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Vol. 13(11), pp. 1295-1302, 12 March, 2014
DOI: 10.5897/AJB2013.13589
ISSN 1684-5315
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African Journal of Biotechnology

Full Length Research Paper

Jabuticaba [*Pliniajaboticaba* (Vell.) Berg] skins decrease lipid peroxidation: Hepatoprotective and antihyperlipidemic effects

LAGE Fabíola Fonseca[1*], SIMÃO, Anderson Assaid[1], GUEDES Mayara Neves Santos[1], RAMOS Vinicius de Oliveira[1], SOUSA Raimundo Vicente de[2] and CORRÊA Angelita Duarte[1*]

[1]Chemistry Department, Biochemistry Laboratory, Universidade Federal de Lavras - UFLA, PO Box 3037, Zip Code 37200.000, Lavras, MG, Brazil.
[2]Veterinary Medicine Laboratory, Universidade Federal de Lavras - UFLA, Zip Code 37200.000, Lavras, MG, Brazil.

Received 20 December, 2013; Accepted 25 February, 2014

The effect of Jabuticaba [*Plinia jaboticaba* (Vell.) Berg] Skin Flour (JSF) was studied on peroxidation, plasma and hepatic lipid profiles of female rats, as well as quantification and characterization of phenolic compounds. The animals were divided into four groups of eight rats. The groups received 0 (control); 0.5; 1.5 and 3.0 g JSF per 100 g diet. The diet with 3.0% JSF increased the HDL level by 20.23% compared to the control. The groups that received JSF had lower AST and ALT activities, when compared to the control group. There was a decrease of macro vesicular steatosis in the liver of animals fed the diet supplemented with 3.0% JSF. The diets containing 1.5% and 3.0% JSF reduced lipid peroxidation in the liver by about 50%. JSF was effective in protecting against dyslipidemia, because it increased the serum level of HDL cholesterol, showed a good antioxidant activity and demonstrated hepatoprotective effect.

Key words: *Plinia jaboticaba*, phenolic compounds, HDL cholesterol, antioxidant action, HPLC.

INTRODUCTION

Jabuticaba [*Plinia jaboticaba* (Vell.) *Berg*] is a typical Brazilian fruit that features pleasant sensory characteristics, with its soft, juicy and bittersweet pulp (Danner et al., 2006). When consumed fresh, the skin is discarded as waste. The fruit has high water content and sugars, which makes it highly perishable, with a short life after harvest. Aiming to minimize significant economic losses, several studies have been conducted for a better

*Corresponding authors: Email: fabiolaflage@gmail.com, angelita@dqi.ufla.br. Tel: +55 (35) 38291272; Fax: +55 (35) 38291271

Abbreviations: ABTS, 2,2′-Azinobis-(3-etilbenzotiazolin-6-sulphonic) acid; **ADC,** average daily consumption; **ADG,** average daily weight gain; **ALT,** alanine aminotransferase; **AOAC,** association of official analytical chemists; **AST,** aspartate aminotransferase; **DM,** dry matter; **FER,** feed efficiency ratio; **GGT,** gamma glutamyl transferase; **HDLc,** high-density lipoprotein cholesterol; **HPLC,** high-performance liquid chromatography; **JSF,** jabuticaba skin flour; **LDLc,** low-density lipoprotein cholesterol; **MDA,** malondialdehyde acid; **NFE,** nitrogen-free extract; **TBARS,** thiobarbituric acid reactive substances.

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use of the fruits (Alves et al., 2013; Asquieri et al., 2004). Jabuticaba skin contains bioactive compounds with potential to promote health benefits. Research has shown that they have an antiproliferative effect against leukemia and prostate cancer cells (Leite-Legatti et al., 2012). Jabuticaba Skin Flour (JSF) is rich in soluble and insoluble fiber [27.03 and 6.77 g 100 100 g^{-1} dry matter (DM), respectively] and has a high content of total phenolic compounds (11.99 g 100 g^{-1} DM), including anthocyanins (2.06 g 100 g^{-1} DM), responsible for its characteristic color (Lima et al., 2008, 2011b). High-performance liquid chromatography (HPLC) analyses detected the anthocyanins cyanidin-3-glucoside and delphinidin-3-glucoside in JSF (Leite-Legatti et al., 2012). It presents antioxidant potential, probably due to the high content of phenolic compounds (Lima et al., 2008, 2011a). The consumption of fiber and phenolic compounds can beneficially affect the population health, and are known to be important in the prevention and treatment of diseases.

