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ILEX PARAGUARIENSIS ST. HILL (YERBA MATE) IMPROVES THE METABOLIC PROFILE OF AND PROTECTS AGAINST OXIDATIVE STRESS IN EXERCISED AND NON-EXERCISED RATS

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ABSTRACT

Ilex paraguariensis (St. Hil) is a native plant consumed mainly in South America in the form of tea and mate and it contains different compounds. Physical exercise is characterized by increased metabolic demand, increased production of reactive oxygen species (ROS). The aim of this study was to evaluate the effect of Ilex paraguariensis (St. Hil) use extract on the metabolic profile and oxidative stress. We used 24 Wistar rats, were divided into four experimental groups (n=6), the extract was orally administered (1.5 ml/animal), and a swimming training protocol was performed for 8 weeks. Was found that the animals that received Ilex showed a significant decrease in plasma glucose, TG and HDL levels. By evaluating the lipoperoxidation, we observed a significant decrease in the groups that received Ilex, and a significant increase in SOD enzyme. Ilex paraguariensis has antioxidant properties that acted reducing oxidative damage in this experimental model.

KEYWORDS: Exercise; *Ilex paraguariensis;* Oxidative Stress.

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INTRODUCTION

Ilex paraguariensis - St. Hil (yerba mate) is a South American native plant widely used in folk medicine and by herbalists. The leaves of yerba mate are used to prepare a beverage consumed in southern Brazil and in neighboring countries such as Argentina, Paraguay and Uruguay. From the leaves, a product for the preparation of beverages such as tea-mate or mate tererê is obtained. This product is also used in pharmaceutical preparations and incorporated into other drugs. Because of its therapeutic properties, it is recommended as a stimulant, anti-inflammatory, antirheumatic, tonic and diuretic. Yerba mate contains several different bioactive compounds, as described in the literature[1,2,3,4,5]. Among the identified compounds are methylxanthines (caffeine, theophylline and theobromine), saponins, and phenolic compounds, such as flavonoids (quercetin, rutin and kaempferol-3-O-rutinoside) and phenolic acids, particularly chlorogenic acid. The amount of polyphenols in yerba mate is more than the amount found in green tea or red wine[6]. The antioxidant activity of yerba mate, which is due to the presence of phenolic acids that protect biological systems from oxidative processes, is its main action. Due to its lipolytic properties, it is speculated that *Ilex paraguariensis* lowers cholesterol. Additionally, *Ilex* appears to be involved in the inhibition of free radicals, an activity that is considered beneficial to the organism and liver. *Ilex* extract contains polyphenols (the largest class being flavonoids) that enable it to scavenge free radicals[7].

Physical exercise is characterized by increases in muscle activity and energy demand, leading to increased oxygen consumption and increased production of reactive oxygen species (ROS). These physiological changes may or may not lead to an oxidative stress situation, depending on the balance between the generation of free radicals and the effectiveness and adaptation of these antioxidant mechanisms. It is known that exercise is a form of stress and chronic exposure is capable of triggering adaptations in response to higher production of free radicals[8,9,10,11,12]. Studies on oxidative stress conducted with experimental animals and humans have shown that increased metabolic activity favors the occurrence of oxidative damage to biomolecules[13,14]. As training and exercise promote an increase in metabolic activity, injuries can take on even greater dimensions in these conditions. The aim of this study was to evaluate the effects of *Ilex paraguariensis* St. Hill (yerba mate) on the metabolic profile and oxidative stress in rats with and without physical exercise.

MATERIALS AND METHODS

Animals

Twenty-four male Wistar rats, each weighing 250 g, were used. The experimental procedures complied with the rules established by the Research Ethics Committee of the Lutheran University of Brazil, Canoas, under protocol nº 2010-16 P, PPGGTA course, research project filed and approved on December 10, 2010. During all the experiments, the animals were kept in the Animal Breeding Plant Biotechnology Institute of the University of Campaign – Bagé-RS, in plastic boxes (made by Techniplast) measuring 47x34x18 cm, lined with wood chips, under a 12-hour dark/light cycle (light from 7 a.m. to 7 p.m.) at a temperature of 22 ± 4°C. The rats were fed 16 g per animal/day with rat chow *Sellecta* and water *ad libitum*.

Experimental groups

The animals, the average weight of per group is 250 g, were divided in four experimental groups (n=6 for each group): control (CO), which were not exercised and received water; control+Ilex (CI), which were not exercised and received yerba mate extract; exercise (E), which were exercised and received water; and exercise+Ilex (EI), which were exercised and received yerba mate.

