Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
20(6): 956-961, Dez. 2010

Received 25 Nov 2009; Accepted 22 Apr 2010; Available online 26 Nov 2010.

Article

Effect of erva-mate (*Ilex paraguariensis* A. St.-Hil., Aquifoliaceae) on serum cholesterol, triacylglycerides and glucose in Wistar rats fed a diet supplemented with fat and sugar

***Franciele Przygodda,*[*,1] *Zenilde N. Martins,*[1] *Ana Paula A. Castaldelli,*[1] *Thais V. Minella,*[1] *Luciana P. Vieira,*[1] *Kathia Cantelli,*[1] *Jordana Fronza,*[1] *Maristela J. Padoin*[1]**

[1]*Laboratório de Neurociências e Comportamento, Centro de Ciências Biológicas e da Saúde, Universidade Estadual do Oeste do Paraná, Rua Universitária, 2069, Jardim Universitário, 85819-110 Cascavel-PR, Brazil.*

RESUMO: "Efeito da erva-mate (*Ilex paraguariensis* A. St.-Hil., Aquifoliaceae) sobre o colesterol, triacilglicerídeos e glucose em ratos Wistar com dieta alimentar suplementada com lipídeos e glicídeos" *Ilex paraguariensis* A. St.-Hil., Aquifoliaceae, é uma espécie nativa das regiões subtropicais e temperadas da América do Sul, usada em bebidas por infusão como chá, chimarrão e tererê. Para verificar os efeitos fisiológicos que a *I. paraguariensis* pode causar sobre o metabolismo de lipídeos e glicídeos em ratos Wistar, após a ingestão de chá de erva-mate, analisou-se quatro grupos experimentais: Grupo Lipídeo Controle (receberam água e dieta hiperlipídica); Grupo Lipídeo Ingestão (extrato de *I. paraguariensis* e dieta hiperlipídica); Grupo Glicídeo Controle (receberam água e dieta hiperglicídica); e Grupo Glicídeo Ingestão (extrato de *I. paraguariensis* e dieta hiperglicídica). Os animais receberam a dieta por 60 dias, de acordo com o grupo que pertenciam, sendo pesados semanalmente. Após esse período, foram avaliadas as concentrações de colesterol, glicose e triacilglicerídeos sanguíneos, e ainda, peso da gordura visceral. Os dados foram analisados estatísticamente. O nível de significância aceito foi p<0,05. Os resultados mostraram que a ingestão de erva-mate atua sobre o peso corpóreo, gordura visceral e taxas de glucose, colesterol e triacilglicerídeos plasmáticos.

Unitermos: *Ilex paraguariensis,* metabolismo de lipídios, metabolismo de glicídios.

ABSTRACT: *Ilex paraguariensis* A. St.-Hil., Aquifoliaceae, is a species native to the subtropical and temperate regions of South America, used in beverages prepared by infusion such as teas, chimarrão and tererê. To investigate the physiological effects of *I. paraguariensis* on the metabolism of fats and sugars in Wistar rats, following the ingestion of erva-mate tea, four experimental groups were constructed: Lipid Control Group (receiving water and high-fat diet); Lipid Tea Group (extract of *I. paraguariensis* and high-fat diet); the Sugar Control Group (water and high-sugar diet); and Sugar Tea Group (extract of *I. paraguariensis* and high-sugar diet). The animals received their particular diet for 60 days, and were weighed weekly. After this period, the plasma concentrations of cholesterol, glucose and triacylglycerides were determined, together with the weight of visceral fat. The data were subjected to statistical analysis with a significance level of p<0.05. The results show that the ingestion of erva-mate affected body weight, visceral fat and plasma glucose, cholesterol and triacylglyceride levels.

Keywords: *Ilex paraguariensis,* fat metabolism, sugar metabolism.

