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Sapucaia nuts (*Lecythis pisonis*) modulate the hepatic inflammatory and antioxidant metabolism activity in rats fed high-fat diets

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Lecythis pisonis **Cambess is commonly known as "sapucaia" nut. Previous studies show that it is rich in unsaturated fatty acids and in antioxidant minerals. The aim of the present study was to assess the antioxidant and anti-inflamatory effects of this nut after its introduction into a control (AIN-93G) or high-fat diet in Wistar rats. The animals were divided into four groups: a control diet, the same control diet supplemented with sapucaia nuts, a high-fat diet or the high-fat diet supplemented with sapucaia nuts and were fed with these diets for 14 or 28 days. The gene expression of the markers tumor necrosis factor (TNF)-α NFκB (p65) zinc superoxide dismutase (ZnSOD) and heat shock protein 72 (HSP72) was determined by the chain reaction to the quantitative reverse transcription-polymerase chain reaction (q-PCR). The antioxidant activity was also measured as thiobarbituric acid reactive substances (TBARS) through the activity of the SOD enzyme. The groups treated with "sapucaia" nuts presented reduced lipid peroxidation values and increased ZnSOD and HSP72 gene expression, as well as decreased TNF-α and NFκB (p65) gene expression levels. The significant results showed that "sapucaia" could serve as a potential source of antioxidants and as a protector agent for the examined animals.**

Key words: Sapucaia nuts, inflammation, oxidative stress, gene expression.

INTRODUCTION

Excessive consumption of food associated or not with sedentary lifestyle contributes to the onset of metabolic disorders linked to increased body weight and systemic insulin resistance due to chronic inflammatory condition (Carobbio et al., 2011). Experimental data demonstrates that Wistar rats can develop obesity when they are fed with high-fat diets (Burneiko et al., 2006; Estadella et al., 2004; Kretschmer et al., 2005). The intake of these high-fat diets increases the total amount of body lipids, and thus, raises the oxidative stress, which is strongly related with inflammation (Brunetti et al., 2009; Pérez-Echarri et al., 2009). The increase of body fat reserves stimulates the macrophage infiltration into adipose tissue that could activate a chronic inflammation cascade, which may

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expand to other tissues and cause several health disorders (Alemany, 2013).

High-fat diets can affect the redox balance in the body Fispecies (ROS) (Vial et al., 2011). On the other hand, human and animal organisms have developed a very effective antioxidant defense system, which mainly consist of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), and glutathione-S-transferase (GST) enzymes. These enzymes co-operate to protect cells from free radicals (Domínguez-Avila et al., 2015).

In addition to the enzymatic antioxidant defense, there are also highly conserved proteins that help in protecting the body, such as heat shock proteins (HSP). Different stimuli can interfere with the gene expression of these proteins and could result in the intracellular accumulation of denatured proteins. An increased gene expression of the HSP 70 protein family generates an anti-inflammatory effect, since these proteins down-regulate the Kappa Beta Transcription Factor (NFκB). Consequently, some inflammatory expression markers such as the tumor necrosis factor alpha (TNF-α) and the interleukin 1 beta (IL 1β) are inhibited (De la Fuente et al., 2015; Kim et al., 2015).

Literature shows that the consumption of nuts has beneficial effects on human health due to their nutritional components. Nuts are rich in unsaturated fatty acids, sulfur amino acids and minerals, such as selenium, magnesium, manganese, zinc, iron and copper. At the same time, they contain satisfactory levels of vitamin C and E (Dominguez-Avila et al., 2015; Sabaté et al., 2010; Serrano et al., 2007). The combined effects of these acids, minerals and vitamins may influence specific processes related to the regulation of cell differentiation, DNA protection and inflammatory responses due to their protective role in oxidative stress (Casas-Agustench et al., 2009; Cassidy et al., 2014; Domínguez-Avila et al., 2015; González and Salas-Salvadó, 2006; Ross, 2010; Sabaté et al., 2010).