Plant Extract

The *Ilex paraguariensis* used in this research was donated by Baron Trade and Industry Ltda Company (RS-Brazil). The *Ilex paraguariensis - St. Hil* aqueous extract (IPAE) was made in Bagé/RS. The infusion of the tea was prepared

daily using 100 ml H_20 in a beaker heated to 80ºC. Five grams (5% by weight of the aqueous extract) of *Ilex* was added in sheet form. After 15 minutes, the tea was filtered with a paper filter. We used a gavage cannula to administer 1.5 ml of the extract to the animals in the appropriate experimental groups daily for 8 weeks. The animals not receiving yerba mate received an equal volume of water.

Adaptation and Exercise protocol

For the experimental protocol, we used the methodology described by Cooper [15], for swimming exercises and adaptation to the water for mice. In the first week, the animals were adapted to the liquid medium. They were placed in a tank of width 40x60x50 cm. A water column with 10 cm of water was maintained at a temperature of 31-32 °C, and the rats were kept in it for 30 minutes per day. During the experiment the animals swam in a tank of 40 cm water width. The experimental protocol started after a week of adaptation.

Termination of animal experiments

At the end of training, the animals were weighed and then were anesthetized with a mixture of ketamine (100 mg / kg) and xylazine (10 mg / kg) injected intraperitoneally. Next, the animal's blood was collected using the technique of puncturing the retro-orbital venous plexus with a capillary tube. The blood was centrifuged at 3000 rpm for 10 minutes to obtain plasma for evaluation of the glucose and lipid levels. The concentrations of plasma glucose (G), total cholesterol (TC), HDL, LDL, triglycerides (TG) [16] and alanine aminotransferase (ALT) were determined through enzymatic kits specific to each substance. The readings were made on an automated COBAS MIRA analyzer (Roche ®). The gastrocnemius muscle and liver were removed and stored at - 80 °C for subsequent biochemical analyses. The animals were sacrificed by exsanguination under deep anesthesia.

Muscle and liver homogenates

The muscle and liver were weighed and cut with scissors and homogenized for 40 seconds in an Ultra-Turrax (IKA-WERK) at 4 ºC in the presence of 1.15% KCL (5 mL per gram of muscle or 9 mL per gram of liver) and phenylmethylsulfonyl fluoride (PMSF) at a concentration of 100 mM in isopropanol (10 µL per mL of KCl was added). Then, the homogenates were centrifuged for 10 minutes at 3000 rpm in a refrigerated centrifuge (SORVALL Super T21 – Condensed Operating Kendro Laboratory Products – USA). The supernatant was pipetted into Eppendorf flasks, and the precipitate was discarded. The samples were stored again at - 80 ºC for subsequent analyses[17].

Protein

We used the Bradford[18] method to quantify protein, with bovine albumin as the standard (SIGMA). The samples were measured spectrophotometrically at 595 nm, and the concentrations, expressed in mg/mL, were used to calculate the amounts of TBARS (thiobarbituric acid-reactive substances) and antioxidant enzymes.

Muscle and Liver Lipoperoxidation (LPO)

The amount of aldehydes generated by lipid peroxidation was measured by the TBARS method, which measures the amount of substances reacting with thiobarbituric acid. The samples were incubated at 100 ºC for 30 minutes after addition of 500 µL of 0.37% thiobarbituric acid in 15% trichloroacetic acid. The samples were then centrifuged at 3,000 rpm (1612.8 x g) for 10 minutes at 4 ºC. The absorbance was determined spectrophotometrically at 535 nm[19].

Antioxidant (AOX) enzyme analysis

The superoxide dismutase (SOD) analysis was based on the inhibition of the reaction of the superoxide radical with adrenaline. The values are expressed in U/mg protein[20].

Statistical analysis

All data are presented as the means ± SE. Statistical significance was calculated using GraphPad InStat version 3.0 for Windows. We used analysis of variance (ANOVA) and the Student-Newman-Keuls method for multiple analyses, adopting a significance level of 5% ($P< 0,05$).

RESULTS AND DISCUSSION

According to the World Health Organization, 65% to 80% of the world's population who live in developing countries make use of plants as a first medical treatment due to poverty and lack of access to allopathic medicine[21]. Therefore, it is important to study these products that possibly have antioxidant activity. The results of the assessment of the glycemic profiles showed that IPAE consumption contributed to a statistically significant decrease in the glucose levels in groups CI, E and EI. The group that received only the IPAE showed a significant reduction in blood glucose when compared with the controls. In the groups subjected to physical exercise, we observed that exercise by itself reduced blood glucose, and the group treated with IPAE and exercise (EI) had a further decrease compared with the other groups (Table 1).

