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Yerba Maté (*Ilex paraguariensis*) Aqueous Extract Decreases Intestinal SGLT1 Gene Expression but Does Not Affect Other Biochemical Parameters in Alloxan-Diabetic Wistar Rats

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Yerba maté (*Ilex paraguariensis*) is rich in polyphenols, especially chlorogenic acids. Evidence suggests that dietary polyphenols could play a role in glucose absorption and metabolism. The aim of this study was to evaluate the antidiabetic properties of yerba maté extract in alloxan-induced diabetic Wistar rats. Animals ($n = 41$) were divided in four groups: nondiabetic control (NDC, $n = 10$), nondiabetic yerba maté (NDY, $n = 10$), diabetic control (DC, $n = 11$), and diabetic yerba maté (DY, $n = 10$). The intervention consisted in the administration of yerba maté extract in a 1 g extract/kg body weight dose for 28 days; controls received saline solution only. There were no significant differences in serum glucose, insulin, and hepatic glucose-6-phosphatase activity between the groups that ingested yerba maté extract (NDY and DY) and the controls (NDC and DC). However, the intestinal SGLT1 gene expression was significantly lower in animals that received yerba maté both in upper ($p = 0.007$) and middle ($p < 0.001$) small intestine. These results indicate that bioactive compounds present in yerba maté might be capable of interfering in glucose absorption, by decreasing SGLT1 expression.

KEYWORDS: *Ilex paraguariensis*; polyphenols; chlorogenic acid; diabetes mellitus; glucose-6-phosphatase; SGLT1

INTRODUCTION

Yerba maté (*Ilex paraguariensis*) is a plant from the subtropical region of South America, widely consumed in Brazil, Argentina, Paraguay, and Uruguay. The leaves are dried or dried and roasted to prepare different beverages (*1*). This plant has been reported to have various biological activities, mostly related to antioxidant activity, which is attributed to its high polyphenol content (*2*−*10*).

Phenolic compounds present in yerba maté are mainly chlorogenic acids (CGAs) and hydroxycinnamic acids (*11*−*13*), which are also the main polyphenols in coffee (*14*−*16*). Besides polyphenolic compounds, yerba maté leaves contain several other bioactive substances that show important biological activities, such as xantines and saponins (*1*).

Mounting evidence suggests that dietary polyphenols may modulate glucose absorption and metabolism (*14*−*31*). Cohort studies have reported that high coffee consumption might be associated with a lower risk of type 2 diabetes, and this effect may be attributed to CGAs (*20*). Indeed, pure 5-caffeoylquinic acid (5-CQA) and some synthetic derivatives were able to reduce blood glucose in animals (*21*−*25*).

The mechanisms of action of CGAs in glucose metabolism are not completely elucidated. Some synthetic derivatives of the 5-CQA have been shown to inhibit glucose-6-phosphatase (G-6-Pase) *in vitro* and *in vivo* after intravenous or intraperitoneal administration, reducing the glycemic peak (*21, 22*). However, it is not clear whether they would display the same effect when administered orally (*25*). G-6-Pase plays a key role in catalyzing the last step of glycogenolysis and gluconeogenesis (*26*).

Another proposed mechanism is associated with the inhibition of glucose transport through intestinal cells. Recently, it was shown that aqueous extracts of plants rich in CGAs reduced

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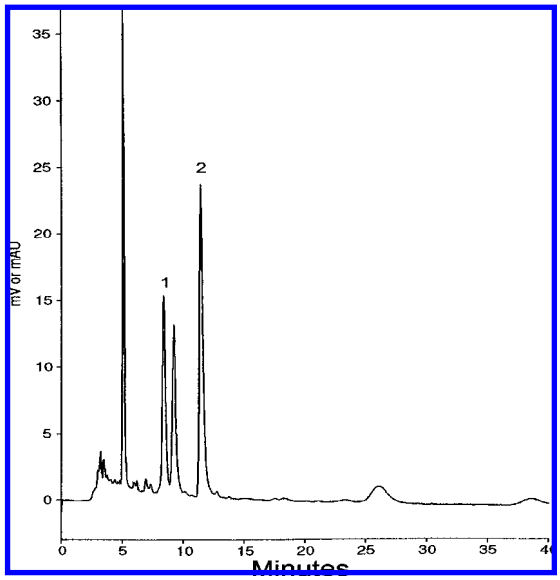