The vast Brazilian biome remains almost untouched when it comes to nut extraction. Only the cashew and the Pará nuts are commercially available. The “sapucaia” nut (*Lecythis pisonis* Cambess) for human consumption remains commercially unexplored, although some local citizens consider it as the best Amazon nut (Clay et al., 2000). revious studies using “sapucaia” nuts conducted in our laboratory have shown that their use in diets can favor health. The composition of this nut makes it a significant source of unsaturated fatty acids, proteins and minerals such as manganese, magnesium, iron and calcium. Besides, “sapucaia” serves as a potential protective agent against different metabolic disorders (Carvalho et al., 2012). Thus, the aim of the current study is to verify the sapucaia nut ability to reduce oxidizing agent production and to modulate the gene expression of pro and anti-inflammatory molecules in the liver tissue of Wistar rats fed with a high-fat diet.

MATERIALS AND METHODS

Plant

The “sapucaia” nuts used in the present study were collected from five trees at the Federal University of Viçosa campus, which is located in the Zona da Mata Mineira region (20°76’S and -42°86’W), Southeastern of Minas Gerais State. Five fruits were collected from each tree and ten nuts were taken from each of them. The current study uses information about the chemical composition of these nuts indicated by Carvalho et al. (2012).

Animals and diets

The experiment followed the standards established by Law 11.794 and the CONCEA/MCTI Regulatory Resolutions, 2008. The study was approved by the Ethics Committee for Animal Experimentation of Federal University of Viçosa (CEUA-UFV) (Protocol 77/2014). Forty eight male albino rats (Rattus norvegicus, Wistar) were used in the present experiment. The newly weaned animals were obtained from the Central Animal Laboratory of the Biological Sciences Center (CCB) at Federal University of Viçosa, Minas Gerais State - Brazil. The weight of the animals was recorded and they were, then, randomly allocated to eight groups, each with six animals, and housed in individual cages with a controlled environment under temperatures between 22 and 25°C and light-dark cycle of 12 h per day. Experimental diets and water were offered *ad libitum* either for 14 or 28 days. Calculations were performed to simulate the human standard consumption of three nuts a day. The nutritional information of the “Sapucaia” nut (SAP) was obtained by the study of Carvalho et al. (2012). A reference intake of 2000 kcal per day, which corresponds to 4.65% of the total calories of three Sapucaia nuts (93.08 kcal for three nuts), was adopted; thus, the diets contained 4.65% of sapucaia nut.

The animals were fed with four different diets for 14 or 28 days, as follows: Group 1, was fed with a standard diet (AIN-93G) (Reeves et al., 1993); Group 2, was fed with the standard diet supplemented with sapucaia nuts (24 g/kg; 4.65% of total calories) (AIN-93G + SAP), although caloric density (3.95 kcal/g) remained the same (Table 1); Group 3, was fed with a high-fat diet (HFD); and Group 4, was fed with the high-fat diet supplemented with sapucaia nuts (HFD + SAP) (52.2 g/kg; 4.65% of total calories), although again the caloric density remained unaffected (6.92 kcal/g) (Table 2). The rats had *ad libitum* access to the diets and water. The diets were prepared weekly and stored at 4°C to prevent oxidation. The animals were euthanized with ketamine (25 mg/kg IM) and xylazine (2 mg/kg IM) at the end of each study period (14 or 28 days), after fasting for 12 h. The liver tissue of the euthanized animals was collected, frozen in liquid nitrogen and stored in an Ultrafreezer (-80°C).

Gene expression

The total RNA extraction was performed in Trizol reagent (Invitrogen, CA, USA) using 100 mg of liver tissue, according to the recommendation of the manufacturer. The concentration and purity were assessed in a spectrophotometer (Multiskan Go, Thermo Scientific DE, USA) and the integrity of the mRNAs was checked through agarose gel electrophoresis. The recovered mRNA was treated with RNase-free DNase (Promega). The cDNA synthesis was performed using the M-MLV Reverse Transcriptase kit (Invitrogen, CA, USA), according to the protocol of the manufacturer. cDNA was used to determine the expression of mRNAs for the markers TNF-α, NFκB, ZnSOD, HSP72 and the reference house keeping glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

Table 1. Diet compositions (g/1000 g of diet).