INTRODUCTION

Ilex paraguariensis A. St.-Hil., Aquifoliaceae, is a native species of the subtropical and temperate regions of South America, found more specifically in Argentina, Uruguay, Brazil and Paraguay (Gnoatto et al., 2007). Its importance centres on its use in drinks prepared as infusions, such as teas, chimarrão, tereré and juices (Carvalho, 1994). The plant makes a significant contribution to the national economy of some of the countries where it is found (Gnoatto et al., 2007; Croce, 2002), and it also plays a valuable socio-economic role since it is widely commercialised by small producers (Maccari Junior & Santos, 2000).

**E-mail: mjpadoin@unioeste.br, Tel. + 55 45 3220 3142.*

ISSN 0102-695X
DOI: 10.1590/S0102-695X2010005000045

The observed physiological effects of *I. paraguariensis* may be due to its reported chemical composition. According to Mazzafera (1997) and Bastos et al. (2006), beverages based on erva-mate contain numerous bioactive compounds, such as methylxanthines (caffeine and theobromine), saponins and phenolic compounds, among them flavonoids (quercetin and rutin) and phenolic acids (chlorogenic acid).

The methylxanthines are known for their stimulant (Nehlig, 1999), lipolytic and thermogenic effects (Acheson, 2005). Meanwhile, saponins have hypocholesterolemic potential, since they interfere in the metabolism of cholesterol and slow the absorption of fats by inhibiting pancreatic lipase (Han et al., 2002; Bastos et al., 2007). In the case of phenolic compounds, in addition to an antioxidant function there is evidence that the class of chlorogenic acids can decrease the hepatic production of glucose (Hemmerle et al., 1997) and its absorption (Johnston et al., 2003), resulting in a lower glycemic index (Cliford, 2004).

The physiological effects of these compounds from erva-mate have been described in a very limited number of studies, while much research has been carried out in relation to the chemical characterisation of *I. paraguariensis* due to the quality demanded by the consumer market. Therefore, there is a clear need for studies that extend understanding of the benefits and hazards of products based on erva-mate that are consumed regularly among the population, increasing the range of information and guaranteeing safe consumption, without risks to health. Taking this into account, the objective of this work was to study the effects of the chronic ingestion of *Ilex paraguariensis* tea on weight, body fat distribution and plasma concentrations of cholesterol and triglycerides in Wistar rats fed high-fat and high-sugar diets.

MATERIAL AND METHODS

Erva-mate

The erva-mate (*Ilex paraguariensis* A. St.-Hil., Aquifoliaceae) was processed and prepared by a local herbalist, by the traditional method for commercial supply, with the plants used being obtained from the municipality of Cascavel-PR, in the area around Rio do Salto, GPS coordinates 250 04' 20'' S and 530 19' 54'' W. A sample was identified by Dr. Lívia Godinho Temponi, of the Universidade Estadual do Oeste do Paraná (Unioeste), Cascavel Campus, and a voucher specimen was deposited in the herbarium of the Universidade do Oeste do Paraná (HUNOP), under registration number 4234.

Animals

The study was carried out in 96 Wistar rats (male and female), aged 60 days at the beginning of the experiment, obtained from the central animal facility of the institution where the work was carried out. The animals were kept in cages, with access to water and standard rat chow (Nuvital®) *ad libitum*, at a temperature of approximately 25 °C and a photoperiod of 12 h light/12 h dark.

Animal procedure

The animals were allocated to four groups, each comprising twelve males and twelve females. Animals fed a high-fat diet formed the control group (LC), while the test group for lipids (LT) received the erva-mate tea in addition to the high-fat diet. Similarly, a group of animals fed the high-sugar diet were designated the control group (SC), and those that received the erva-mate tea and the high-sugar diet comprised the test group for sugar (ST).

Preparation of the erva-mate tea

The tea was prepared twice a day, early in the morning and at night, and used on the day of preparation, due to the duration of action of compounds in erva-mate. Five grams of *Ilex paraguariensis* were added to 100 mL of boiling water, in an adaptation of the method of Schinella et al. (2000). The erva-mate was left to infuse in the boiling water for approximately 5 min, before filtering the liquid part. After cooling, the tea was transferred to the feeding bottles of each cage. The animals were given access to water or *Ilex paraguariensis* tea *ad libitum*. The mean liquid intake for groups LT and ST was 25 mL/day/animal, while that of control groups (LC and SC) was 35 mL/day/animal.