Figure 1. HPLC bioactive compound profile of the yerba maté extract, at 272 nm. 1, 5-CQA; 2, caffeine.

glucose absorption *in vitro* (*27*). Isolated 5-CQA displayed the same effect, and it was suggested that it dissipates the Na^+ electrochemical gradient, which provides the driving force for active absorption of glucose (*28*).

SGLT1 is the main glucose cotransporter in small intestine, and its gene expression can be regulated by the amount of carbohydrates and sodium ions in the intestinal lumen (*29*). Some phenolic compounds, such as quercetin, phlorizin, and the green tea catechins, are capable of interacting with SGLT1, inhibiting glucose transport, probably by competitive mechanisms (*29*−*31*). Yerba maté is a rich source of polyphenols, especially CGAs, and we hypothesized that it could display antidiabetic properties. The aims of this study were to evaluate the effect of a 28 day diet supplementation with yerba maté on biochemical parameters of alloxan-diabetic rats (serum glucose and insulin) and to verify the effect on two possible mechanisms of action in glucose metabolism: hepatic G-6-Pase activity and intestinal Na^+/glucose cotransporter SGLT1 gene expression.

RESEARCH DESIGN AND METHODS

The procedures used for the manipulation of animals were in agreement with the Ethical Principles in Animal Research, adopted by the Brazilian College for Animal Experimentation, according to the American Psychological Association Guidelines for Ethical Conduct in the Care and Use of Animals. The study protocols were approved by the Ethical Committee of the Tropical Medicine Institute, São Paulo University.

Plant Material. The yerba maté beverage was prepared by dissolving instant yerba maté powder (spray dried material from green yerba maté infusion) in saline solution (100 mg/mL) using an homogeneizer and was prepared fresh each day. The aqueous yerba maté and vehicle (saline solution) were administrated by intragastric gavage, to guarantee total ingestion. The animals were treated for 28 consecutive days and received 1 g of instant yerba maté/kg body weight, which represents the ingestion of approximately 1.5 L of yerba maté infusion (*10*).

The instant yerba maté contained 420 mg of total phenolics/g, determined by the Folin−Ciocalteau method. 5-CQA was used as a standard for the calibration curve because the mono- and dicaffeoylquinic acids are the main polyphenols in yerba maté leaves (*2, 11*−*13*). The caffeine and 5-CQA contents were 17.5 and 60.5 mg/g, respectively, and were both determined by reverse-phase high-performance liquid chromatography (HPLC) (*32*−*34*). The chromatogram is shown in **Figure 1**.

Animals and Study Design. Male Wistar rats (8 weeks old, ∼336 g body weight), obtained from the animal facilities of the Pharmaceutical Sciences School, São Paulo University, were housed in groups of up to five per cage in a controlled temperature room with a 12/12 light/dark cycle with free access to water and food. Diabetes was induced by a single intravenous dose (38 mg/kg body weight) of alloxan solution (A7413-10G, Sigma-Aldrich, St. Louis, MO) to overnight fasted rats. The nondiabetic animals received the vehicle only. After the induction, they received a sucrose/water solution for 24 h, to avoid death by hypoglycemia. Diabetes was identified 48 h after injection of alloxan by polyuria (>25 mL/day) and nonfasting glucose level, measured on blood collected from the tail vein (Accu-Chek Advantage, Roche Diagnostics, Mannheim, Germany). Those animals whose glycemic levels were below 200 mg/dL were rejected.