Table 1

Serum glucose (G) mg/dL and lipid profile mg/dL (Triglycerides (TG), total cholesterol (TC)), HDL, LDL and VLDL) and alanine aminotransferase (ALT) in the different groups Control group (CO), Control + Ilex (CI), Exercise group (E) and Exercise + Ilex (EI).

	CO	CI	E	EI
Glucose (mg/dL)	101.54 ± 0.79	66.54 ± 9.068#	75.6 ± 5.8#	54.22 ± 4.54*
TG (mg/dL)	112.5 ± 0.8	95.24 ± 4,27#	107 ± 1.6	74.61 ± 3.5*
TC (mg/dL)	83.89 ± 0.3	61.19 ± 3.48	58.45 ± 5.26	60.83 ± 3.78 NS
HDL (mg/dL)	6.16 ± 0.12	14.49 ± 0.7*	11.01 ± 1.25#	16.81 ± 1.2*
LDL (mg/dL)	15.05 ± 0.03	27.25 ± 3.52*	25.45 ± 2.67*	27.12 ± 2.01*
ALT (U/L)	59.43 ± 0.25	73.73 ± 2.11	57.41 ± 9.09	51.36 ± 4.8 NS

Mean ± standard error of mean (n = 6); Statistical significance p< 0,05
Glucose: * Significant difference between EI compared with CO and E groups. # Significant difference between CI and E compared with CO. TG: * Significant difference between EI and all groups. # Significant difference between CI compared with CO and E groups. HDL: * Significant difference between CI and EI groups compared with E and CO groups. #Significant difference between E and CO. LDL: * Significant difference between CI, EI and E groups compared with CO. NS: No significant difference.

Mello[22] reported that chromium is one of the nutritional components of yerba mate. This mineral actively participates in the metabolism of carbohydrates. In particular, it acts together with insulin to improve glucose tolerance. We suggest that in the groups in this study that received IPAE, CI and EI, the lower concentrations of glucose are due to the presence of chromium in IPAE. Chromium facilitates the interaction of insulin with tissues by forming a complex with the disulfide chain hormone. This complex facilitates the transport of glucose into cells and thereby reduces the levels of circulating glucose[23,24]. Oliveira[25], in an experiment on animals with diabetes in which IPAE was delivery gavage, observed a significant difference in glucose transporter SGLT1 expression in the bowel, suggesting that the bioactive compounds of Ilex are able to reduce the absorption of glucose. We can see a similar reduction of absorption of glucose in the results of the groups that received Ilex. Based on the literature, one would expect blood glucose levels to decrease with exercise. McArdle, Katch, and Katch[26] reported that plasma glucose levels suffer a sudden drop that may persist for several days, due to the increased insulin sensitivity of the active muscles. This decrease in glucose levels was found in our study, groups E and EI showed a drop in their serum levels.

The results showed a significant difference in the levels of TG in the CI group compared with the control (Table 1). In the exercise group, IPAE contributed to a significant reduction in circulating triglyceride levels. Many studies have reported that physical activity has beneficial effects on the lipid profile[22]. Horowitz[27] reported that light or moderate physical activity over long periods promotes greater uptake of circulating triglycerides by increasing the activity of muscle contraction. This same effect causes a decrease of triglycerides in the plasma of the exercised experimental animals. Mello[22] treated rats with a hypercholesterolemic diet and found a decrease in triglyceride levels in animals that received IPAE. This extract has components that slow the absorption of fats and thereby decrease the levels of circulating

triglycerides, as observed in this study. The polyphenol content of yerba mate is greater than that of green tea and equivalent to that of wine[6]. In addition to the recognized antioxidant activity of the phenolic and methylxanthine stimulants in yerba mate, the saponins present in it also have beneficial effects because they interfere with the metabolism of cholesterol and slow fat absorption by inhibiting pancreatic lipase[28,4,29].

Although many studies report that IPAE lowers serum cholesterol, in this study, we did not observe a significant difference in cholesterol between the groups. In the study by BERNARDES et al[30], rats that swam for 3 to 6 weeks had no significant changes in cholesterol levels. This result was in contrast to the other results of, who reported that in a 4 week program of exercise on a cycle ergometer, associated with diet, diabetic subjects showed a reduction in body mass and in levels of TG, TC and LDL. However, there were no changes in HDL in these subjects. In this experiment we observed significantly higher HDL levels in the groups that received IPAE and in the group that exercised and received no IPAE (CI, EI and E) compared with the control group (CO). There was no significant difference in HDL between the CI and EI groups, suggesting that administration of Ilex triggers an increase in HDL independent of physical exercise. However, it is important to note that the group that exercised but received no IPAE showed significantly increased HDL compared to the CO group. Therefore, we suggest that administration of IPAE caused a greater effect on the serum concentrations of HDL in the CI and EI groups compared to the control group. Therefore, IPAE promotes an increase in HDL.