Afonso et al. (2013) suggested that phenolic compounds attenuate oxidative stress and reduce cholesterol levels in the blood of rats. Food that is rich in phenolic compounds, such as green tea and blueberry, show an inhibitory effect on hepatic steatosis (Park et al., 2011; Liu et al., 2011). The occurrence of non-alcoholic hepatic steatosis has been associated with the excess accumulation of lipids in the liver, liver injury and dyslipidemia. Hepatic steatosis causes elevations in serum aminotransferases, and the analysis of these enzymes is one of the forms to diagnose its occurrence. Diets containing antioxidants have been used for the prevention and as a strategy to limit the accumulation of lipids and liver damage (Park et al., 2011; Bruno et al., 2008). Jabuticaba skins have chemical characteristics that demonstrate their potential as functional and/or nutraceutical food; however, studies on possible applications in health promotion are scarce. In this context, the objective of this study was to analyze the effect of JSF on peroxidation, plasma and hepatic lipid profiles of female rats, and quantification and characterization of its phenolic compounds.

MATERIALS AND METHODS

Preparation of the jabuticaba skin flour (JSF)

P. jaboticaba (Vell.) Berg fruits, Sabará genotype, were hand-picked on São José do Ismeril Farm, in the municipality of Coqueiral, MG, Brazil, transported to the laboratory, where they were selected, washed in tap water, sanitized with sodium hypochlorite solution (200 mg kg^{-1}), by a 10 min immersion; they were then squeezed and the skins were weighed and separated into three lots of approximately 2.9 kg. The jabuticaba skins were dried in a food dehydrator, in mesh metallic material baskets, at a temperature of 45°C, with a 1 m s^{-1} air flow over a period of 36 h. The skins were then ground and the resulting JSF was packaged in hermetically sealed flasks in three replicates, wrapped in aluminum foil, stored at room temperature and subjected to analysis. This JSF was classified as fine grain.

Proximate composition

The proximate composition (moisture, ether extract, crude protein (N X 6.25), ash, dietary fiber and nitrogen-free extract) was performed, based on the methodology described by the Association of Official Analytical Chemists (AOAC, 2005).

Chromatographic study of phenolic compounds

The extraction of phenolic compounds was performed using 50% methanol in the ratio 1:25 (w/v). Chromatographic analyses were performed using an Agilent HPLC equipment model 1100, and the best response was obtained at a wavelength of 280 nm. The extract of phenolic compounds and the standards were injected, in three replicates, into an Ascentis C_{18} column (250 mm × 4.6 mm × 5 µm), attached to a Supelguard Ascentis C_{18} pre-column (20 mm × 4.0 mm × 5 µm). The mobile phase was composed of the solutions: 2% acetic acid (A) and methanol:water:acetic acid (70:28:2 v/v/v) (B). The flux used in all analyses was 1.00 ml min^{-1}; the injection volume was 20 µL. Analyses were performed in a total time of 65 min at 15°C in a gradient-type system: 100% solvent A for 5 min, 70% solvent A for 20 min, 60% solvent A for 18 min, 55% solvent A for 7 min, 0% solvent A for 10 min. Until the end of the run, solvent A was increased to 100%, in order to balance the column. Addition of standards to the extracts was also used as an identification parameter. Quantitation was performed using external standardization with concentrations of standard stock solutions: Gallic, p-coumaric, ferulic, ellagic, 3,4-dihydroxybenzoic, syringic and salicylic acids, as well as the gallocatechin, catechin, epigallocatechin gallate and resveratrol (Sigma-Aldrich - St. Louis, MO, USA).

