values ( $n=8$ ). The statistical significance among the groups was evaluated using ANOVA and Tukey test (\* $P<0.05$ ; \*\* $P<0.01$ ; and \*\*\* $P<0.001$  when compared to G0). NS= non-significant.

et al., 2012; Alezandro et al., 2013).

Polyphenol-rich fruits, as jaboticaba, is strongly effective in increasing insulin secretion. This could explain the plasma glucose reduction found in the present study, possibly caused by the increase in insulin secretion in FJP-fed animals (Lenquiste et al., 2012). The fibers from FJP (Table 1) could also be responsible to enhance hypoglycemic effect, acting in synergy with polyphenols from the peel (Kim et al., 2010). In addition, studies have shown that the intake of jaboticaba peel is related to an increased insulin sensitivity in liver and adipose tissue (Dragano et al., 2013; Lenquiste et al., 2012). Studies with polyphenol and fiber-rich meals corroborate this result: reduction in plasma glucose levels by 13.04% (Esteves et al., 2011).

The intake of 1% FJP caused a significant decrease (48.69%) in the total cholesterol and triglycerides levels of G1 animals. Although, the rats that consumed 4% of FJP showed an unexpected increase in total cholesterol, suggesting that there is no dose-response trend. However, other studies using different animal models, but the same doses of FJP did not confirm this data (Lenquiste et al., 2012). Corroborating this study, Alezandro et al. (2013) showed that daily 1 and 2g kg<sup>-1</sup> doses of aqueous extract of jaboticaba peel were responsible for plasma triglycerides and cholesterol lowering levels.

In another way, as in other studies, no significant differences in HDL-cholesterol levels were found in the experimental groups in comparison to the control (Alezandro et al., 2013). Kwon et al. evaluated the effect of black soy anthocyanins addition on an hyperlipidic diet of rats and observed 23 and 20% reduction in triglycerides and cholesterol levels, respectively, and an increase of 37% in the HDL-cholesterol fraction (Kwon, et al., 2007). In contrast to the present, a recent study showed that FJP could enhance HDL-cholesterol in obese rats (Lenquiste et al., 2012).

Like polyphenols, the fibers of jaboticaba may have also played the hypocholesterolemic role. The polyphenol and fiber-rich diets was mainly related to an up-regulation of CYP7A1 expression, suggesting an increase in the conversion of hepatic cholesterol to bile acid, resulting in a decrease in the plasma cholesterol levels (Kim et al., 2010; Villanueva et al., 2011). The intake of these diets also indicate a decreased hepatic cholesterol synthesis by down-regulation of CYP 51, and higher fecal lipid excretion, corroborating the plasma cholesterol-lowering effect (Kim et al., 2010).

Kalt et al. verified that pigs fed with rich fibers diet supplemented with 2% freeze-dried blueberries showed a reduction of 11.7% for total cholesterol and 15.1% for LDL-cholesterol (Kalt et al., 2008). In our study, the addition of 1% FJP was sufficient to reduce total cholesterol to 70%. In view of the increase in total cholesterol and LDL-cholesterol fractions in G2 and G3 groups, we may suppose that 2% FJP should represent

an upper limit for dietary intake. Thus, even that other studies showed good results with the same doses (Dragano et al., 2013; Lenquiste et al., 2012), more investigations are necessary in order to elucidate the mechanisms that may cause undesirable effects.

Aminotransferases (ALT and AST) are enzymes present in high levels in the muscle, liver and brain. Increased levels of ALT are therefore relatively specific to hepatobiliary disease, although the AST levels are likely to be higher increased in the diseases of other organs. Increases over 10 times above the upper limit of normal variation normally are considered hepatic or biliary pathologies (Motta, 2003). The slight increase of ALT in the present study is therefore irrelevant and not related to a possible liver damage, since the AST level remained unchanged.

## Conclusions

Freeze-dried jaboticaba peel showed high amounts of fibers and polyphenols, as ellagic, gallic acid and anthocyanins. Significant reductions in the glucose levels were observed in rats fed diets containing 2% FJP. The rats fed 1% FJP also showed reduced levels of total triglycerides, cholesterol and LDL cholesterol. Thus, in the same conditions of this study, we can conclude that FJP do not represent toxicity to the liver, since the ALT and AST activity was similar among the groups. In addition, the 2% FJP in diet is the upper limit dose in which we have observed functional role without any health undesirable effects. Further investigations about higher and lower doses of phenolic compounds in the human diet are needed. It is, therefore, very important to investigate the metabolism of such compounds *in vivo* and their effective dosages.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

The authors are grateful to CNPq (#470648/2010-4), CAPES, FINEP and FAPESP (#2010/05262-6) for their financial support.

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