The animals were assigned to four groups: nondiabetic control (NDC, $n = 10$), nondiabetic with yerba maté (NDY, $n = 10$), diabetic control (DC, $n = 11$), and diabetic with yerba maté (DY, $n = 10$). The yerba maté groups received 1 g/kg of yerba mate extract diluted in saline solution (100 mg/mL), and the control groups received saline solution only. Oral gavage was performed once per day for 28 days, and the volume was adjusted to achieve 1 g/kg body weight (recorded twice a week). After this period, the animals were anesthetized with pentobarbital (50 mg/kg body weight; intraperitoneally) for the blood and tissue sampling.

Tissue and Blood Sampling. Blood was drawn from the inferior vena cava and centrifuged immediately, and the serum was collected. Liver was removed and washed with saline solution. An intestinal segment of 15 cm in length was taken. The pylorus was the starting point, and 2 cm pieces of isolated loops were cut at both the upper and middle small intestine. They were cleared of food residue by washing with saline solution. All samples were frozen immediately in liquid nitrogen and stored at −70 °C.

Serum Glucose and Insulin. Serum glucose was quantified by the glucose/oxidase method with a colorimetric kit (Labtest, São Paulo, Brazil). Insulin levels were measured with a rat radioimmunoassay kit (Linco Research, St. Charles, MO). The assays were performed in duplicate.

G-6-Pase Activity in Hepatic Tissue. Rat liver (2 g) was homogenized in 10 mL ice-cold sucrose solution (0.25 M). The homogenate was centrifuged at 11000*g* for 30 min at 4 °C, after which the supernatant was recentrifuged at 105000*g* for 60 min at 4 °C. The microsomal pellet was suspended in 1 mL ice-cold sucrose/ethylenediaminetetraacetic acid (EDTA) solution (0.25 M/1 mM) and then homogenized for 10 s. Aliquots of the microsomal suspension were stored at −70 °C. Assay of G-6-Pase activity was determined by the measurement of inorganic phosphate liberated from glucose-6-phosphate, after 5 min of incubation at 37 °C, according to the method described by Baginski and co-workers (*35*). Protein content of liver microsomal suspension was measured by the Bradford methodology (*36*), and the activity of the enzyme was expressed as nanomoles of phosphate released per minute of incubation per milligram of protein. The assays were performed in duplicate.

Quantitative Real-Time Polymerase Chain Reaction (PCR) for Intestinal SGLT1 Expression. This analysis was performed in intestinal samples of five animals from each group. From each segment sample (upper and middle small intestine), a segment weighing 0.1 g was homogenized in TRIZOL reagent (Invitrogen, CA). RNA was isolated using RNeasy tissue kit (Qiagen, Hilden, Germany). The single-stranded cDNA was synthesized using the high-capacity cDNA archive kit (Applied Biosystems, Foster City, CA), following the protocol of the manufacturer. Quantitative real-time PCR assays were performed using a 7300 real-time PCR system (Applied Biosystems, Foster City, CA), and threshold cycle numbers were determined using RQ Study Software (Applied Biosystems). Reactions were performed in triplicate, and results were normalized by β-actin expression. The 50 μL reaction mixture was prepared as follows: 25 μL of platinum quantitative PCR SuperMix-UDG (Invitrogen), 10 μM of each primer (SGLT1, FW 5′-GACTGATTCTCGGCTTCCTG-3′, RV 5′- CAGATGATCTTGGGGCAGTT-3′; and β-actin, FW 5′-AGCCATGTACGTAGCCATCC-3′, RV 5′-ACCCTCATAGATGGGCACAG-3′), and 10 μL of cDNA (100 ng). The reaction was cycled with preliminary UDG treatment

