



## *Ilex paraguariensis* extract prevents body weight gain in rats fed a high-fat diet

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### ABSTRACT

Studies have shown that drinks containing *Ilex paraguariensis* extract can promote many benefits in animals and in humans. The present study aimed to evaluate *in vivo* effects of *Ilex paraguariensis* extract on metabolic profile, obesity prevention and expression of genes related with adipogenesis and lipogenesis in *Wistar* female rats fed a high-fat diet. For this experiment 32 *Wistar* female rats with normal weight were used and randomly separated into four groups: diet (standard or high-fat) and treatment (water or *Ilex paraguariensis* extract) for 34 days. The rats receiving *Ilex paraguariensis* extract had lower body weight compared to the control group in both diets. Likewise, there was a reduction in triglycerides in the groups fed high-fat diet and treated with *Ilex paraguariensis* extract. The creatinine levels were lower in the groups treated with *Ilex paraguariensis* and in high-fat diet. It was observed an increased liver gene expression for *Fas* and *Scd1* in the group treated with hyperlipid diet + *Ilex paraguariensis*. It can be concluded that *Ilex paraguariensis* extract decreased body weight gain in both control and high-fat diets, reduced plasma triglycerides and creatinine levels and increased liver expression of genes related to lipogenesis.

**Keywords:** obesity; triglycerides; glucose levels; genes.

**Practical Application:** Control of obesity by the consumption of the extract of the *Ilex paraguariensis*.

### 1 Introduction

*Ilex paraguariensis* A. St. Hil. belongs to the *Aquifoliaceae* family and is a native species from the subtropical and temperate regions of South America. This herb is used regularly in beverages prepared by infusion such as teas, and is a natural product, recognized for having anti-inflammatory and diuretic properties (Przygodda et al., 2010). Likewise, the extract from *Ilex paraguariensis* contain different bioactive constituents, and several *in vitro* studies have shown the important role of polyphenols on the antioxidant activity, suggesting the potential use for the development of natural products aiming to protect biological systems against oxidative stress-mediated damages. Furthermore, others have shown anti-diabetic and antiobesity effects of the extract (Kang et al., 2012).

Obesity is an increasing problem worldwide, resulting in significant morbidity and mortality, as well as a reduced quality of life (Hurt et al., 2010). The unbalance on ingestion of food and loss of energy by exercise causes obesity and visceral adiposity, promoting complications to the personal health such as atherosclerosis, hepatic steatosis, and type 2 diabetes (Berg & Scherer, 2005). Furthermore, the ingestion of a high-fat meal leads to shifts in particle size, numbers, and plasma levels of very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL) (ALNohair, 2014). The potential of a diet or food to increase serum concentrations of cholesterol, especially LDL cholesterol, and promote

atherosclerosis is directly related to its cholesterol, saturated fat and trans fat content (Mente et al., 2009). Also, it was reported that consumption of high-fat diets is associated with a reduction of serum paraoxonase 1 (*Pon1*) activity (Garcia et al., 2016).

The enzyme *Pon1* is a natural antioxidant, and has a primary role in protecting HDL and LDL from lipid peroxidation (Ng et al., 2008). *Pon1* is synthesized in the liver and secreted into the bloodstream bound to HDL (She et al., 2012). In addition to *Pon1*, apolipoprotein A1 (*ApoA1*) has a specific role in fat metabolism (Jaichander et al., 2008), and is a structural protein in HDL, being the main protein component of HDL (Rader, 2002). *ApoA1* is responsible for the activation of lecithin cholesterol acyltransferase (LCAT), stimulating cholesterol flow and the binding of HDL to its receptors (Kontush et al., 2013). In addition to *Pon1* and *ApoA1*, other genes are involved in lipid metabolism. Fatty acid synthase (*Fas*) is the key enzyme required for *de novo* synthesis of fatty acids (Wajant, 2012). Stearoyl coenzyme A desaturase-1 (*Scd1*) is an enzyme that appears to represent a pivotal control point in lipid homeostasis. *Scd1* catalyzes a rate-limiting step in the biosynthesis of monounsaturated fats, which are required for triacylglycerol synthesis and very low density lipoprotein production. In absence of *Scd1*, hepatic lipid storage and very low-density lipoprotein production are impaired, and as default, fatty acids are oxidized (Wajant, 2012). The peroxisome proliferator-activated receptor co-activator-1 (*Pgc1*) is a transcriptional protein co-activator (Handschin, 2010). *Pgc1* was