Preparation of supplemented diets

The supplemented diets were prepared according to Reeves et al. (1993) of the American Institute of Nutrition (AIN), with some modifications made with the aim of producing a diet with the lipid and sugar fractions enriched. Table 1 gives details of the diets used in this study. The diets were prepared every two days or when required, and were kept in a refrigerator until they had been completely consumed. The diets were provided *ad libitum* to the appropriate experimental group.

Determination of plasma cholesterol, glucose and triacylglycerides

At the end of the experimental period of 60 days, the rats were fasted for 12 h and sacrificed by decapitation. Five mL of blood was collected from each animal in heparinised assay tubes; this was then centrifuged and the plasma harvested. The plasma was stored in a freezer at -20 ºC for later biochemical analyses. Cholesterol and triacylglycerides were measured by enzymatic methods using the Colesterol Liquiform and Triglicérides Liquiform (Labtest) reagents according to the manufacturer's instructions. Glucose was measured by collecting a drop of blood from the tail of each animal and transferring it to a test strip. The glucose level was then read using an Accu-chek® Advantage II glucose meter (Roche Diagnostics).

Table 1. Composition of the diets consumed by Wistar rats during the experimental period.

Ingredients	High-fat diet[1]	High-sugar diet[2]
Casein	200	200
Soya oil	40	40
Animal fat	333	0
Starch	232	565
Saccharose	100	100
Fibre	50	50
Vitamin Mix	10	10
Mineral Mix	35	35
Choline	2.5	2.5
tert-Butylhydroquinone (TBHQ)	0.14	0.14
Total in grams	1002.64	1002.64

[1]Experimental groups LC and LT; [2]Experimental groups SC and ST

Removal of adipose tissues

Following sacrifice and blood collection, the peritoneum of each animal was opened and the retroperitoneal (RET) and epididymal (EPI) white adipose tissues were removed; these were then weighed on a precision analytical balance prior to morphometric analyses.

Statistical analysis

The normal distribution of the data obtained was analysed by Shapiro-Wilk and D'Agostino & Pearson tests, and the homogeneity of the variances was analysed by Welch's test. Normality and homogeneity were observed in all groups. Comparisons were made by analysis of variance (ANOVA), followed by Newman-Keuls test, when appropriate. The results were expressed as means±standard deviation. All analyses were carried out using the program *Graphpad Prism 4.0,* and a significance level of 5% was adopted ($p<0.05$).

Ethical questions

The experimental protocol was submitted to the Committee for Ethics in Animal Research (Cepeea/Unipar) and approved under licence number 1019/2008.

RESULTS AND DISCUSSION

The data from the Lipid and Sugar Controls, and the Lipid and Sugar Tea groups are presented in Tables 2 and 3. There was no apparent evidence of toxic effects associated with the tea, and no anatomopathological abnormalities were noted during dissection of the animals.

It can be seen from Tables 2 and 3 that there were statistically significant differences ($p<0.01$) between the weight gain in males of the Lipid Control Group (LC) and that observed in the Lipid Tea Group (LT), as well as between the Sugar Control Group (SC) and the Sugar Tea Group (ST).

With regard to the deposition of fat in the epididymal white adipose tissue in males, the LT presented significantly lower mean values than the LC ($p<0.01$). In addition, there was a trend ($p<0.089$) towards a reduction in the deposition of fat in the retroperitoneal white adipose tissue among males of the ST; however, it would be necessary to increase the number of animals per group or the duration of ingestion in order to confirm whether this difference between the groups is statistically significant. The retroperitoneal fat in the Lipid Group ($p=0.53$) and epididymal fat in the Sugar Group ($p=0.19$) were not significantly different among the males.