Ingredient	AIN93G (g)	AIN93G+Sapucaia
Caseine (81.50%)	200	192.2
Dextrinized starch	132	123
Saccharose	100	100
Soy oil	70	54
Microcrystalline cellulose	50	50
Mineral mixture	35	35
Vitamin mix	10	10
L-cystine	3	3
Coline	2.5	2.5
Sapucaia nuts	-	29.4
Corn starch	397.5	400.9
Caloric density (kcal/g)	3.95	3.96

Table 2. High hat diet (HFD) compositions with sapucaia nuts (g/1000 g of diet).

Ingredient	AIN93G (g) [HFD]	AIN93G+Sapucaia (g) [HFD+SAP]
Ham pâté	222.22	215.7
Potato sticks	111.11	104.61
Bacon	111.11	104.61
Bologna	111.11	104.61
Sweet biscuit cornstarch	111.11	104.61
Chocolate powder	111.11	104.61
whole milk powder	111.11	104.61
Commercial Diet	111.11	104.61
Sapucaia nuts	-	52.2
Caloric density (kcal/g)	6.94	6.92

gene.

The relative quantification of the gene expression was performed through real-time polymerase chain reaction (qPCR) using the Sybr Green Reagent 2X Master Mix (Applied Biosystems, CA, USA). The final volume of each reaction was 10 µl: 2 µl of the cDNA, 0.8 µl of the primers mixture (2.5 µM) (sense and antisense), 5.0 µl of 2X Master Mix Sybr Green reagent and 2.2 µl of ultrapure water in each tested gene. The used qPCR reaction protocol was: 15 min at 95°C, then 40 cycles at 95°C (15 s), 60°C (30 s) and 72°C (30 s) followed by melting curve analysis. Samples were analyzed in four biological repetitions and two technical repetitions and quantified in independent runs.

The negative controls (NTC) were made in two technical repetitions by replacing the cDNA samples by the same water volume in the reaction. "AB Step One Real Time PCR System" (Applied Biosystems) equipment was used to run the experiment. The relative quantification of the gene expression was analyzed through the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

The pairs of oligonucleotides used to amplify the genes of interest were: NFκB (p65) (Fw 5'-CTTCTGGGCCATATGTGGAGA-3') and (Rw 5'-TCGCACTTGTAACGGAAACG-3'), TNF-α (Fw5′-GCCGATTTGCCATTTCATACC-3′) and (Rw 5′-GGACTCCGTGATGTCTAAGTAG-3′), HSP 72 (Fw 5'-AGGCCAACAAGATCACCATC-3') and (Rw 5'-TAGGACTCGAGCGCATTCTT-3'), ZnSOD (Fw 5'-GAGCAGAAGGCAAGCGGTGAA-3') and (Rw 5'-CCACATTGCCCAGGTCTC-3'), GAPDH (Fw 5'-GGTTGTCTCCTGTCACTTC-3') and (Rw 5'-CTGTTGCTGTAGCCATATTC-3'). The oligonucleotides were designed based on the gene sequences of the Wistar *Rattus norvegicus* found in the GenBank, using Primer 3 plus software. The experiment followed the MIQE guidelines established for studies that use the qPCR technique in real time (Bustin et al., 2009).

Preparation of liver homogenate

Liver samples (200 mg) were collected from each rat and after their thawing, were homogenized in Tris-HCl 0.01 M buffer, pH 7.4 at the ratio of 5 ml buffer per 500 mg of tissue and finally, centrifuged at 10.000 g for 15 min at 4°C. The supernatants were used to determine the total protein content, the superoxide anion dismutase antioxidant activity, and hepatic lipid peroxidation.

Enzymatic activity of the hepatic SOD

Superoxide dismutase (SOD) activity was determined in relative units. Each SOD unit was defined by the amount of enzyme able to

Table 3. Mean body weight gain, daily diet consumption and hepatosomatic index in the initial and final stages of the treatment.