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initially characterized in adipose tissue and it is now known that this molecule plays an important role in oxidative metabolism, in mitochondrial biogenesis and hepatic gluconeogenesis. Based on this, evaluation of liver expression of these genes represents an important hallmark of the effect of the high-fat diets.

Thus, to evaluate the efficacy of potential compounds in the prevention and treatment of obesity, several animal models have been used (Kang et al., 2012). It has been reported that rodents fed with a high-fat diet are a good model of obesity, where the dietary environment is a major contributor (Bullo et al., 2007). Based on these evidences, this study aimed to evaluate the effects of *Ilex paraguariensis* extract on metabolic profile, body weight gain and liver gene expression related with adipogenesis and lipogenesis of *Wistar* female rats fed to a high-fat diet.

## 2 Materials and methods

### 2.1 Experimental conditions and monitoring

The experimental protocol was approved by the Animal Welfare Commission from the Federal University of Pelotas (Rio Grande do Sul State, Brazil), under the number 1641, and all procedures were conducted according to the guidelines of laboratory animal use in research. For this study 32 female *Wistar* rats (*Rattus Norvegicus*), 60 days old were used. The rats were kept in groups of four at polypropylene boxes in ventilated cabinets, with controlled temperature and relative humidity conditions (23 °C ± 1 °C and 65-75%), and exposed to a 12 hour light/dark cycle. After five days of adaptation, the rats were randomly divided into four groups (n= 8 rats per group), as follows: standard diet (4% fat) + water *ad libitum* (SW); standard diet (4% fat) + *Ilex paraguariensis* extract *ad libitum* (SIP); high-fat diet (25% fat, 1% cholesterol and 0.1% cholic acid) + water *ad libitum* (HFW); high-fat diet (25% fat, 1% cholesterol and 0.1% cholic acid) + *Ilex paraguariensis* extract *ad libitum* (HFIP). The standard (4% fat content) and high-fat diets (25% fat content, 1% cholesterol and 0.1% cholic acid) were prepared in the laboratory, as recommended by the American Institute of Nutrition - AIN93- M for rodents (Reeves et al., 1993) (Table 1). The same brand and batch of *Ilex paraguariensis* was used throughout the experiment period, guaranteeing product homogeneity. The *Ilex paraguariensis* extract was prepared in a 10% concentration and 70 °C temperature,

resembling conditions of the human ingestion. The infusion of the extract was performed for 20 minutes and after it was sieved.

The body weight was measured weekly using an electronic scale (JH2102/Bioprecisa, Curitiba, Brazil) and food intake was monitored daily during the 34 days of the study. Rats were euthanized at day 34, after a 12 hours fasting, following the ethical principles in animal experimentation used by the Brazilian College of Animal Experimentation.

### 2.2 Sample collection and biochemistry analysis

Blood was collected and centrifuged at 1000rpm for 10 minutes (Centrifuge 5415, Eppendorf, Westbury, New York, USA). The serum was transferred to a microtube and frozen at -20 °C until analysis.

The determination of total cholesterol, HDL, triglycerides and glucose levels in serum were performed using commercial kits (Triglycerides Liquiform and Glucose Liquiform, respectively, Labtest®, Minas Gerais, Brazil). Samples reading were performed in a spectrophotometer (Ultraspec 2000, Pharmacia Biotech) at 500 nm. The results from total cholesterol, HDL cholesterol, triglycerides and glucose levels were expressed in mg/dL. The intra assay coefficients of variation found between 11.4% and 17.0%.

