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Effect of yerba mate (*Ilex paraguariensis*) extract on the metabolism of diabetic rats

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ABSTRACT

The relationship between metabolic disturbances and clinical events related to diabetes is well known. Yerba mate has presented a potential use as preventive and therapeutic agent on diabetes. The aim of this study was to evaluate the effect of yerba mate on different tissues of diabetic rats, focusing on energetic metabolism. Diabetes was induced by streptozotocin, followed by daily yerba mate treatment. After 30 days, the animals were euthanized to evaluate metabolic parameters on liver, adipose tissue, muscle and serum. The results showed mate treatment promoted a decrease in retroperitoneal adipose tissue in healthy animals. Muscle weight returned to control levels in diabetic rats treated with mate. There was improvement on serum glucose, creatinine, urea and total protein levels associated with mate treatment. Muscle parameters, such as glucose uptake and carbon dioxide production, were improved by mate treatment to control levels. The results evidenced the beneficial actions mate can have on metabolic disturbances of diabetes.

1. Introduction

Yerba mate (*Ilex paraguariensis*) belongs to Aquifoleaceae family and is a native species from South America. Its leaves and stems are used to prepare a tea-like beverage widely consumed by the local population. Studies have shown that yerba mate has potential properties, performing improvements on oxidative imbalance and lipid metabolism [1,2]. These properties are mainly related to its bioactive fractions, represented by methylxanthines, saponins and polyphenols.

Large quantities of phenolic compounds been found in yerba mate [3]. This fraction has been presenting protective effect against oxidative damage to lipids and DNA [1]. The lipid peroxidation, particularly in blood vessels, is mitigated by treatment with mate in face of high-fat diet [4,5].

Alkaloids, such as xanthines and methylxanthines, represented by caffeine and theobromine, are also found in yerba mate [6,7]. According to Yamada et al. [8], the stimulating action of caffeine is related to the activation of sympathetic autonomic nervous system. Also, Pang et al. [9] observed decrease in caloric intake and inflammatory markers, and an increase in signaling molecules of satiety (leptin).

Saponins also comprise one of *I. paraguariensis'* fractions [10]. Resende et al. [11] investigated its specific action *in vivo*, demonstrating that this fraction has effects on lipid metabolism. Indeed, they found increase in fecal excretion of lipids and, at the same time, stimulation of adipogenesis in adipose tissue.

Metabolic unbalance events have their association with diabetes already described: change in sympathetic nervous system response, oxidative stress, metabolic syndrome, dyslipidemia and atherosclerosis. According to the World Health Organization (WHO), the overall prevalence of diabetes mellitus was estimated in 9% in 2014 and rising [12]. So, faced the alarming prevalence of diabetes and the potential use of *I. paraguariensis'* extract as additional therapy. The aim of this study was to evaluate the muscle and liver metabolism of diabetic rats after treatment with yerba mate.

2. Materials and methods

2.1. Animals and experimental design

Male Wistar rats (8 weeks old) were divided into four groups:

Abbreviations: C group, Control group; CM group, Control with treatment group; D group, diabetes group; DM group, diabetes with treatment group; STZ, streptozotocin; RAT, retroperitoneal adipose tissue; EAT, epidydimal adipose tissue; SM, soleus muscle; L, liver; ^{14}C, carbon-14; KRB, Krebs Ringer bicabornate; [U-^{14}C]-glucose, uniformly labeled carbon-14 glucose; [^{14}C-2-DG], 2-deoxi-D-[1-^{14}C]-glucose; GLP-1, similar to glucagon peptide; PEPCK, phosphoenolpyruvate carboxykinase enzyme; ApoA-I, apolipoprotein A1 structure; AST, aspartate aminotransferase; ALT, alanine aminostransferase; HMG-CoA, 3-hidroxi-3-methyl-glutaril-CoA reductase; GLUT, glucose transporter

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control (C), control treated with mate (CM), diabetes (D) and diabetes treated with mate (DM) (nine individuals per group).

The diabetic groups (D and DM) received Streptozotocin (STZ) (65 mg/kg) in citrate Buffer (0.1 M, pH 4,5) intraperitoneally. Control groups (C and CM) received vehicle. Hyperglycemia was confirmed in diabetic individuals (above 250 mg/dL).

2.2. Plant materials and aqueous extract preparing

Yerba mate was obtained from commercial market (Ervateira Barão de Cotegipe, RS, Brazil/ Lot /12/14/3), heated up to 80 °C (70 g/L of water), left for 15 min, filtered, cooled and offered as tea immediately after being prepared. The treatment was performed for 30 days. All groups received 1 L of aqueous extract (70 g/L) for *ad libitum* consumption, and DM group received it diluted three times (23,3 g/L), since diabetic individuals tend to ingest it about three times more than controls when *ad libitum* consuming.

2.3. Euthanasia and tissue proceedings

Tissues were collected and processed according to specific protocols. Morphometric parameters were expressed as tissue index (tissue weight / body weight): retroperitoneal adipose tissue (RAT), epididymal adipose tissue (EAT), soleus muscle (SM), and liver (L). All euthanasia proceedings were approved by the Research Ethics Committee (protocol number: 09-055) and performed in the Physiology Department (Federal University of Rio Grande do Sul).