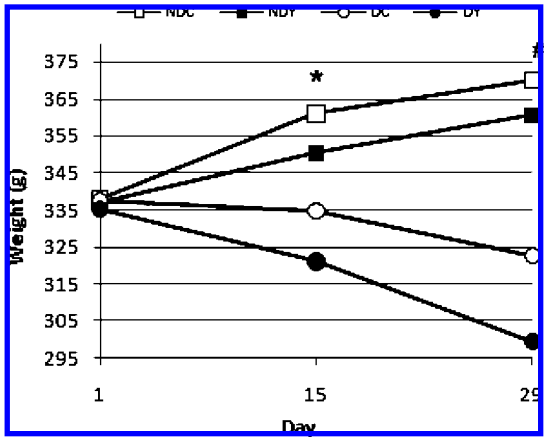

Figure 2. Mean animal weight evolution during the experiment (*) $p = 0.01$ (NDC and NDY versus DC and DY). (#) $p < 0.001$. NDC, nondiabetic control; NDY, nondiabetic yerba maté; DC, diabetic control; DY, diabetic yerba maté.

Table 1. Serum Glucose, Insulin, and Hepatic G-6-Pase Activity after 28 Days with or without Yerba Maté Supplementation[a]

parameter	NDC ($n = 10$)	NDY ($n = 10$)	DC ($n = 11$)	DY ($n = 10$)
glucose (mmol/L)	7.6 ± 0.8 b	7.1 ± 1.3 b	27.1 ± 9.6 c	28.9 ± 6.6 c
insulin (pmol/L)	153.6 ± 126.9 b	143.6 ± 71.8 b	38.4 ± 28.4 c	48.4 ± 48.4 c
G-6-Pase activity [nmol of Pi min^{-1} (mg of Ptn)$^{-1}$]	254.9 ± 83.9 b	262.0 ± 64.2 b	545.7 ± 206.6 c	518.9 ± 208.9 c

[a] Data are expressed as means ± SD. Different letters in the same row indicates significant differences ($p < 0.05$). NDC, nondiabetic control; NDY, nondiabetic yerba maté; DC, diabetic control; DY, diabetic yerba maté.

for 2 min at 50 °C and a denaturation for 2 min at 95 °C, followed by 45 cycles of denaturation at 95 °C for 15 s, annealing for 15 s, and primer extension at 72 °C for 15 s. This was followed by melting point analysis of the double-stranded amplicons consisting of 40 cycles of 1 °C decrement (15 s each) beginning at 95 °C. The $\Delta\Delta C_t$ method of relative quantification was used to determine the fold change in SGLT1 expression, according to the formula $2^{(-\Delta\Delta C_t)}$ (*37*).

Statistical Analysis. Data are expressed as mean ± standard deviation (SD). Serum glucose, insulin, and hepatic G-6-Pase activity values did not show homoscedasticity and were logarithmically transformed before analyses. Group differences were analyzed by two-way analysis of variation (ANOVA) with the presence of diabetes and the treatment (yerba mate/control) as the two main factors. Values for $p < 0.05$ were considered significant.

RESULTS

The body weight of diabetic animals (~330 g) at the end of the study was lower ($p < 0.001$) than that of nondiabetic ones (~368 g) (**Figure 2**). The yerba maté ingestion had no effect in weight in either diabetic and nondiabetic groups.

Yerba maté ingestion at 1 g/kg did not change the serum biochemical parameters (**Table 1**). The rats in the diabetic group developed serum glucose levels approximately 4 times higher than nondiabetics. As expected, the insulin was lower ($p < 0.001$) in animals with diabetes (DC and DY) than nondiabetic ones (NDC and NDY).

Similarly, the hepatic G-6-Pase activity did not differ between the groups that ingested yerba maté and the controls ($p = 0.85$; **Table 1**), but the difference between animals with and without diabetes was significant ($p < 0.001$).

The SGLT1 gene expression was reduced in both upper and middle small intestine samples in the groups that received yerba maté extract (**Figure 3**). The relative expression (arbitrary units, AU) in the nondiabetic group that ingested yerba maté (NDY) was 53.8% lower in upper small intestine and 26.6% lower in middle small intestine compared to the nondiabetic control (NDC). In the diabetic group that ingested yerba maté (DY), there was a 19.3% reduction in upper small intestine and a 46.2% reduction in the middle small intestine samples compared to the diabetic control (DC).