The *Pon1* activity was measured by its arylesterase activity as previously established (She et al., 2012). The arylesterase activity was measured by the phenol formation rate through monitoring the increase in absorbance at 270 nm and 25 °C. The working reagent consisted of 20 mM Tris/HCl, pH 8.0, containing 1mM of CaCl<sub>2</sub> and 4 mM phenylacetate as substrate. The samples were diluted 1:3 in a 20 mM Tris/HCl buffer and were added to the working reagent and the change in absorbance recorded for 60 sec. The activity the *Pon1* was expressed in U/L, based on the phenol extinction coefficient. The intra assay coefficients of variation found were 14.7%.

The determination of the concentration of transaminases glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) in the serum was performed by colorimetric method using a commercial kit (Dolles®, Goiânia - GO, Brazil) and readings obtained spectrophotometrically at 505 nm and the results expressed as IU/L.

The determination of creatinine concentrations in serum were performed using commercial kits (Creatinine K®, Labtest Diagnostica SA, Lagoa Santa, Brazil) based on the Jaffé reaction. For the measurements, 50 uL of serum sample was mixed with 50 uL of alkaline picrate. Subsequently, the reading was held in spectrophotometer at 520 nm after 0 and 60 seconds. The results were expressed in mg/dL.

### 2.3 Gene expression

To determine gene expression, samples of liver were collected and immediately frozen in liquid nitrogen and stored at -80°C. The samples were homogenized with Qiazol (Qiagen, Valencia, USA), and total RNA was isolated and purified following the Qiazol protocol. The quality of RNA was assessed by electrophoresis in agarose gel. The reverse transcription reactions were performed using 1 µg of RNA with a reverse transcription kit containing RNase inhibitor (Applied Biosystems, Foster City, USA) in a volume of 10 µL. Real-time PCR was performed to assess the

**Table 1.** Diet composition according to AIN93-M (Reeves et al., 1993).

	Standard Diet	High-fat Diet
	g.kg <sup>-1</sup>	g.kg <sup>-1</sup>
Maize starch	466	256
Casein	140	140
Saccharose	100	100
Dextrinated starch	155	155
Soy oil	40	40
Fiber	50	50
Minerals	35	35
Vitamins	10	10
L-cistina	1.8	1.8
β-Colina	2.5	2.5
Tetra Butilh	0.008	0.008
Lard	----	120
Overall	1000	1000

expression of the target genes *Apoa1*, *Pon1*, *Scd1*, *Foxo*, *Fasn* and *Pgc1* and the internal control *Actb* (Table 2).

PCR reactions were performed in duplicate in a volume of 12 µL using SYBR Green Mastermix (Applied Biosystems) and the fluorescence was quantified in the Eco Real Time (Illumina, San Diego, California, USA). For each test, 40 cycles were carried out and a dissociation curve was included at the end of the reaction in order to verify the amplification of a single PCR product. Data is reported as folds over the minimum according to Masternak et al. (2005). Each assay plate included a negative control with water.

### 2.4 Statistical analysis

Data was analyzed using two-way analysis of variance (Two-way ANOVA) and Tukey's test at 5% significance level for comparison of means, using Graphpad Prism 5.0 (GraphPad, La Jolla, CA, USA). The effects of the diet, supplementation with *Ilex paraguariensis*

extract and their interaction were tested. When the interaction was significant individual groups were compared by t-test.

### 3 Results

The results from feed intake indicate that the groups fed high-fat diet had lower intake ( $p < 0.01$ , Table 3) when compared to the standard diet groups, although there was no effect of *Ilex paraguariensis* extract supplementation nor interaction between the diet and treatment ( $p > 0.05$ , Table 3). Despite no difference in feed intake when submitted to the same diet, rats supplemented with *Ilex paraguariensis* extract presented smaller weight gain ( $p < 0.01$ ). No effect of diet or interaction between *Ilex paraguariensis* extract and diet was observed ( $p > 0.05$ , Table 3).