Among the females, statistically significant differences ($p<0.01$) were observed between the weight gain in Lipid Group and that in Sugar Group, with a greater weight gain in the control groups. When the fat deposition in the retroperitoneal white adipose tissue was analysed in the females, groups LT and ST exhibited significantly lower mean values compared to their respective controls ($p<0.01$).

These results are in agreement with those reported by Andersen & Fogh (2001), who found that erva-mate, when combined with other herbs such as guaraná (*Paullinia cupana*) and damiana (*Turnera diffusa* var. *aphrodisiaca*), could delay gastric emptying and help with weight reduction, increasing the duration of satiety and leading to a correspondingly lower food intake.

Furthermore, the results of this work agree with those of Pang et al. (2008), who investigated the efficacy of an extract of erva-mate in reducing the accumulation of visceral fat, reporting that the extract of *I. paraguariensis* can decrease the appetite and prevent obesity, causing a reduction in the weight of visceral fat, the size of adipocytes and the gain in body weight. However, in this work the changes in the body weight of the animals treated with erva-mate cannot be attributed directly to the inhibition of appetite, since there was no control over the quantity of the diet ingested by the groups.

These results are also in agreement with the broader literature, since research in animals and epidemiological studies indicate that the consumption of caffeine can reduce body weight and adiposity, by increasing thermogenesis, lipid oxidation and lipolysis. One suggested mechanism for the thermogenic and lipolytic effects of caffeine is an increase in the circulating concentration of epinephrine, which accelerates the metabolism and stimulates the mobilisation of free fatty acids from the tissues or intramuscular stores. Another mechanism proposed for the lipolytic action involves the inhibition by caffeine of phosphodiesterase, which reduces the intracellular breakdown of cyclic AMP (cAMP), resulting in an increase in the concentration of cAMP and a subsequent increase in lipolysis (Acheson, 2005).

With regard to the biochemical parameters investigated, lower mean values were observed in the serum concentrations of glucose and triglycerides in males from groups LT and ST, treated with erva-mate, when compared to their respective controls. However, in the case of serum cholesterol a difference ($p<0.05$) was found only in males of the LT group when compared to LC, and there was no significant difference ($p=0.82$) between the concentrations in groups ST and SC, as shown in Tables 1 and 2.

By contrast, among the females (Tables 1 and 2), the plasma levels of all the parameters analysed were found to be significantly different in Lipid and Sugar Groups. Groups LT and ST presented lower mean serum concentrations of glucose (Lipid Group $p<0.01$; Sugar Group $p<0.01$), cholesterol (Lipid Group $p<0.01$; Sugar Group $p<0.05$) and triacylglycerides (Lipid Group $p<0.05$; Sugar Group $p<0.01$) when compared to their respective control groups.

In a preliminary study, we treated two groups of animals with a standard diet without the addition of supplements. One of the groups was given water (Control Group 'GCO'), while the other drank an infusion of *I. paraguariensis* tea (Tea Group 'GI'). Among the parameters analysed it was found that the level of triglycerides in GI females was lower than in GCO females ($p<0.05$). However, the glucose concentration in GI females was higher than that in GCO females ($p<0.05$). Meanwhile, the level of cholesterol was not significantly different between the two groups. In the males the level of cholesterol was lower in the GCO animals compared to the GI group ($p<0.03$). However, the other two parameters measured, triacylglycerides and glucose, were not significantly different (Castaldelli & Padoin, 2008).

The results of the present study agree with those of Stein and collaborators (2005), who showed that animals who received a normal diet had a total cholesterol level of 88.9±1.2 mg/dL and a level of triacylglycerides of 68.8±5.2 mg/dL. These values are much lower than those seen in animals from the group on a hypercholesterolemic diet: 161.0±12.9 mg/dL total cholesterol and 180.7±23.8 mg/dL triacylglycerides. Meanwhile, among the animals maintained on a hypercholesterolemic diet and treated with *I. paraguariensis* it can be seen that there was a decrease in both parameters in comparison with the animals given a hypercholesterolemic diet alone, with a mean total cholesterol level of 117.5±5.0 mg/dL and a mean triacylglycerides concentration of 71.5±2.2 mg/dL, although the levels were still high in relation to the animals in the control group. Pang et al. (2008) also showed that the extract of *I. paraguariensis* decreased the blood and liver concentrations of lipids, as well as levels of glucose. Differences between males and females in the effect of *I. paraguariensis* on cholesterol levels may have been due to the females having double the renal capacity to convert mevalonate into CO_2, compared to that of males, while the latter have an increased capacity to convert plasma cholesterol (Feingold et al., 1980).