Group/Treatment	Weight gain (g)	Daily diet consumption (g)	Hepatossomatic Index
14 days			
AIN-93G	95.10±19.89[a]	26.60±7.02[b]	4.9±0.44[a]
AIN-93G+SAP	96.85±16.46[a]	29.54±4.36[b]	4.7±0.51[a]
HFD	97.82±14.46[a]	32.73±6.05[a]	4.7±0.62[a]
HFD+SAP	94.32±11.86[a]	25.40±5.12 [b]	4.6±0.71[a]
28 days			
AIN-93G	152.27±12.49[b]	26.56± 6.24[b]	4.8±0.63[a]
AIN-93G+SAP	160.89±8.14[b]	30.21± 7.23[a]	4.6±0.48[a]
HFD	186.67±26.88[a]	32.62±9.39[a]	4.6±0.52[a]
HFD+SAP	187.38±11.54[a]	32.21±5.06[a]	4.7±0.73[a]

Means (n = 6) followed by different letters in the same column are statistically different (Tukey test, p <0.05). AIN-93G, AIN-93G diet; AIN-93G + SAP, AIN-93G + sapucaia nuts diet; HFD, high-fat diet; HFD + SAP, high-fat diet + sapucaia nuts diet, liver somatic index (liver's relative weight = liver weight / body weight).

inhibit 50% of the pyrogallol oxidation rate. The used reaction medium contained 30 µl of liver homogenate and 15 µl of pyrogallol 24 mmol/L and 15 µl of catalase prepared with 2.4 mg in 2 ml of distilled water. The volume was completed with Tris-HCl buffer 50 mM 270 µl up to 300 µl, pH 8.2 and 1 mM of EDTA. A standard 100% pyrogallol oxidation was conducted in 15 µl of catalase and 15 µl of pyrogallol 24 mmol/L. The blank solution contained 15 µl of catalase and 285 µl of the buffer. Initially, the readings were performed at 420 nm absorbance. The reaction was then incubated at 37°C for 5 min and the readings were performed again at 420 nm. The absorbance found before and after the 5 min incubation was subtracted to minimize the presence of interferents. The results were expressed as U SOD/mg of protein (Marklund, 1985). Calculations were made based on the standard absorbance value, by considering that such value had zero SOD units. Protein quantification was performed according to Bradford (1976) using a standard curve, which was based on bovine serum albumin callibration.

Hepatic lipid peroxidation analysis

This was conducted through the measurement of malondialdehyde (MDA), which is produced by the oxidation of fatty acids through the reaction of thiobarbituric acid reactive substances (TBARS) (Kohn and Liversedge, 1944). The lipid peroxidation was measured by means of a reaction containing 400 µl liver homogenate, 1 ml trichloroacetic acid (20%) and 400 µl thiobarbituric acid (1.6%). The mixture was incubated at 95°C for 1 h, 1.6 ml n-butanol was added and it was then centrifuged at 3000 rpm/10 min. The reading was performed at 510, 532, and 560 nm. Equation proposed by Pyles et al. (1993) was used to calculate the final values in order to minimize interferences from pigments of heme in the hemoglobin in the MDA dosage:

$$MDA_{532}=1.22[(A_{532}) – (0.56)\ (A_{510}) + (0.44)\ (A_{560)}]$$

Malondialdehyde concentration was determined through the coefficient of molar absorptivity E 0 = 1.56 × 105 L mol^{-1} cm^{-1} (Buege and Aust, 1978) and the results were expressed in nmoles of MDA per mg of protein.

Statistical analysis

ANOVA analysis was performed to assess the distribution of variables in the experiments. It was followed by the Tukey test (Graphpad Prism 5) in all treatments. The significance level was 95%, p <0.05. The results were expressed as mean ± standard deviation. The comparisons were conducted between the AIN-93G and AIN-93G groups + SAP and between HFD and HFD + SAP groups by considering the caloric density difference between the administered diets.

RESULTS

Weight gain and diet consumption

Weight assessments comparing animals subjected to HFD/HFD + SAP and those fed with AIN-93G/AIN-93G + SAP showed that the weight gain of animals was significantly different after 28 days of treatment. Daily feed intake per animal was higher in the HFD group at day 14 and lower in the AIN-93G group at day 28 of the experiment compared to the other groups (Table 3).

Lipid peroxidation values and antioxidant activity

Lipid peroxidation (MDA) values and the antioxidant activity expressed in SOD units (Table 4) were determined with the intention to assess the impact of the high-fat diet on the oxidative stress in the liver of rats and to discover the possible protective effect of sapucaia nut supplementation. All groups treated with “sapucaia” nut-enriched diets showed lower MDA concentration compared to the other groups in both tested diets (HFD and AIN-93G).MDA level measurements showed that the AIN-93G diet had similar values at days 14 and 28 of the

Table 4. Means of the hepatic malondialdehyde (MDA) concentrations (nmol MDA / mg STP) and superoxide dismutase units (SOD) (U SOD/mg LWA) at the 14th and 28th day in each diet (AIN-93G, AIN-93G + SAP, HFD, HFD + SAP) administered to the animals.