*Ilex paraguariensis* extract supplementation was effective to reduce plasma triglycerides on groups submitted to both diets (standard or high-fat diet) ( $p < 0.05$ , Table 4). The analysis from

**Table 2.** Primers used in the analysis of gene expression by real-time PCR.

Primers	Gene	Sequence (5'-3')	Length (pb)
<i>Actb</i> (forward)	β-actin	TCACCACCACAGCCGAGAGA	72
<i>Actb</i> (reverse)		CGAAATCCAGTGCACGTAGC	
<i>Pon1</i> (forward)	<i>Pon1</i>	CAAGAACCATCGGTCTTCCT	197
<i>Actb</i> (reverse)		CAGGCTTACTGGGATCGAAA	
<i>Apoa1</i> (forward)	<i>Apoa1</i>	CAAGAACCATCGGTCTTCCT	828
<i>Apoa1</i> (reverse)		CAGGCTTACTGGGATCGAAA	
<i>Scd1</i> (forward)	<i>Scd1</i>	CACATCAACTTCACCACGTTCTTC	74
<i>Scd1</i> (reverse)		GAAACTTTCTCCGGTCGTAAGC	
<i>Foxo3</i> (forward)	<i>Foxo3</i>	TCCCAGATCTACGAGTGGATGG	42
<i>Foxo3</i> (reverse)		CCTTCATTCTGAACGCGCAT	
<i>Fasn</i> (forward)	<i>FAS</i>	CTGGACTCGCTCATGGGTG	111
<i>Fasn</i> (reverse)		CATTTCTGAAGTTCCGCAG	
<i>Pgc1</i> (forward)	<i>PGC1</i>	CCGAGAATTCATGGAGCAAT	114
<i>Pgc1</i> (reverse)		TTTCTGTGGGTTTGGTGTGA	

**Table 3.** Analysis of food intake and average weight of female rats in control (SW and HFW) and *Ilex paraguariensis* extract groups (SIP and HFIP). The values were expressed as mean (M) ± standard error (SE).

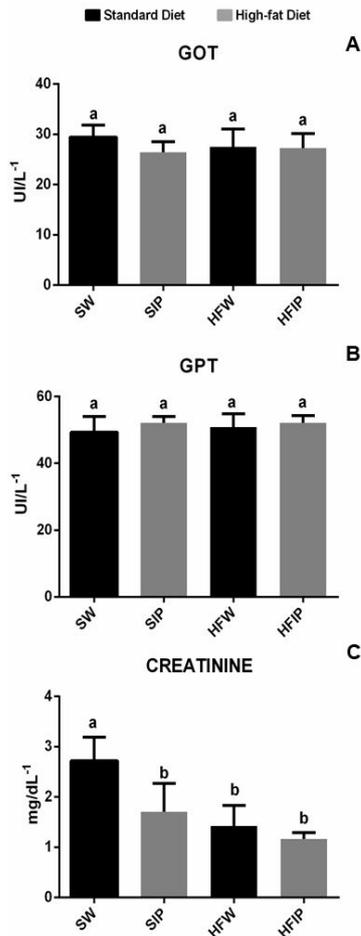
Groups	Feed intake (g)	Total caloric intake	Starting weight (g)	Final weight (g)	Weight gain (g)
	---- M ± SE ----	---- Kcal ----		---- M ± SE ----	
SW*	22.31 ± 0.39 <sup>ns</sup>	3624.8	194.89 ± 0.43	233.38 ± 0.21	38.49 ± 0.23 <sup>ns</sup>
SIP	21.85 ± 0.27 <sup>a</sup>	3624.8	191.25 ± 0.36	222.58 ± 0.26	31.33 ± 0.31 <sup>b</sup>
HFW	19.36 ± 0.34 <sup>b</sup>	3907.1	192.28 ± 0.54	238.94 ± 0.78	46.66 ± 3.03 <sup>a</sup>
HFIP	19.85 ± 0.24 <sup>b</sup>	3907.1	192.37 ± 0.41	225.41 ± 0.62	33.04 ± 0.45 <sup>b</sup>
CV(%)	11.12	----	12.35	11.97	11.20