Caffeine, one of the chemical constituents of IPAE, stimulates catecholamines and thus mobilizes LDL to provide an energy substrate. This increase in LDL was observed in the CI and EI groups. As described in some studies, *IPAE* has a lipolytic effect because it promotes an increase in the basal metabolism rate. This increase in the basal metabolism rate is caused by caffeine, which causes an increase in norepinephrine activity[31]. Nacif et al, states that the lipolytic effect of caffeine, that is present in mate, may be responsible for increased lipids serum concentrations such as found in this work[40]. This lipolytic effect promotes increased mobilization of free fatty acids from stocks in muscles or other tissues, causing an increase in LDL and HDL as noted in Table 1. Therefore, in group E, exercise mobilized LDL and HDL via the action of catecholamines because of the metabolic demand observed in this group that was exercised and consequently spent a greater amount of energy

AST and ALT are enzymes present in the hepatic cells that permeabilize to the blood circulation when there is damage on the hepatic cell. ALT is a more specific indicator of hepatic inflammation, while AST may appear elevated on diseases of other organs, such as heart or muscle. Therefore, when evaluating ALT, indicator of hepatic alterations, there was no statistical difference between any of the groups, suggesting that the extract does not have hepatotoxic effects in this protocol. Schneider et al.[32] state that training extends regular aerobic endurance capacity and increases antioxidant defenses. In the results of this experiment, exercise promoted an increase in lipoperoxidation in muscle and liver tissue, as observed in Figure 1. The use of IPAE in animals exposed to physical exercise has the protective effect of reducing the LPO in both liver and muscle tissues, as observed in this experiment (Figure 1).

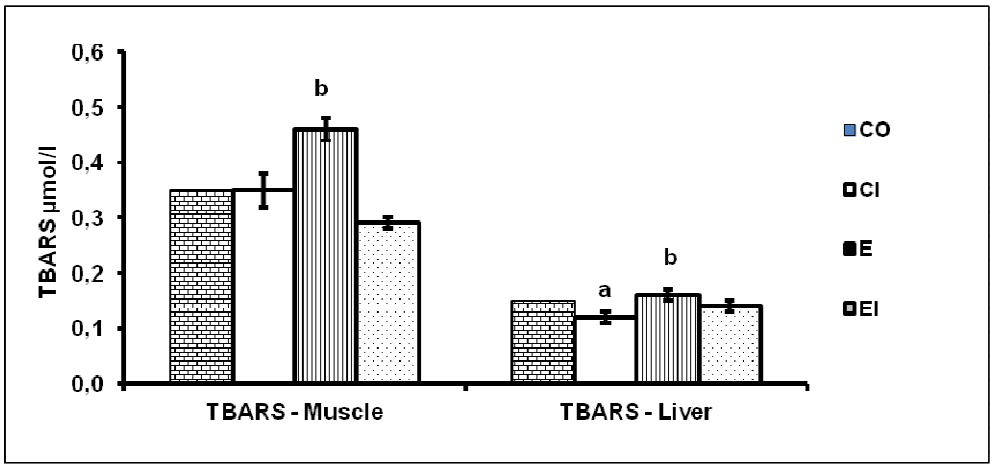

Figure 1

Lipid peroxidation by thiobarbituric acid reactive substances (TBARS) in gastrocnemius muscle and liver tissue of Wistar rats. The mean ± standard error of the mean (n = 6). Statistical significance p< 0,05. Both TBARS from Liver and Muscle follow the same group sequency CO, CI, E and EI, being that groups (n=6 for each group): control (CO), which were not exercised and received water; control+Ilex (CI), which were not exercised and received yerba mate extract; exercise (E), which were exercised and received water; and exercise+Ilex (EI), which were exercised and received yerba mate. Muscle: [b] Significant difference between EI and all groups. Liver: [a] Significant difference between CI and all groups. [b] significant difference between EI compared with E groups.