Group	MDA		SOD	
	14 days	28 days	14 days	28 days
AIN-93G	1.10 ± 0.04^{aA}	1.16 ± 0.02^{aA}	8.10 ± 0.44^{bA}	8.45 ± 0.72^{bA}
AIN-93G+SAP	0.62 ± 0.05^{bB}	1.03 ± 0.03^{cB}	11.78 ± 1.14^{aB}	13.65 ± 0.50^{aB}
HFD	1.39 ± 0.01^{aC}	1.72 ± 0.05^{bC}	6.30 ± 0.48^{aA}	10.35 ± 0.47^{bC}
HFD+SAP	0.65 ± 0.05^{bB}	0.96 ± 0.02^{aB}	14.28 ± 0.81^{bB}	14.86 ± 0.62^{bB}

Means (n=6) followed by different letters in the same column are statistically different (Tukey test, $p < 0.05$). AIN-93G, AIN-93G diet; AIN-93G + SAP, AIN-93G + sapucaia nuts diet; HFD, high-fat Diet; HFD + SAP, HFD + sapucaia nuts diet, PTN - Proteins.

experiment, although a significant increase in the MDA levels was observed in the other groups. The antioxidant enzyme activity expressed in SOD units was significantly higher in the sapucaia supplemented groups both at days 14 and 28.

Antiinflamatory and antioxidant hepatic gene expressions

Relative mRNAs gene expression was determined to help understand the effects caused by the inclusion of the “sapucaia” nuts in the diets. The experiment compared the diets with or without sapucaia nuts to find the genes involved in the inflammatory process and the hepatic antioxidant activity by real time RT qPCR (Figure 1). The gene expression of TNF-α was lower in animals fed with the sapucaia nuts than in those of the controls. There were no significant differences in both AIN-93G + SAP and HFD + SAP in the TNF-α gene expression in all treatment periods.

It was also observed that the gene expression of the NFkB (p65) was significantly lower in the group of rats that consumed the diets supplemented with “sapucaia” nuts. The gene expression of ZnSOD, in turn, was relatively higher in sapucaia supplemented groups than in the controls.

There was also an increased gene expression in the hepatic HSP72 gene in both diets. However, the HFD + SAP showed more significant amounts.

DISCUSSION

High-fat diets administered to rats can induce inflammation and oxidative stress (Burneiko et al., 2006; Brunetti et al., 2009; Estadella et al., 2004; Kretschmer et al., 2005; Pérez-Echarri et al., 2009; Scoaris et al., 2010). Rats fed with the hyperlipidic diet (HFD) did not show a significantly higher hepatosomatic index (HIS; relative liver weight) compared to the other groups. Sapucaia dietary supplementation and the duration of the experiment appeared not to affect HIS value. Although, diets enriched with “sapucaia” nuts did not have any effect on weight gain and on HSI, our findings showed that sapucaia enrichment contributed in the prevention of the redox imbalance caused by high-fat diets due to its interference in both the MDA concentrations and in the SOD enzyme activity. As it was expected, more oxidizing agents in animals fed with high-fat diet were observed. However, this negative effect was diminished after sapucaia supplementation as shown by the significant increase in the SOD activity and of the decrease in MDA values.

Carvalho et al. (2012) showed that 100 g of sapucaia nuts cover the daily needs for manganese and magnesium and the half of them for iron, according to the reference values. Other studies using “sapucaia” nuts found high magnesium, iron, selenium and phosphorus concentration levels (Kerdell-Vargas, 1966; Souza et al., 1996; Vallilo et al., 1999), as well as a desired protein digestibility (Denadai et al., 2007). Such findings about the nutritional value of the the sapucaia nut (*L. pisonis*), reinforce the hypothesis that are several benefits in the metabolism of animals fed with the sapucaia enriched diets.