\*SW: standard diet (4% fat) + water *ad libitum*; SIP: standard diet (4% fat) + *Ilex paraguariensis* extract *ad libitum*; HFW: high-fat diet (25% fat content, 1% cholesterol and 0.1% cholic acid) + water *ad libitum*; HFIP: high-fat diet (25% fat content, 1% cholesterol and 0.1% cholic acid) + *Ilex paraguariensis* extract *ad libitum*; CV: coefficient variation; <sup>ns</sup>Average values followed by the same letter in the column show no significant statistical difference between them with the Tukey test at 1% probability of error ( $p < 0.01$ ).

**Table 4.** Analysis of total cholesterol, HDL cholesterol, triglycerides levels, blood glucose and *Pon1* activity of female rats in the control and treatment groups. The values were expressed as mean (M) ± standard error (SE).

Treatment	Total cholesterol	HDL cholesterol	Triglyceride	Glucose	<i>Pon1</i>
			----- mg/dL -----		----- U/L -----
SW*	73.45 ± 04.90 <sup>ns</sup>	53.58 ± 07.05 <sup>ns</sup>	117.76 ± 05.72 <sup>ns</sup>	464.76 ± 37.19 <sup>ns</sup>	134.10 ± 10.73 <sup>ns</sup>
SIP	81.74 ± 08.73 <sup>ns</sup>	60.68 ± 05.67 <sup>ns</sup>	92.55 ± 02.31 <sup>ab</sup>	399.29 ± 25.69 <sup>ns</sup>	151.95 ± 10.62 <sup>ns</sup>
HFW	80.99 ± 07.23 <sup>ns</sup>	60.19 ± 06.90 <sup>ns</sup>	89.50 ± 03.18 <sup>ab</sup>	370.72 ± 16.48 <sup>ns</sup>	125.30 ± 13.81 <sup>ns</sup>
HFIP	78.80 ± 11.32 <sup>ns</sup>	51.39 ± 04.01 <sup>ns</sup>	64.10 ± 01.80 <sup>b</sup>	390.72 ± 35.12 <sup>ns</sup>	132.10 ± 10.19 <sup>ns</sup>
CV(%)	11.35	16.78	11.55	16.99	14.71

\*SW: control diet (4% fat) + water *ad libitum*; SIP: control diet (4% fat) + *Ilex paraguariensis* extract *ad libitum*; HFW: fat diet (25% fat content, 1% cholesterol and 0.1% cholic acid) + water *ad libitum*; HFIP: fat diet (25% fat content, 1% cholesterol and 0.1% cholic acid) + *Ilex paraguariensis* extract *ad libitum*; CV: coefficient variation; <sup>ns</sup>not significant; <sup>ns</sup>Average values followed by the same letter show no significant statistical difference between them with the Tukey test at 1% probability of error ( $p < 0.01$ ).



**Figure 1.** Analysis of the GOT (A) and GPT (B) enzymes, and creatinine (C) determined by colorimetric method. Results were expressed in mean ± standard error. SW: standard diet + water; SIP: standard diet + *Ilex paraguariensis* extract; HFW: hyperlipidic diet + water; HFIP: hyperlipidic diet + *Ilex paraguariensis* extract.

total cholesterol, HDL cholesterol and glucose concentration indicates no significant changes induced by diet, *Ilex paraguariensis* or its interaction ( $p > 0.01$ , Table 4). Likewise, *Pon1* activity was not affected by diet, *Ilex paraguariensis* or its interaction ( $p > 0.05$ , Table 4).