The findings of the present investigation also agree with results reported by Melo and colleagues (2007) and Arçari (2009), where intervention with *I. paraguariensis* in mice maintained on a high-fat diet lead to an improvement in parameters such as body weight, glycemia, liver weight, response to insulin, cholesterol, triacylglycerides, LDL-cholesterol and the increase in HDL-cholesterol, therefore exerting a positive influence on several biochemical markers related to obesity. The mechanism by which these markers influence physiological processes is related to the action of saponins, which interfere in the metabolism of cholesterol and reduce the absorption of fats derived from the diet, acting mainly via inhibition of pancreatic lipase (Han et al., 2002; Bastos et al., 2007).

Other authors have suggested that chlorogenic acid can reduce glycemia by inhibiting the action of glucose-6-phosphatase (Bassoli et al., 2008). This enzyme is involved in the final step of gluconeogenesis and glycogenolysis, resulting in free glucose that is exported to the blood stream, thereby regulating blood homeostasis (Arion et al., 1998). Furthermore, the polyphenols present in erva-mate may interfere in the intestinal absorption of glucose by reducing gene expression of the glucose co-transporter SGLT1, due to the dissipation of sodium ions on the intestinal membrane (Hossain et al., 2002). More specifically, flavonoids may reduce the absorption of glucose, since they bind competitively to a site on SGLT1, impeding the transport of glucose (Cermak et al., 2004).

Oliveira (2008) investigated the influence of erva-mate on parameters related to diabetes mellitus and glucose metabolism, and did not find a significant reduction in glycemia. This may have been partly a result of the animal model used, which exhibits high glycemic levels. The activity of the enzyme hepatic glucose-6-phosphatase was also unchanged by the treatment with erva-mate, although there was a reduction in gene expression for SGLT1 in the small intestine, suggesting that the bioactive compounds in erva-mate can reduce the absorption of glucose.

Thus, the results presented in this work suggest that the ingestion of erva-mate significantly influences several physiological parameters, reducing the body weight, the amount of visceral fat and plasma levels of glucose, cholesterol and triglycerides.

Table 2. Effect of erva-mate tea on animals fed with the high-fat diet.

Parameters	Males			Females		
	LC n=12	LT n=12	*p**	LC n=12	LT n=12	*p**
Mean weight gain (g)	196±17.05	152±34.88	*p*<0.01	111±17.16	65±31.60	*p*<0.01
Retroperitoneal fat (g)	12.75±3.63	11.56±5.24	*p*=0.53	20.08±7.73	11.46±4.73	*p*<0.01
Epididymal fat (g)	7.37±2.88	13.24±1.48	*p*<0.01	----	----	----
Glucose (mg/dL)	101.4±0.71	89.8±0.77	*p*<0.01	112.9±10.0	86.5±8.0	*p*<0.01
Total cholesterol (mg/dL)	113.1±63.2	64.8±32.2	*p*<0.05	110.1±40.5	62.1±24.2	*p*<0.01
Triacylglycerides (mg/dL)	181.1±41.6	148.5±51.8	*p*<0.01	194.7±45.2	131.8±64.9	*p*<0.05

The values are expressed as mean±standard deviation. LC (Lipid Control Group): animals received the high-fat diet and water; LT (Lipid Tea Group): animals received the high-fat diet and erva-mate tea.*Probability value for ANOVA.

Table 3. Effect of erva-mate tea on animals fed with the high-sugar diet.