The enzyme system is the first line of antioxidant defense of the organism. The constituents of the nuts associated with the gene stimulation of the enzymes can play an important role as exogenous antioxidants, such as tocopherol, ascorbate, carotenoids, and phenolic compounds, due to their mineral components. This set of nutrients can cause many benefitial health effects against metabolic disorders such as obesity and diabetes (Borges and Lessa, 2015; Casas-Agustench et al., 2009; Cassidy et al., 2014; Dominguez-Avila et al., 2015; Fernandes et al., 2015; Shahidi and Ambigaipalan, 2015).

The liver has multiple functions and is directly involved in the oxidative processes (Rodríguez-Hernández et al., 2013; Stadler et al., 2004). Changes in the hepatic redox balance can interfere in the translocation of proteins

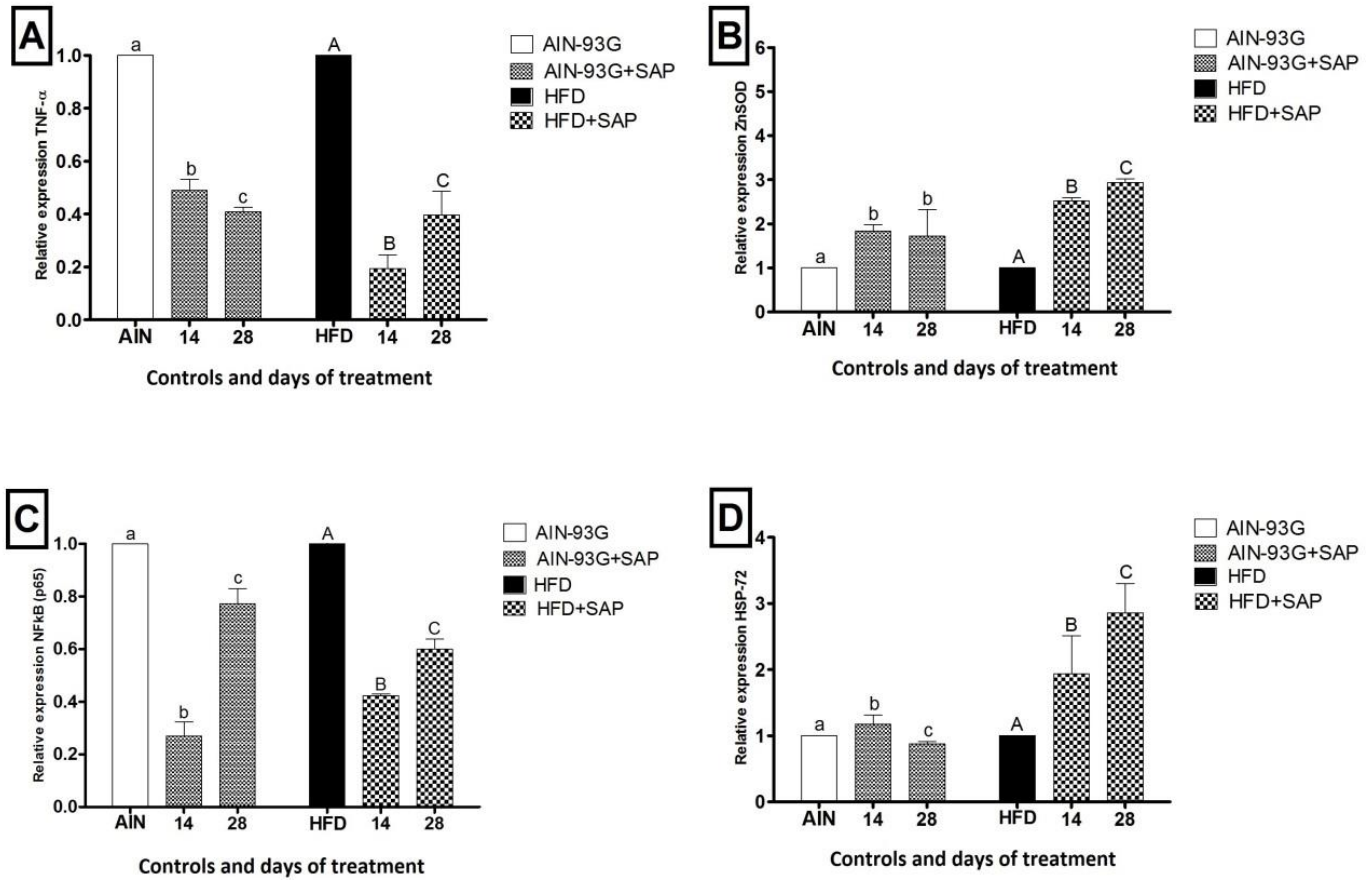

Figure 1. Averages of the mRNA relative gene expression (calibrator sample, y=1) of TNF-α (A), ZnSOD (B), NFkB (p65) (C) and HSP-72 (D) byqPCR for the animals fed with AIN-93G + SAP and HFD + SAP for 14 and 28 days. Means followed by different letters (lowercase for AIN-93G and capital to HFD) are significantly different according to the Tukey's test ($p<0.05$).

sensitive to oxidative stress from the cytoplasm to the cell nucleus (Cassidy et al., 2014; Maritim et al., 2003; Sadi et al., 2008). Thus, the oxidative stress induced by high-fat diets tends to increase the gene expression of TNF-α and NFkB (p65), as a response to the process triggered by the oxidative imbalance associated to the high content of saturated fatty acids. In addition, the HSP70 HSP family is also affected by the oxidative stress caused by dietary fats (Jangale et al., 2013; Lightfoot et al., 2015; Yaglom et al., 2003; Gabai and Sherman, 2002).

There has been a significant difference in the HSP-72 gene expression between rats treated with both HFD and HFD + SAP and those fed with AIN-93G and AIN-93G + SAP. Rats treated with the sapucaia nut-enriched diets showed significantly higher HSP-72 gene expression than those fed just with the control diets (HFD and AIN-93G), except AIN-93G at day 28. As it is concluded, "sapucaia" nut could play an important role in the modulation of this gene expression. The HSP-72 is part of the HSP-70 proteins family, which inhibits NFkB (p65) activation by reducing the production of pro-inflammatory cytokines (Dokladny et al., 2010; Shi et al., 2006; Tanaka et al., 2014). Therefore, increased HSP-72 expression seen in groups fed with the sapucaia nuts-enriched diets (HFD + SAP and AIN-93G + SAP) could be correlated with the decrease in the NFkB activation cascade activity (p65).