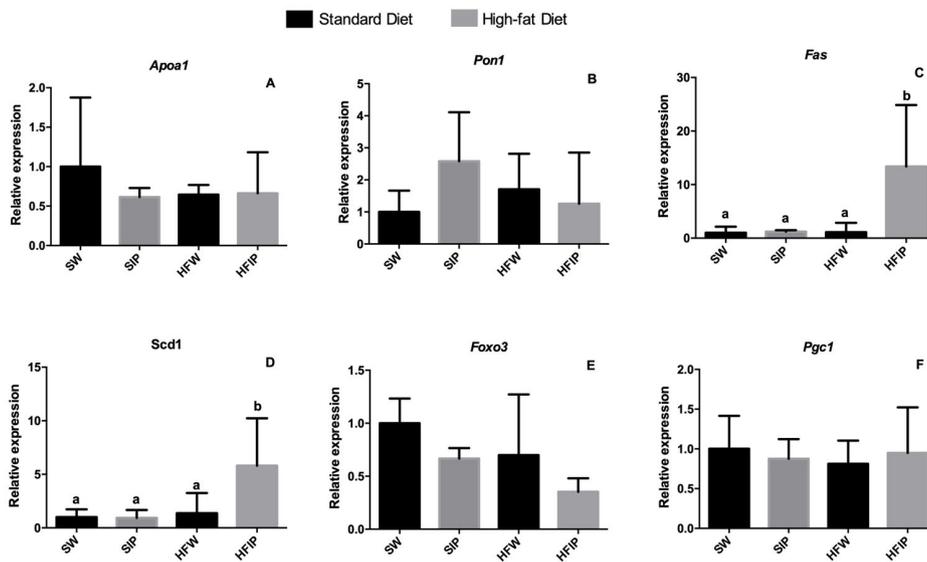
There was no difference in transaminase enzymes GOT (Figure 1A) and GPT (Figure 1B) activity between groups ( $p > 0.05$ ), indicating no change in liver function for groups treated with *Ilex paraguariensis* extract.

Creatinine levels (Figure 1C) were lower in the group treated with standard diet + *Ilex paraguariensis* extract when compared to the standard diet + water group ( $p < 0.01$ ). When only the rats fed the high-fat diet were analyzed, no difference ( $p > 0.05$ ) was observed between the groups treated with water or *Ilex paraguariensis* extract.

Regarding liver gene expression, *Apoa1* (Figure 2A), *Pon1* (Figure 2B), *Foxo3* (Figure 2E) and *Pgc1* (Figure 2F) expression were not different between groups. However, *Fasn* (Figure 2C) and *Scd1* (Figure 2D) were higher ( $p < 0.01$ ) in the high-fat + *Ilex paraguariensis* group.

#### 4 Discussion

We observed lower food intake in the high-fat compared to the standard groups. Kojima & Kangawa, (2005) reported that consumption of the high-fat diet induced a satiating effect, which is reflected in the reduced levels of the appetite-stimulating peptide ghrelin. Thomàs-Moyà et al. (2007) reported that rats fed with high-fat diet reduced their food intake, thus maintaining their energy intake and their body weight closer to those of the control rats. However, a marked increment of adipose depots was observed, which was greater in males than females (Thomàs-Moyà et al., 2007). The reduced intake in rats fed a high-fat diet is well known, and Hariri et al. (2010) found



**Figure 2.** *Ilex paraguariensis* extract intake effect of gene expression of *Apoa1* (A), *Pon1* (B), *Fas* (C), *Scd1* (D), *Foxo3a* (E), *Pgc1* (F), in the liver of female rats supplemented with standard and high-fat diet. SW: standard diet + water; SIP: standard diet + *Ilex paraguariensis* extract; HFW: hyperlipidic diet + water; HFIP: hyperlipidic diet + *Ilex paraguariensis* extract.

similar results regarding food intake, when feeding a high-fat diet to rats for a period of 26 days, in order to analyze the effect of diet as a facilitator to excessive weight gain.