Vanillic acid and m-and o-cumaric acids (Fluka - St. Louis, MO, USA). Stock standard solutions were prepared in dimethylsulfoxide and/or methanol (Merck). Each solution was injected three times on the HPLC system, with the purpose of obtaining concentration means and retention times.

Animals and treatments

All procedures were performed in accordance with the ethical principles in animal experimentation, adopted by the Ethics Committee on Animal Use of the Universidade Federal de Lavras (Protocol 009/11, approved on 09/01/2011). Thirty two female Fischer rats were used, with a body weight of approximately 140 g, divided into four groups with eight animals in each group. The animals were kept in individual cages, in a room with a temperature of 25 ± 3°C (light/dark cycle of 12 h) with access to water and feed *ad libitum* for a period of 28 days. The experimental diets were prepared according to AIN-93G (Reeves et al., 1993) modified by the addition of crystalline cholesterol (0.5 g 100 g^{-1} diet) and sodium cholate (0.25 g 100 g^{-1} diet). The four groups were divided according to the amount of JSF added to the diet: 0 (control); 0.5, 1.5 and 3.0 g 100 g^{-1} diet (Table 1). Feed consumption and animal weight were monitored weekly, in order to calculate the average daily consumption (ADC), the average daily weight gain (ADG) and the feed efficiency ratio (FER). At the end of the experiment, the animals were fasted for about 12 h, and then were anesthetized with thiopental sodium, intraperitoneally.

Blood was removed from the heart and then centrifuged at 2,500 x *g* for 5 min for the collection of the plasma, which was stored at -20°C. The liver was removed by median laparotomy, washed with 0.9% saline solution, weighed and stored at -25°C for further analyses.

Blood analyses

The analyses were performed with blood plasma. For all tests, Lab

Table 1. Composition of the experimental diets.

Ingredient	Diet (g 100 g^{-1} diet)			
	Control (0)	0.5	1.5	3.0
Starch	40	40	40	40
Casein	20	20	20	20
Sucrose	10	10	10	10
Oil	10	10	10	10
Cellulose	5	5	5	5
Mineral mixture	3.5	3.5	3.5	3.5
Vitamin mixture	1	1	1	1
Methionine	0.5	0.5	0.5	0.5
Cholesterol	1	1	1	1
Sodium cholate	0.25	0.25	0.25	0.25
JSF[1]	0	0.5	1.5	3.0
Kaolin	8.75	8.25	7.25	5.75
Caloric value (cal g^{-1})	3,022.32	2,975.31	2,912.32	3,019.97

[1]JSF, Jabuticaba skin flour.

Test kits were used. Analyses of total cholesterol and of the HDL-c fraction were performed, as well as triacylglycerols, cholesterol in VLDL + LDL fractions, activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT) were also determined.

Analyses in the liver

Moisture, lipid and total cholesterol

The livers were lyophilized until constant weight and finely ground. Moisture and lipid content were determined using the methods proposed by AOAC (2005). The extraction of cholesterol was carried out with isopropanol (Haug and Hostmark, 1987) and the dosage was performed in the same way for the blood analyses.

Thiobarbituric acid reactive substances

The peroxidation of lipids isolated from the liver of animals was determined by the formation of thiobarbituric acid reactive substances (TBARS), according to Winterbourn et al. (1981). The pigment produced by the colorimetric reaction was read in a spectrophotometer at 535 nm. The TBARS concentration was calculated from the standard curve of 1,1,3,3 tetraethoxypropane. The results were expressed as n moles of malondialdehyde acid (MDA) g^{-1} protein.

Histopathological analysis

A liver fragment from each animal was fixed in 10% formalin. The fragments were soaked in paraffin, sectioned (5 µm) and stained with hematoxylin and eosin (HE method). The slides were evaluated under a microscope and identified for the presence of hepatic steatosis, considering mild (+), moderate (+ +) or severe (+ + +) lesion.