These results agree with many studies that have suggested that physical activity promotes increased LPO. The LPO may be related to the intensity and frequency of the exercise the animals undergo. Additionally, the use of an antioxidant and adaptation to exercise may reduce the damage. The use of *IPAE* decreased lipid peroxidation in both muscle and liver tissue. Recent discoveries about free radicals have stimulated the interest of a large number of researchers in the action of antioxidants, which are naturally present in some foods. Different types of antioxidants can act as protectors of living organisms against oxidative processes, in our case, exercise. Some studies show a direct relationship between physical exercise and increased oxidative stress in the liver. This relationship can be observed in the results shown in Figures 1 and 2.

Leeuwenburgh et al[33] have suggested that exercise-induced oxidative stress can trigger adaptations in response to training and that such adaptations are tissue-specific, suggesting a complex regulatory mechanism. The activity of the antioxidant enzyme SOD increased significantly in groups CI and EI, in both muscle and liver. This result shows that IPAE has an antioxidant action that is achieved by promoting increased activity of this enzyme. In the exercise group, in both muscle and liver, we found decreased SOD activity. We assume this decrease is happening because, in this group, SOD is being consumed because there is not another antioxidant. In contrast, IPAE is acting directly as an antioxidant in the group that received it, thus saving SOD from being consumed. The antioxidant mechanism is possibly related to the presence of substances (phenolic acids) in IPAE that are capable of chelating metals and scavenging free radicals. These substances are formed in both the initiation step of and during propagation of the oxidative process. The detoxification site of these compounds is the liver[34,35,27].

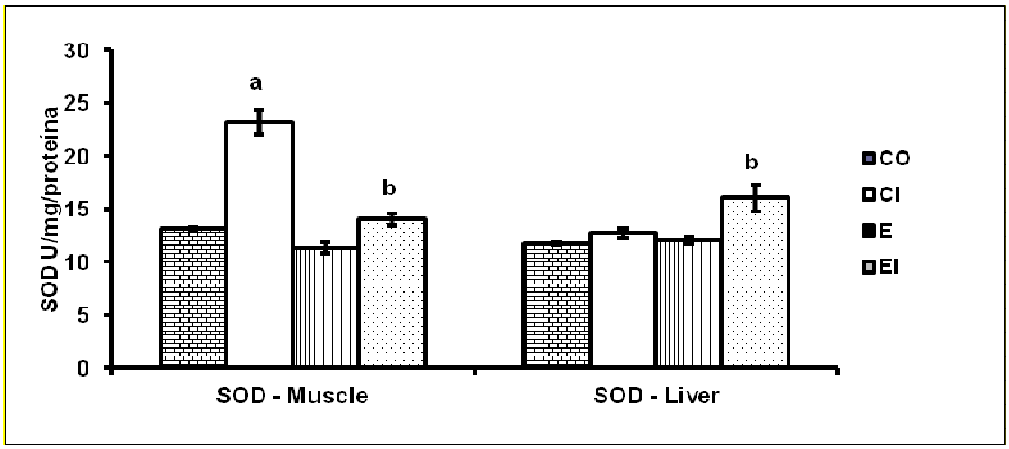

Figure 2

The mean ± standard error of the mean (n = 6). Activities of antioxidant enzymes: superoxide dismutase – SOD for Muscle and Liver. A significant difference was found between CI and each of CO and E. Both TBARS from Liver and Muscle follow the same group sequency CO, CI, E and EI, being that groups (n=6 for each group): control (CO), which were not exercised and received water; control+Ilex (CI), which were not exercised and received yerba mate extract; exercise (E), which were exercised and received water; and exercise+Ilex (EI), which were exercised and received yerba mate. A significant difference was found between EI and E. Significance level: p<0,05. Muscle: [a] significant difference between CI and all groups. [b] significant difference between EI compared with E groups. Liver: [b] Significant difference between EI and all groups.

In this context, we suggest that the antioxidant activity of IPAE phytochemicals can act through mechanisms other than by acting directly in muscle, chelating free radicals. Another possible mechanism would be by acting indirectly, for example by modulating the activity of antioxidant enzymes. Such modulation may result in modification of the cellular redox state[36]. Therefore, we can observe from this work that physical exercise in rats increased LPO, whereas in the group that received Ilex paraguariensis, we observed a reduction in LPO. Therefore, we suggest that IPAE may contribute effectively to antioxidant activity with exercise and even without exercise.

CONCLUSION

The results presented in this study show that the use of IPAE contributed to the improvement of biochemical parameters. For example, it decreased levels of blood glucose and triglycerides and increased levels of HDL cholesterol. Based on our evaluation of oxidative stress in the experimental model described here, we suggest that the antioxidant action of IPAE is due to an increase in the level of antioxidant enzyme superoxide dismutase, which reduces lipid peroxidation in both muscle and liver of these animals.

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