Body weight gain was lower in rats supplemented with *Ilex paraguariensis* extract. Similar data was observed by Pang et al. (2008), indicating that dietary supplementation with *Ilex paraguariensis* extract administered to obese rats induced by high-fat diet was able to significantly reduce body weight gain, plasma triacylglycerides, glucose, along with reduction on anti-inflammatory markers (Luz et al., 2016), and antidepressant-like effects (Reis et al., 2014). According to Hetzler et al. (1990) the amount of caffeine present in *Ilex paraguariensis* could be responsible for the significant decrease in the amount of epididymal and abdominal fat. The caffeine has been shown to be able to cross the blood brain barrier and to increase the circulating concentrations of catecholamine (epinephrine) in humans, which is known to increase thermogenesis and lipolysis (Silva et al., 2011). In studies with mice fed a high-fat diet, *Ilex paraguariensis* has been suggested to promote satiety through several mechanisms, including induction and/or enhancement of intestinal glucagon-like peptide-1 (GLP-1), modulation of serum leptin levels and a possible direct central satiety-stimulatory effect (Resende et al., 2012). Data obtained from experiments conducted in diet-induced obesity models have shown that *Ilex paraguariensis* suppresses body weight gain and visceral fat accumulation and decreases serum levels of cholesterol, triglycerides, LDL cholesterol, glucose, insulin, pancreatic lipase and leptin (Gambero & Ribeiro, 2015). Therefore, our study further support the idea that *Ilex paraguariensis* extract treatment is able to reduce body weight gain in rats fed a high-fat diet.

We also observed that serum triglycerides were lower for rats fed high-fat diet and receiving *Ilex paraguariensis* extract than rats fed high-fat diet and receiving only water. Silva et al. (2011) reported, for rats that consumed the extract of gross mate, an increase in triglyceride levels of 21.4% compared to the control group. Increased triglyceride levels may be related to the lipolytic effect, resulting in increased mobilization of fatty acids from adipose tissue or intramuscular fat depots. However, Paganini-Stein et al. (2005) found in their study that animals treated with *Ilex paraguariensis* had a decrease in triglyceride levels compared to controls animals, in agreement with our current findings. Another study with mice treated with high-fat diet and *Ilex paraguariensis* also showed that *Ilex paraguariensis* reduced plasma triglycerides (Kang et al., 2012). Therefore, in addition to reducing body weight gain, *Ilex paraguariensis* results in decreased triglycerides levels which can be beneficial in the prevention of heart diseases.

The present study indicated that total cholesterol, HDL and *Pon1* activity were not affected by diet, *Ilex paraguariensis* extract or its interaction. Recently, Bravo et al. (2014) studying the effect of *Ilex paraguariensis* on serum lipids and antioxidant status of normocholesterolemic and hypercholesterolemic rats demonstrated that in the normocholesterolemic rats, the *Ilex paraguariensis* had no effect on serum lipids or antioxidant status. Despite that in the hypercholesterolemic rats, *Ilex paraguariensis* treatment also had no effect on HDL-c or protein carbonyls, it

showed a marked hypolipidemic action, decreasing triglycerides, total cholesterol and LDL-c, and serum malonaldehyde levels. These parameters had been increased after consumption of a high cholesterol diet, pointing out that the potential beneficial effect of *Ilex paraguariensis* on risk factors for cardiovascular diseases seems to be restricted to already hyperlipidemic animals. Paganini-Stein et al. (2005) were the first to report a significant reduction in serum total cholesterol and triglycerides of cholesterol-fed rats after administration of *Ilex paraguariensis* aqueous extract. Thomàs-Moyà et al. (2007) also reported that *Ilex paraguariensis* extract decreased the VLDL-LDL fraction in obese rats. A probable mechanism for the LDL-C lowering ability of *Ilex paraguariensis* is the blocking of cholesterol absorption in the small intestine and/or the inhibition of cholesterol synthesis in the liver, which can be attributed to the presence of saponins, phenolic compounds, flavonoids, and/or caffeine in the mate infusion (Morais et al., 2009).