Serum parameters were determined using commercial kits (Labtest ®, Minas Gerais, Brazil). Insulin levels were assayed using ELISA kit (Millipore ®).

2.4. ^{14}C glucose incorporation into carbon dioxide ($^{14}CO_2$)

The ^{14}C glucose incorporation into $^{14}CO_2$ was performed according to Torres et al. [13]. Tissues were incubated at 37 °C for 60 min in Krebs–Ringer buffer (KRB, pH 7.4), 0.1 μCi [U-^{14}C] glucose (55 mCi/mmol, Amersham, Little Chalfont, UK), and glucose 5 mM. To capture the $^{14}CO_2$, a 3 MM-Whatman paper were placed above the incubation medium ($^{14}CO_2$ wells). The oxidation assay was stopped by trichloracetic acid 50% (v/v) into tissue well, and NaOH (2.0 M) into the $^{14}CO_2$ wells. The paper contents were transferred liquid scintillation mixture and the radioactivity was measured (1209 Liquid Scintillation Counter). Results expressed as nmol of ^{14}C glucose incorporated into CO_2 per g of tissue per hour.

2.5. ^{14}C-glucose incorporation into glycogen and lipid assay

Samples from ^{14}C glucose incorporation into $^{14}CO_2$ assay were used to glycogen and lipid production measurement. Radioactivity was assessed in scintillation liquid mixture.

To glycogen content, samples were placed into KOH 30% (w/v) solution and boiled for 15 min. Ethanol (96°GL) was added for glycogen precipitation and the samples were washed two times. Data described as nmol ^{14}C glucose converted to glycogen per g of tissue per hour.

Lipid content was measured adding chloroform:methanol (2:1, v/v), followed by extraction performed according to Folch et al. [14]. Results expressed as nmol ^{14}C glucose converted to lipid per g of tissue per hour.

2.6. Determination of glycogen, triacylglycerol and cholesterol liver concentrations

Glycogen content was measured through KOH (30%) solution boiling, alcoholic precipitation, washing, HCl (4 M) boiling, and glucose detection by colorimetric kit (Labtest®). Determination of hepatic triacylglycerol was enzymatically assessed using colorimetric kits (Labtest), and the cholesterol was measured according to Folch et al. [14].

2.7. Glucose uptake

Soleus muscle was incubated (37 °C/1 h) into tubes containing KRB (pH 7.4) and 0.1 μCi of 2-deoxi-D-[1-^{14}C]-glucose (^{14}C-2-DG) (55 mCi/mmol, Amersham). Tissue was disrupted in distilled water, then samples from incubation medium and tissue fluid were collected. Results expressed as dpm/ml of tissue fluid per g of tissue divided by dpm/ml of incubation medium (T/M ratio).

2.8. Statistical analyses

Data expressed as mean and standard deviation (SD). Differences among groups were tested by *two-way* ANOVA and Bonferroni post hoc test (Prism® software, 6 edition). Values of $p < 0.05$ were considered significant. Nonparametric data were tested by Kruskal Wallis (KW) and Dunn's post hoc, data expressed as median and 25/75 interquartile range (IQR).

3. Results and discussion

3.1. Morphometry

Regarding the mate treatment, a reduction in the retroperitoneal adipose tissue weight, but not in epididymal adipose tissue, was observed in control group (Table 1). Lafontan [15] and Mauriege et al. [16] described peculiarities and different responses that fat depots can display according to the number and type of catecholamines' receptors. This heterogeneity could explain the observed effect probably through the lipolytic action of methylxanthines and saponins. There were no effects related to treatment in diabetic group (DM).

Resende et al. [11], showed that the fraction rich in methylxanthines was able to improve the lipid profile and increase lipolysis in

Table 1
Morphometric parameters on Yerba Mate treated diabetic rats.

Parameter	Experimental Group				ANOVA
	C	CM	D	DM	
Body weight (g)	406.8 ± 36.37 ^{a}	410.2 ± 24.84 ^{a}	273.7 ± 26.58 ^{b}	277.9 ± 34.29 ^{b}	# (P < 0.0001)
RAT Index *10^4	75.46 ± 15.35 ^{a}	62.25 ± 10.85 ^{b}	6.29 ± 1.96 ^{c}	7.84 ± 4.58 ^{c}	# (P < 0.0001) & (P = 0.0347)
EAT Index *10^4	9.70 ± 1.49 ^{a}	8.86 ± 1.20 ^{a}	3.37 ± 1.40 ^{b}	3.91 ± 1.87 ^{b}	# (P < 0.0001)
L Index *10^2	3.33 ± 0.36 ^{a}	3.27 ± 0.13 ^{a}	4.03 ± 0.27 ^{b}	4.12 ± 0.14 ^{b}	# (P < 0.0001)
SM Index *10^3	0.91 ± 0.17 ^{a,b}	1.01 ± 0.06 ^{a,b}	0.87 ± 0.27 ^{b}	1.12 ± 0.20 ^{a}	# (P = 0.0070)

Two-way ANOVA and Bonferroni post hoc. Sources of variation are represented as model (#), treatment (*) and model-treatment interaction (&). Data expressed by mean and standard deviation (SD). Same letters indicate equivalence. $p < 0.05$ considered significant. n = 9 per group. RAT, retroperitoneal adipose tissue; EAT, epididymal adipose tissue; SM, soleus muscle; L, liver.