DISCUSSION

In our study, the animals ingested 1 g of instant yerba maté kg^{-1} day^{-1}, which provided for the adult Wistar male mice (~340 g body weight) 143 mg of phenolic compounds/day. The mean daily intake of total polyphenols in the human diet may range from 100 mg to 2 g (*14*).

The ingestion of 1 g/kg body weight of instant yerba maté during 28 days did not exert a significant effect on serum glucose levels nor did it alter the insulin secretion of the animals. We believe that the very high glycemic levels developed by the animals prevented any minor effect to achieve significance. Otherwise, it is known that barely 10% of the poplyphenols ingested are absorbed (*14*), and the use of pharmacological doses or other routes of administration were not the aim of this research.

In a recent paper, other group (*38*) showed that supplementing the diet of normoglycemic Sprague−Dawley rats with yerba maté for 60 days decreased the blood glucose level by 12%. However, the insulin levels also decreased, suggesting that yerba maté does not directly stimulate insulin release. Corroborating this hypothesis, Sotillo and Hadley (*39*) suggest that CGAs do not stimulate insulin secretion, implying that it is unlikely for these compounds to cause sustained hypoglycemia. They might act, instead, as either an insulin sensitizer or a hypoglycemic agent, such as metformin, by decreasing glucose output by the liver.

Although the single glucose measurement, as used in our research, is the most common method because it is easy, quick, and of low cost, the glucose tolerance test gives a much better picture of the handling of elevated glucose by the body when a test compound is present (*40*). Therefore, further studies that evaluate the glucose tolerance test are needed to confirm the hypothesis that yerba maté could act as a hypoglycemic agent.

The ingestion of yerba maté did not alter hepatic G-6-Pase activity (**Table 1**). The difference between diabetic and nondiabetic rats was expected, because there is an increase in the activity of this enzymatic complex during insulin absence or resistance (*26*).

Studies with CGA (5-caffeyolquinic acid) (*25, 41, 42*) and its synthetic derivatives (*21, 43*) have demonstrated their ability to inhibit hepatic G-6-Pase activity *in vitro*. The synthetic derivatives also reduce the glucose release *in vivo* in animal models, by both gluconeogenesis and glycogenolysis, leading to a decrease in blood glucose (*21, 22, 42*). However, single or fractioned doses equal or higher than 100 mg/kg were administered intravenously or intraperitoneally, and it is known that the potency of these synthetic derivatives is more than 1000 times greater than the parent compound (*43*). When orally administered, CGAs are extensively metabolized in the intestinal tract (*14, 44*) and they might not attain plasmatic and intracellular levels sufficient enough to inhibit G-6-Pase in the same way as the synthetic derivatives by the other administered routes.

There is evidence that some polyphenols have trophic effects in the small intestine, resulting in an altered pattern of intestinal glucose uptake (*14, 15*). The rate of absorption

