

## Full Length Research Paper

# ***Camellia sinensis* extract inhibits *in vitro* pancreatic lipase and has preventive effect on obesity in female rat fed a high-fat diet**

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The aim of this study was to evaluate eight plants extract for porcine pancreatic lipase inhibition, characterizing biochemically the extract with high inhibitory activity and its effects on preventing weight gain in female rat fed a high-fat diet (HFD). *In vitro* pancreatic lipase inhibition was carried out in *p*-nitrophenyl-laurate substrates and a double-reciprocal plot was used for inhibition mechanism identification. *In vivo* experiments, female rats were fed with a standard diet or high fat diet (HFD), HFD+orlistat, HFD+22.5 mg/ml GT, and HFD+112.5 mg/ml GT. Feed intake, weight body gain, fecal lipid excretion and biochemistry parameters were analyzed. *Camellia sinensis* extract had the highest inhibitory lipase activity ( $76.65 \pm 2.04\%$ ) with a non-competitive inhibition. *C. sinensis* administration, equivalent to 112.5 mg/ml, promoted weight loss, while 22.5 mg/ml increased fecal excretion of lipids in 31.41%. *C. sinensis* extract is certainly a promising alternative for preventive obesity treatment, since biochemical parameters analyzed showed significantly, reduction in the serum triglycerides levels and significantly decreased the LDL-cholesterol basal levels when compared with animals that did not receive a fat diet.

**Key words:** Enzyme inhibition, plant extracts, animal, preventive obesity.

## INTRODUCTION

Lipids are important components in human nutrition, but their increased intake contributes to the development of obesity and can lead to multiple long-term complications

(Slanc et al., 2009). Life-style modifications is obviously the most appropriate approach, but therapeutic strategies such as anti-obesity agents and surgery are much sought

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by obese patients. There are limited options for medical therapy of obesity at present; in most countries, only orlistat is available as oral medication. Sibutramine (an amphetamine derivative) and rimonabant (a cannabinoid receptor blocker) have been removed from the market due to the increased cardiovascular risk associated with sibutramine and the association of depression, anxiety and suicidal ideation with rimonabant. Glucagon-like peptide (GLP)-1 receptor agonists also have potential as weight-loss agents, but so far they are only approved for the treatment of type 2 diabetes and not yet for obesity (Cameron et al., 2012). Lorcaserin, a serotonin 5-HT<sub>2C</sub> receptor agonist, and phentermine plus topiramate have been approved in the USA for the treatment of obesity as an adjunct to lifestyle modifications in obese adults (body mass index [BMI]  $\geq 30$  kg/m<sup>2</sup>), or overweight adults (BMI  $\geq 27$  kg/m<sup>2</sup>) with at least one weight-related, co-morbid condition (for example, dyslipidemia, hypertension, type 2 diabetes) (Cameron et al., 2012; Gallwitz, 2013).

The potential of natural products or herbs for the treatment of obesity is still largely unexplored and might be an excellent alternative strategy for the development of safe and effective antiobesity drugs (Souza et al., 2011; Birari and Bhutani, 2007). Plant extracts, defined as raw or refined products derived from plants or parts of plants (for example, leaves, stems, buds, flowers, roots, seeds or tubers) are frequently used for the treatment of diseases (Boqué et al., 2012). Among plant extracts, grape seed extract was used as treatment to limit dietary fat absorption and accumulation of fat in adipose (Moreno et al., 2003). *Baccharis trimera* leaves is popularly used in the treatment of hepatic and digestive problems, for malaria, ulcers, diabetes, anemia, diarrhea, urinary inflammations, tonsillitis, worms, Hansen disease and weight reduction (Souza et al., 2011). *Cymbopogon citratus* leaves was used as aqueous extract for hypoglycemic and hypolipidemic study in rats (Adeneye and Agbaje, 2007). Flavonoids extracts from *Solanum melongena* showed significant hypolipidemic action in normal and cholesterol fed rats (Sudheesh et al., 1997). *Fragaria ananassa* extracts has ellagitannins that were the main active components for amylase inhibition, however, the polyphenols have been found to inhibit lipase activity *in vitro* at low levels (Boath et al., 2012). *Camellia sinensis* is an herb used for green tea and white tea. To produce green tea, the young leaves are rolled and steamed to minimize oxidation. White tea is prepared from very young tea leaves or buds covered with tiny, silvery hairs, which are harvested only once a year in the early spring (Rusak et al., 2008). Green tea is a richer source of phenolics than is white tea, but the extraction efficiency of these compounds strongly depends on the time of extraction, the solvents used and it is much slower than is the extraction of the same compounds from green tea (Rusak et al., 2008).