Statistical analysis

The experimental design was completely randomized with four treatments, which were the control group (0%) and the groups containing 0.5, 1.5 and 3.0% JSF, with eight replicates, and each animal represented an experimental plot. For the analyses of ADC, ADG and FER, split plots in time were used. The software Sisvar (Ferreira, 2003) was used to perform the analysis of variance and, when significant, the regression analysis was performed, with $p \leq 0.05$.

Table 2. Proximate composition[1] of the jabuticaba skin flour (g 100 g^{-1}).

Constituent	Content
Moisture	21.69 ± 0.20
Lipids	1.59 ± 0.41
Crude protein (N × 6.25)	5.53 ± 0.18
Ash	5.46 ± 0.57
Insoluble fiber	27.51 ± 1.08
Soluble fiber	6.05 ± 0.78
Total dietary fiber	33.56 ± 1.45
NFE [2]	32.17 ± 1.30

[1]Data are the mean of triplicate ± standard deviation. [2]NFE, nitrogen-free extract.

RESULTS AND DISCUSSION

The proximate composition of JSF is presented in Table 2. The lipid content was low, but higher than that found by Leite et al. (2011) and Lima et al. (Lima 2011b) in lyophilized jabuticaba skin samples (1.27 and 1.16 g 100 g^{-1} DM, respectively). Lima et al. (2011a) reported contents of crude protein and ash (1.16 and 4.40 g 100 g^{-1} DM, respectively) for Sabará skins, lower than those found in this study. On the other hand, the contents of soluble (7.73 g 100 g^{-1} DM) and insoluble fiber (35.13 g