Parameters	Males			Females		
	SC n=12	ST n=12	*p**	SC n=12	ST n=12	*p**
Mean weight gain (g)	194±24.30	129±31.15	*p*<0.01	98±14.99	72±24.51	*p*<0.01
Retroperitoneal fat (g)	11.42±6.67	6.74±6.65	*p*<0.089	20.49±2.58	13.18±4.56	*p*<0.01
Epididymal fat (g)	7.33±4.02	6.28±1.69	*p*=0.19	----	----	----
Glucose (mg/dL)	94±11.8	57.6±10.7	*p*<0.01	101.8±13.5	77.8±10.9	*p*<0.01
Total cholesterol (mg/dL)	51.7±24.9	50.6±24.8	*p*=0.82	79.9±35.7	56.4±22.2	*p*<0.05
Triacylglycerides (mg/dL)	128.4±59.8	62.3±28.0	*p*<0.01	161.2±77.1	58.5±14.8	*p*<0.01

The values are expressed as mean±standard deviation. SC (Sugar Control Group): animals received the high-sugar diet and water; ST (Sugar Tea Group): Animals received the high-sugar diet and erva-mate tea. *Probability value for ANOVA.

REFERENCES

Acheson KJ 2005. Caffeine and insulin sensitivity. *Metab Syndr Relat Disord 3*: 19-25.

Andersen T, Fogh J 2001. Weight loss and delayed gastric emptying following a South American herbal preparation in overweight patients. *Hum Nutr Dietet 14*: 243-250.

Arçari DP 2009. *Efeitos biológicos do consumo de chá-mate* (Ilex paraguariensis) *frente a obesidade em camundongos*. São Paulo, 74p. Master's degree dissertation, Postgraduate Programme in Nutrition in Health, Universidade de São Paulo.

Arion WJ, Canfield, WK, Ramos FC, Su ML, Burger HJ, Hemmerle H, Schubert G, Below P, Herling AW 1998. Chlorogenic acid analogue S 3483: a potent competitive inhibitor of the hepatic and renal glucose-6-phosphatase systems. *Arch Biochem Biophys 351*: 79-285.

Bassoli BK, Cassolla P, Borba-Murad GR, Constantin J, Salgueiro-Pagadigorria CL, Bazotte RB, Da Silva RS, De Souza HM 2008. Chlorogenic acid reduces the plasma glucose peak in the oral glucose tolerance test: effects on hepatic glucose release and glycemia. *Cell Biochem Funct 26*: 320-328.

Bastos DHM, Fornari AC, Queiroz YS, Torres EAFS 2006. Bioactive compounds content of chimarrão infusions related to the moisture of yerba maté (*Ilex paraguariensis*) leaves. *Braz Arch Bio Tech 49*: 399-404.

Bastos DHM, Saldanha LA, Catharino RR, Sawaya ACHF, Cunha IBS, Carvalho PO, Eberlin MN 2007. Phenolic antioxidants identified by ESI-MS from yerba mate (*Ilex paraguariensis*) and green tea (*Camelia sinensis*) extracts. *Mol 12*: 423-432.

Carvalho PER 1994. *Ilex paraguariensis* Saint-Hilaire; erva-mate. In: Carvalho, PER. *Espécies florestais brasileiras: recomendações silviculturais, potencialidades and uso da madeira*. Colombo: EMBRAPA-CNPF; Brasília: EMBRAPA-SPI, p. 280-287.

Castaldelli APA, Padoin MJ 2008. *Efeito da erva mate* (Ilex paraguariensis) *no comportamento e fisiologia de ratos Wistar*. Cascavel, 54p. Monograph, Biological Sciences undergraduate degree course, Universidade Estadual do Oeste do Paraná.

Cermak R, Landgraf S, Wolffram S 2004. Quercetin glucoside inhibits glucose uptake into brush-border-membrane vesicles of porcine jejunum. *Br J Nutr 91*: 849- 855.