The present study also analyzed the GOT and GPT enzymes, which were not different among groups. These enzymes have been investigated in order to verify possible liver damages induced by the high-fat diet. They are found within cells in the liver, but when there is some abnormal liver function, lead to an increase in these enzymes, which are released into the blood stream. Usually this increase is asymptomatic and transient, but some diseases caused by elevations of GOT and GPT levels are acute hepatitis A or B, fatty liver, obesity and hepatitis C (Russo & Jacobson, 2012). Despite that, creatinine was lower in the groups treated with *Ilex paraguariensis*. Creatinine has been used as a parameter for the initial evaluation of renal function in daily clinical practice. However, inferring that normal creatinine values always indicate normal kidney function can lead to significant errors, since early changes in glomerular filtration rate can be "hidden" in normal's creatinine values (Pinto et al., 2004). Therefore, higher creatinine level can reveal that renal function is disturbed, indicating that *Ilex paraguariensis* can be beneficial for kidney function.

The effects of *Ilex paraguariensis* extract on the gene expression of antioxidant/inflammatory markers have been studied in animal models (Matsumoto et al., 2009; Arçari et al., 2011). The analysis of liver tissue expression of *Pon1* and *ApoA1*, indicated no difference between diets and treatments. Similar results were demonstrated by Boaventura et al. (2012) in humans, indicating that there was no change in *Pon1* activity after a prolonged ingestion (90 days) of *Ilex paraguariensis* tea. However, Menini et al. (2007) reported increases in serum *Pon1* activity after ingesting 500 mL of *Ilex paraguariensis* infusion in healthy individuals. Bastos & Gugliucci (2009) demonstrated that the chlorogenic acid, the main phenolic constituent of the *Ilex paraguariensis*, can preserve *Pon1* against oxidative degradation *in vitro*. Results found by Morais et al. (2009) in humans corroborate with the data in this study, and also did not observe significant increases in *ApoA1* expression, suggesting that this can be due to the decreased HDL catabolism. The *ApoA1* is indicative of the amount of HDL in plasma or new HDL particles forming the potential to exert its function in reverse cholesterol transport to the liver. When there is an increase in *ApoA1*, it is suggested that there is an increase in hepatic production of nascent HDL particles (Lyssenko et al., 2013).

Diet-induced obesity is largely caused by disorders of fat metabolism, resulting in a massive accumulation of fat in various tissues. Lipid and energy metabolism are regulated by a complex network of signaling processes, therefore we investigated the mRNA expression of key genes regulating lipid metabolism such as *Scd1* and *Fasn* (Yang et al., 2012). *Fasn*, which encodes a rate limiting enzyme in fatty acid biosynthesis to produce palmitic acid; *Scd1*, which converts stearic acid to oleic acid, and glycerol-3-phosphate acyltransferase, which encodes the first committed enzyme in triglyceride and phospholipid synthesis (Horton et al., 2002). The mRNA expression levels of genes encoding lipogenic proteins such as *Scd1* and *Fas* increased in rats receiving *Ilex paraguariensis* and high-fat diet, can be suggest a rapid effect of the high-fat diet on lipogenesis. Expression of *Foxo* gene, which regulates gluconeogenesis, and *Pgc1* gene, a coactivator essential for coordinating gluconeogenesis and fatty acid oxidation, were not different among groups in this study. Additionally, *Pon1* and *Apoa1* gene expression were also not different, confirming the observations that serum levels of *Pon1* were not changed as well as of HDL.

## 5 Conclusion

In summary, the data presented here indicates that the use of *Ilex paraguariensis* extract prevents body weight gain, while improving the lipid parameters in rats fed a high-fat diet. In addition, *Ilex paraguariensis* modulates the expression of genes related in adipogenesis and lipogenesis in the obese state. Thus, the results from this study indicated that *Ilex paraguariensis* extract might be helpful in the treatment against obesity and its comorbidities.

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