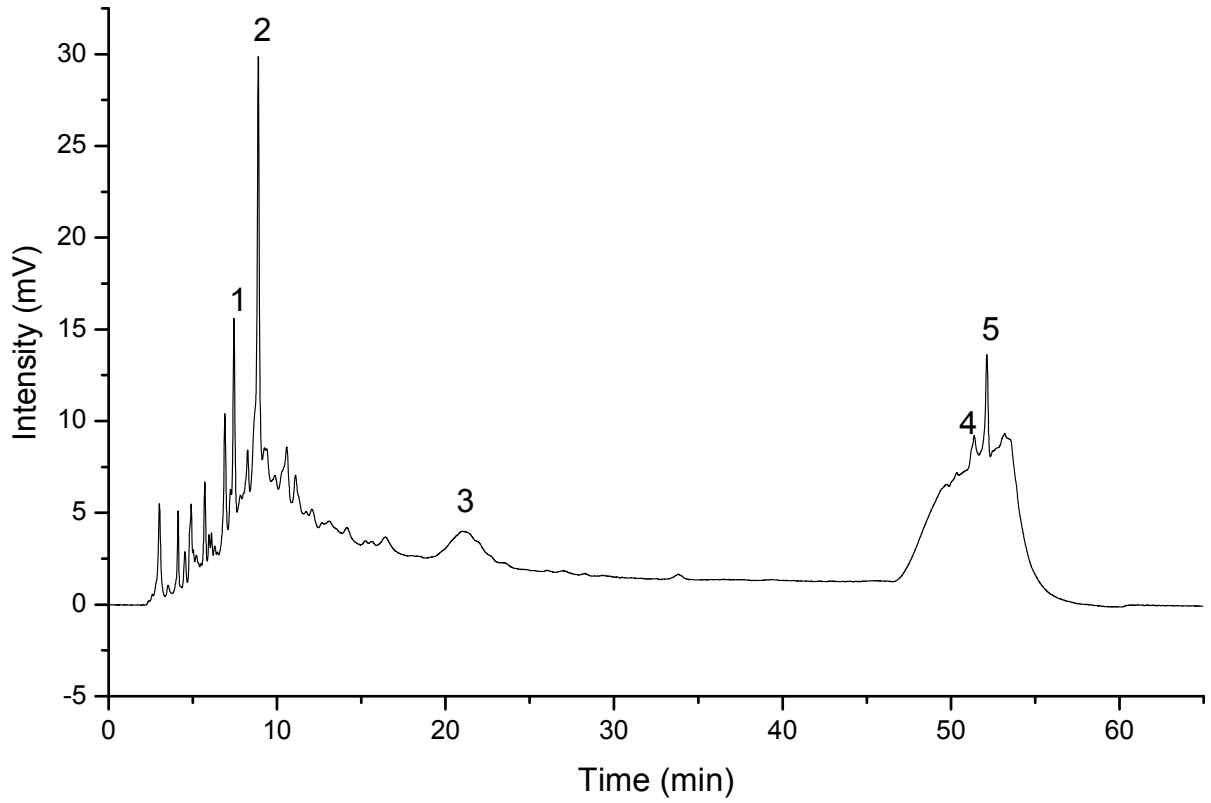

Figure 1. Chromatogram of the JSF extract peak identification: 1- gallic acid, 2- gallocatechin, 3- epicatechin, 4- ellagic acid, 5- salicylic acid.

Table 3. Phenolics average in jabuticaba skin flour, by HPLC.

Phenolic compound	Phenolic content (g 100 g^{-1} DM)
Gallic acid	0.11
Gallocatechin	0.01
Epicatechin	1.9 ±
Ellagic acid	0.12
Salicylic acid	0.54
Total	2.68

100 g^{-1} DM) were higher than those reported by Lima et al. (2011a) (6.80 g 100 g^{-1} DM for soluble fiber and 26.43 g 100 g^{-1} DM for insoluble fiber). The component with the highest content was dietary fiber. These differences are probably inherent in harvest, among other factors. In the chromatographic analyses of the JSF extract, phenolic acids and flavonoids were identified, presenting the following quantitative order: epicatechin > salicylic acid > ellagic acid > gallic acid > gallocatechin (Figure 1) and the total content of phenolic compounds was 2.68 g 100 g^{-1} DM (Table 3). The anthocyanins cyanidin-3-glucoside and delphinidin-3-glucoside were identified in Sabará JSF by HPLC (Leite-Legatti et al., 2012). JSF has proven antioxidant action (Leite-Legatti et al., 2012), probably due to the high content of phenolic compounds. In Table 4, the analyses of ADC, ADG and FER are shown. The analyses of variance for these variables using the split-plot scheme in time showed significant difference at 1% by f test, just for the time. Lenquiste et al. (2012) evaluated the effect of lyophilized jabuticaba skin on rats, in the proportions 0, 1, 2 and 4% added to the diets rich in fat, and did not observe significant statistical differences in the average daily consumption (ADC) and average daily weight gain (ADG) of the animals. These results suggest that the addition of JSF did not affect the palatability of the diets.

The analyses of total cholesterol, triacylglycerols and GGT activity carried in the blood of the animals showed no significant difference and averages are shown in Table 5. The diet supplemented with 3.0% JSF had the highest increase in the level of HDL-c, compared to the control (Figure 2), that was, 20.23%. In a research conducted by Lenquiste et al. (2012), using lyophilized jabuticaba skin, the authors also reported that there was no significant difference for the levels of total cholesterol and triacylglycerols between the diets supplemented with jabuticaba skins and the control and that there were statistical differences for the level of HDL-c. The diets supplemented with 2 and 4% jabuticaba skins showed the lowest values of HDL-c. Phenolic compounds, which

Table 4. Average daily consumption (ADC), average daily weight gain (ADG) and feed efficiency ratio (FER) of animal during the experimental phase.

Parameter	Diet			
	Control (0% JSF[1])	0.5% JSF	1.5% JSF	3.0% JSF
ADC (g)	17.35	17.11	18.55	17.94
ADG (g)	1.43	1.15	1.46	1.43
FER	0.09	0.07	0.08	0.09

[1]JSF, Jabuticaba skin flour.

Table 5. Total cholesterol, triacylglycerols (TAG) and gama glutamyl transferase (GGT) of animal during the experimental phase.