Cliford NM 2004. Diet-derived phenols in plasma and tissue and their applications for health. *Plant Med 70*: 1103-1114.

Croce DM 2002. Caracterização físico-química de extratos de erva-mate (*Ilex paraguariensis* St. Hil.) no estado de Santa Catarina. *Cienc Florestal 12*: 107-113.

Feingold KR, Wiley MH, Searle GL, Machida BK, Siperstein MD 1980. Sex difference in human mevalonate metabolism. *J Clin Invest 66*: 367-366.

Gnoatto SCB, Bassani VL, Coelho GC, Schenkel EP 2007. Influência do método de extração nos teores de metilxantinas em erva-mate (*Ilex paraguariensis* A. St.-Hil., Aquifoliaceae). *Quim Nova 30*: 304-307.

Han S, Zheng Z, Ren D 2002. Effect of *Salvia miltiorrhiza* on left ventricular hypertrophy and cardiac aldosterone in spontaneously hypertensive rats. *J of Huazhong Univ of Sci and Tech 22*: 302-304.

Hemmerle H, Burger HJ, Below P, Schuber G, Rippel R, Schindler PW, Paulus E, Herling AW 1997. Chlorogenic acid and synthetic chlorogenic acid derivatives: novel inhibitors of hepatic glucose-6-phosphate translocase. *J Med Chem 40*: 137-145.

Hossain SJ, Kato H, Aoshima H, Yokoyama T, Yamada M, Hara Y 2002. Polyphenol-induced inhibition of the response of Na^+/glucose cotransporter expressed in *Xenopus oocytes*. *J Agric Food Chem 50*: 5215-5219.

Johnston K, Clifford M, Morgan LM 2003. Coffee acutely modifies gastroinstestinal hormone secretion and glucose tolerance in humans: glycemic effects of chlorogenic acid and caffeine. *Am J Clin Nutr 78*: 728-733.

Maccari Junior A, Santos APR 2000. Parâmetros tecnológicos para a utilização industrial da erva-mate. In: Maccari Junior A, Mazuchowski JZ *Produtos alternativos e desenvolvimento da tecnologia industrial na cadeia produtiva da erva-mate*. Curitiba: SEAB. p. 43-68.

Mazzafera P 1997. Mate drinking: caffeine and phenolic acid intake. *Food Chem 60*: 67-71.

Melo SS, Nunes NSI, Baumgarten C, Tressoldi C, Faccin G, Zanuzo K, Michels MK, Da Cunha N, Specht S, Da Silva MW 2007. Efeito da erva-mate (*Ilex paraguariensis* A. St. Hil) sobre o perfil metabólico em ratos alimentados com dietas hiperlipídicas. *Alim Nutr 18*: 439-447.

Nehlig A 1999. Does caffeine lead to psychological dependence? *Chem Tech 2*: 30-35.

Oliveira DM 2008. *Influência da ingestão de erva-mate* (Ilex paraguariensis) *sobre parâmetros relacionados ao diabetes mellitus e metabolismo de glicose em ratos Wistar*. São Paulo, 78p. Master's degree dissertation, Post-graduate Programme in Public Health, Universidade de São Paulo.

Pang J, Choi Y, Park T 2008. *Ilex paraguariensis* extract ameliorates obesity induced by high-fat diet: Potential role of AMPK in the visceral adipose tissue. *Arch Biochem Biophys 476*: 178-185.

Reeves PG, Nilsen FH, Fahey Junior GC 1993. AIN-93 Purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr 23*: 1939-1951.

Schinella GR, Troiani G, Dávila V, de Buschiazzo PM, Tournier HA 2000. Antioxidant effects of an aqueous extract of *Ilex paraguariensis*. *Biochem Biophys Res Commun 269*: 357-360.

Stein FLP, Schmidt B, Furlong EB, Soares LAS, Soares MCF, Vaz MRC, Baisch ALM 2005. Vascular responses to extractable fractions of *Ilex paraguariensis* in rats fed standard and high-cholesterol diets. *Bio Res Nutr 7*: 146-156.