There is an interaction of NFkB signaling cascade (p65) with the TNF-α gene expression, since TNF-α is a pro inflammatory agent and can be activated by the NFkB (p65) protein (Dokladny et al., 2010; Tanaka et al., 2014). As it was shown in the present study, there was a decrease in the TNF-α gene in animals fed with HFD + SAP compared to the HFD group. Higher ZnSOD gene expression was observed in all groups treated with sapucaia nuts-enriched diets, in addition to all the aforementioned findings. It allows inferring that the presence of these nuts in the diets played an important role, since the gene expression of this enzyme was higher in the HFD + SAP than in the HFD.

The correlation between saturated fat acids consumption and oxidative stress is not simple, because there are many biochemical mechanisms involved. Thus, saturated fat acids consumption can increase oxygen

consumption and generate other oxidizing molecules (Dandekar et al., 2015; Hybertson et al., 2011; Seifert et al., 2009). However, our results showed increased antioxidant and anti-inflammatory capacity due to the lower gene expressions of TNF-α and NFκB. These findings reinforce the hypothesis that dietary antioxidants are particularly important for protection against chronic diseases. Finally, the sapucaia nuts could serve as a potential antioxidant source and their protection is profound even in animals fed with high-fat diets.

Conclusion

The sapucaia nut dietary supplementation stimulated the enzymatic activity of SOD and reduced lipid peroxidation values in the hepatic tissue of rats fed with high-fat diets. These results were confirmed by the observed differencies in the expression of genes directly involved in the metabolic processes. ZnSOD enzyme and HSP72 protein expressions were significantly higher in rats fed with the sapucaia nuts-enriched diets. Diet enriched with sapucaia nuts was found to influence the gene expression of the inflammation markers TNF-α and NFκB; reduction in their expression levels can be linked with the increased antioxidant activity, since these parameters are related. Further studies on sapucaia must be conducted in order to assess the impacts of its consecutive use as food, since is rich in nutrients and could have beneficial effects on health status.

Conflict of Interests

The authors have not declared any conflict of interests.

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