Parameter	Diet			
	Control (0% JSF[1])	0.5% JSF	1.5% JSF	3.0% JSF
Total cholesterol	193,14	165,99	147,56	166,37
TAG	29.72	25,3	26,73	26,10
GGT	4,87	4,46	3,72	3,82

[1]JSF, Jabuticaba skin flour.

are antioxidants, may be responsible for the increase in HDL-c. The accumulation of cholesterol in erythrocytes, leukocytes, platelets and endothelial cells can lead to a reduction in the antioxidant defense systems and cause an increase in the concentration of reactive species (Afonso et al., 2013). Flavonoids act to inactivate free radicals in hydrophilic and lipophilic cellular compartments and have the ability to donate hydrogen atoms, inhibiting chain reactions caused by free radicals (Degáspari and Waszczynscyj, 2004). The flavonoids identified in JSF may have acted as lipophilic antioxidants, attenuating the oxidative stress associated with cardiovascular diseases, which may have increased HDL levels.

One possible mechanism proposed for the reduction of plasma cholesterol levels is the formation of insoluble complexes with bile acids, increasing their fecal excretion, therefore there is no reabsorption of bile acids; cholesterol is then used to synthesize new bile acids, thus decreasing the level of cholesterol (Mäkynena et al., 2013). Studies indicate that phenolic compounds can induce an increase in the fecal excretion of bile acids (Lee et al., 2010). The high content of these compounds in JSF may have increased the fecal excretion of sterols, bile acids and non-fecal cholesterol, contributing to the occurrence of the antihyperlipidemic action of the flour. Although, not significant, a decrease in total cholesterol was observed in the animals. Analyses of the enzymes AST and ALT are used to identify changes in the function of the liver and of the biliary tract, and the enzyme GGT identifies biliary lesions. There was no statistical difference between the control group and the treatments in relation to the enzyme GGT (Table 5). It was observed in Figure 2 that the groups that received JSF showed activities of AST and ALT significantly lower than the control group ($p \leq 0.05$). JSF is probably acting as a hepatoprotective, since all groups received 1.0 g cholesterol, therefore with an accumulation of fat in the liver. The results suggest that this accumulation of fat was less harmful to the animals that received the treatment with JSF.

Total flavonoids extracted from the fruit *Rosa laevigata* Michx showed a significant hepatoprotective effect on mice that suffered liver damage, caused by the ingestion of paracetamol. The results were based on the determination of liver enzymes and histopathological tests (Liu et al., 2001). The authors suggest that this effect is due to the antioxidant potential of flavonoids. The decrease in the levels of AST and ALT, caused by the addition of JSF in the diets, may be due to its high content of phenolic compounds. Confirming these results, the histopathological study revealed a significant decrease in macrovesicular steatosis in the liver of the animals fed the diet containing 3.0% JSF (Figure 3). The increase in cholesterol intake has been associated with lipid peroxidation processes. Analysis of the lipid peroxidation index in the plasma of hamsters treated with hypercholesterolemic diets show an increase in the occurrence of thiobarbituric acid reactive substances (TBARS) (Sánchez-Muniz, 2012). Diets containing 1.5 and 3.0% JSF reduced the production of TBARS by about 50% in the liver of animals with an average weight of 140 g, indicating that JSF conferred protection against oxidative attack (Figure 1). Lenquiste et al. (2012) analyzed the antioxidant potential, by the 2,2′-azinobis-(3-etilbenzotiazolin-6-sulphonic) acid (ABTS) method, of the