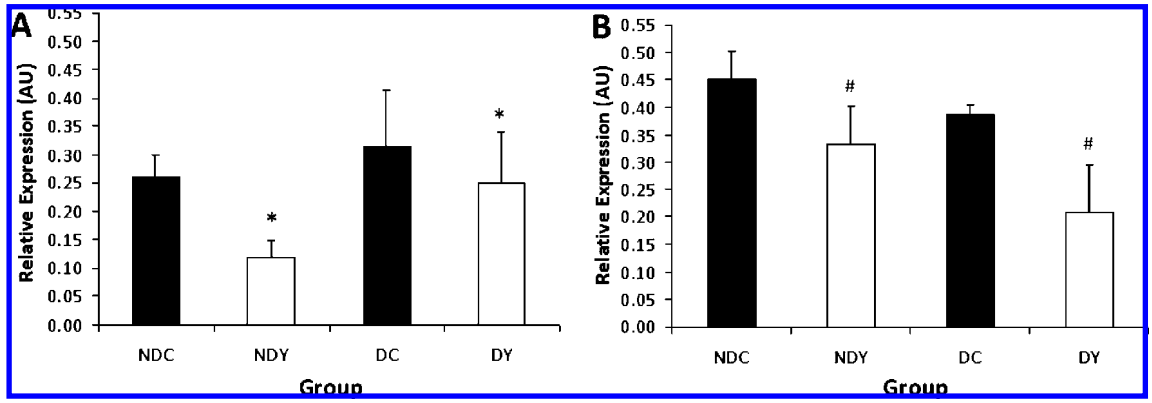

Figure 3. Relative gene expression (AU) of Na^+/glucose cotransporter SGLT1 in (A) upper and (B) medium small intestine of rats after 28 days with or without yerba maté supplementation. Data are means ± SD of relative expression in arbitrary units (AU) ($n = 5$). (A) Upper small intestine. (∗) $p = 0.007$ comparing the groups treated with yerba maté (NDY and DY) to controls (NDC and DC). (B) Middle small intestine. (#) $p = 0.004$ comparing the groups treated with yerba maté to controls. NDC, nondiabetic control; NDY, nondiabetic yerba maté; DC, diabetic control; DY, diabetic yerba maté.

in the intestinal region, where sugars are absorbed, affects the time course of appearance of sugars in the blood and their availability to other body tissues (*29*). The SGLT1 is the main Na^+/glucose cotransporter in the small intestine and is expressed in the glycocalyx, where it responds for the absorption of glucose and galactose from food (*45*). The kinetics for Na^+/glucose cotransport can be described as an ordered reaction scheme, in which two external Na^+ ions bind first to SGLT1, thereby increasing the affinity of the protein for sugar. Upon sugar binding, the protein undergoes a conformational change to deliver the two Na^+ ions and sugar to the other side of the membrane, where first the sugar and then the two Na^+ ions dissociate (*46*).

The role of polyphenols or other food component effects on the gene expression of glucose transporters has not been clarified thus far. Cao and co-workers (*18*) observed a significant decrease in the gene expression of glucose transporters of the GLUT family in liver, muscle, and kidney of rats that consumed a green-tea-supplemented diet.

We evaluated the SGLT1 gene expression in the upper and middle small intestine of rats by means of quantitative real-time PCR, and there was a significant reduction in the tissues of the yerba maté group compared to the control group. These results indicate that the bioactive compounds present in yerba maté are able to decrease glucose absorption by interfering with SGLT1 gene expression. The decreased SGLT1 mRNA levels with yerba maté extract reported here suggest a positive effect on the long-term regulation of glucose absorption, although changes in SGLT1 protein levels need to be confirmed in future studies.

It is known that the amount of carbohydrates and sodium ions in intestinal lumen can regulate the SGLT1 gene expression (*29*), and an *in vitro* study demonstrated that CGA and other polyphenols decreased the rate of glucose absorption by intestinal cells through the dissipation of sodium electrochemical gradient (*28*). Hossain and co-workers (*19*) also demonstrated that polyphenols modify the electrical responses of intestinal bush border membrane. We propose that the polyphenols present in the yerba maté extract might dissipate the sodium ion gradient in the intestinal membrane, leading to a reduction on the SGLT1 gene expression.

The results of the present study shows that the yerba maté ingestion for 28 days at a 1 g extract/kg body weight dose did not promote significant differences in serum glucose and insulin and that the hepatic G-6-Pase activity was not modified either. However, yerba maté ingestion significantly reduced gene expression of the intestinal SGLT1 glucose cotransporter in diabetics and nondiabetics animals, suggesting that the polyphenols present in yerba maté may be capable of interfering with glucose absorption. Further studies are necessary to confirm if this mechanism of action can occur in humans and if it can effectively lead to a decrease in blood glucose and improve glucose tolerance.

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