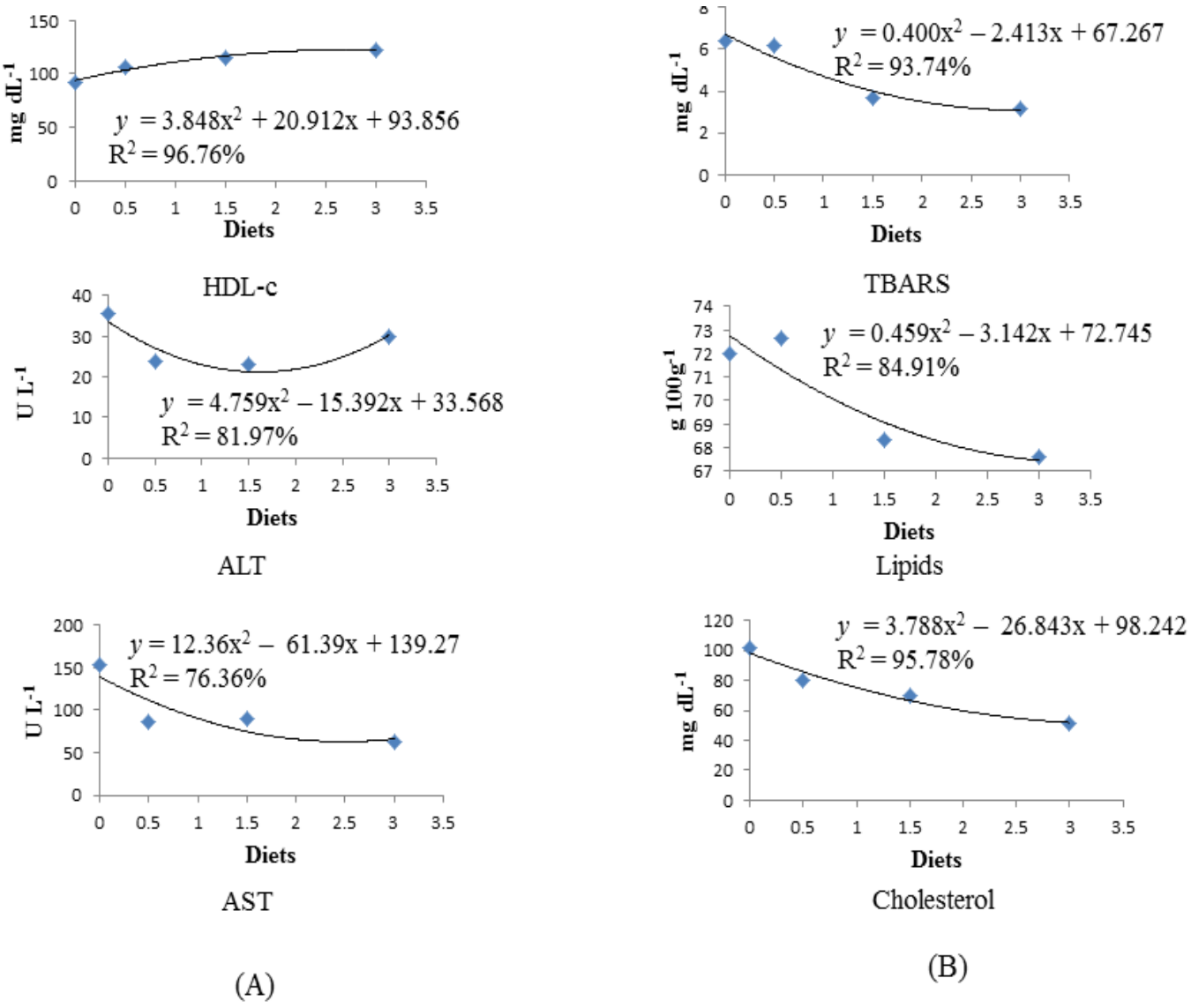

Figure 2. Analyses in the blood (A) and liver (B) of animals, after four weeks with the experimental diets ($p \leq 0.05$).

plasma of rats treated with a diet containing 0, 1, 2 and 4% lyophilized jabuticaba skin, and observed that there was an increase in the levels of ABTS with the diet with up to 2% jabuticaba skin for animals with an average weight of 250 g, also protecting against oxidative attack. The colonic microbiota causes the fermentation of phenolic compounds that occur in matrices rich in fibers, and releases absorbable compounds (Liu et al., 2001).

Regarding flavonoids, colonic bacteria share the heterocyclic ring and degrade flavonoids into phenyl acids that can be absorbed. After absorption, they are conjugated in the liver by glucuronidation, sulfation, methylation, or are metabolized into smaller phenolic compounds. They are able to inhibit cell proliferation and oxidative stress, as well as induce enzyme detoxification, apoptosis and activate the immune system (Guida-Cardoso et al., 2004). The phenolic acids and flavonoids present in JSF, after being metabolized and deposited in the liver of the animals, may be acting to inhibit oxidative stress and cause body detoxification. There was no significant difference for liver moisture, and the average moisture content was 52.79 g 100 g^{-1}. There was a reduction in the level of cholesterol and lipids in the liver of the animals, compared to the control. The high content of fiber and phenolic compounds in JSF may be responsible for these results. Hepatic cholesterol is the result of the balance between the cholesterol acquired through food, the cholesterol synthesized by the body and the cholesterol eliminated by the liver. Cholesterol has a ring structure, which the human body is unable to metabolize into CO_2 and H_2O, and is then eliminated by the liver as unchanged cholesterol into the bile, which

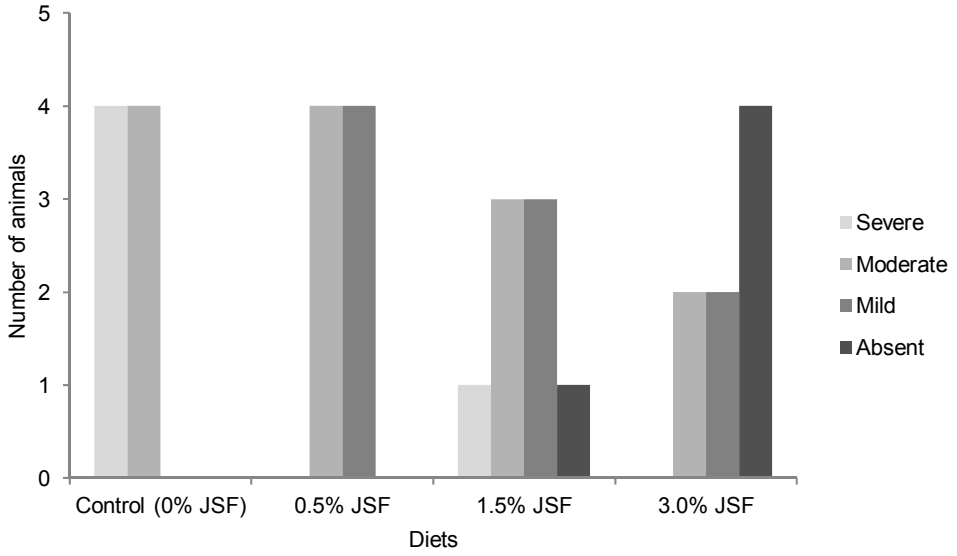

Figure 3. Distribution of macrovesicular steatosis observed in the histopathological analysis of the liver of the animals after four weeks of experiment. JSF, Jabuticaba skin flour.

transports it to the intestine for elimination, either as a component of plasma lipoproteins, or as bile salts, which are excreted in the feaces (Pérez-Jimenéz, 2009).
The animals fed diets supplemented with 3.0% JSF showed a decrease in the cholesterol level of 37.39% in the liver, when compared to the animals fed the control diet (Figure 1). The phenolic compounds in JSF may be acting to cause the decrease in cholesterol synthesis.

Conclusion

The JSF was effective in the protection against dyslipidemia, because it increases the serum level of cholesterol in HDL. The JSF has a high content of phenolic compounds and these components may be responsible for the occurrence of the hepatoprotective effect observed. Furthermore, the JSF has an antioxidant activity, since it protected the liver against lipoperoxidation. The phenolic acids and flavonoids identified epicatechin, salicylic acid, ellagic acid, gallic acid and gallocatechin, probably are responsible for this antioxidant activity.

Conflict of Interests

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

Acknowledgments

The authors thank the development agencies CAPES, FAPEMIG, and CNPq for financial support for the completion of this study.

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