The main compound found in these plant extracts that have pharmacology effects on the weigh low was called

polyphenols. Polyphenols are a class of phytochemicals that are likely candidates as anti-obesity agents and several studies have suggested they can modulate the adipocyte life-cycle (Williams et al., 2013). Vegetables provide a major dietary source of polyphenols with potential anti-obesity properties. These compounds inhibit the action of pancreatic lipase by preventing the lipids from being absorbed by the enterocytes. A variety of natural products, including crude extracts and compounds isolated from plants, have been widely used traditionally in the treatment of obesity (Williams et al., 2013; Jang and Choung, 2013). The predominant constituents of *C. sinensis* (green tea), accounting for up to 35% of dry weight, are polyphenols, which include flavonols, flavones, and flavan-3-ols commonly known as catechin (Mizukami et al., 2007). Epigallocatechin-3gallate is the most abundant catechin of green tea (GT), representing 50 to 80% of the total catechin content, and other minor catechin include epicatechin3-gallate, epigallocatechin, epicatechin and catechin (Rains et al., 2011).

Experimental and clinical studies regarding the action mechanism of GT in the treatment of obesity and overweight are controversial. Diepvens et al. (2005) reported that the catechins in GT may stimulate thermogenesis and fat oxidation through an inhibition of catechol O-methyl-transferase, an enzyme that degrades noradrenaline. Juhel et al. (2000) demonstrated the *in vitro* inhibition of the two digestive lipases by green tea extract (GTE) separately. This experiment was conducted because *in vivo*, triglyceride hydrolysis is first initiated by an excess amount of gastric lipase under acidic conditions and is then completed by an excess amount of pancreatic lipase in neutral conditions. The results showed a reduced gastric and intestinal fat digestion by green tea extract mediated by direct inhibition of lipases as well as a reduction of lipid emulsification process. In studies with humans were observed significant increase of energy expenditure, lowering of body weight and good tolerance (Chantre et al., 2002). Rains et al. (2011) suggested that GT catechin may reduce glucose absorption by inhibiting gastrointestinal enzymes involved in nutrient digestion, in particular,  $\alpha$ -amylase and  $\alpha$ -glucosidase activity. Zhong et al. (2006) reported that GT induced carbohydrate malabsorption of 25% of the carbohydrate but did not cause triacylglycerol malabsorption or any significant increase in symptoms. Finally, Jurgens et al. (2012) related that the modest size of the reduction in weight produced by GT preparations make it then unlikely to be clinically relevant.

This study, initially, presents the screening of several methanolic plant extracts for *in vitro* inhibition of pancreatic lipase and to determine the kinetic parameters of the inhibition of green tea extract using p-nitrophenyl laurate as substrate. Furthermore, the preventive effects of green tea extract on obesity development and changing levels of lipid and lipoproteins were also

analyzed in female rats fed a high-fat diet.

## MATERIALS AND METHODS

### Plant

Fresh *B. trimera* and *C. citratus* were obtained from Hermínio Ometto Foundation – Uniararas' garden. Dehydrated *E. macrophyllus*, *C. sinensis* (green tea), *C. sinensis* (white tea) and *Vitis vinifera* (seed) were obtained from a compounding pharmacy, and fresh *F. ananassa* and *S. melongena* were obtained from a local market.

### Methanolic extract preparation

Fresh materials were maintained in a 2.5% sodium hypochlorite solution for 30 min and washed in deionized water and picked at 1 cm<sup>2</sup>. Methanolic extracts (1:10, w/v) were prepared using fresh and dehydrated materials from static maceration for 24 h, filtered, and the solvent was evaporated in bath at 45°C. Deionized water was added to residues (ratio 1:10, w/v), and then clarified at 10,000 rpm, for 20 min, at 4°C and supernatant stored at -18°C. *S. melongena* and *C. sinensis* (green tea) extracts were diluted three-times and *C. citratus*, *B. trimera* and *C. sinensis* (white tea) extracts were diluted five-times for further analysis.

### Total polyphenols

Polyphenols compounds were determined by the Folin-Ciocalteu method (Singleton and Rossi, 1965). Total polyphenols content, expressed in µg/ml, was calculated using catechin as the standard for the calibration curve. Results were expressed as catechin polyphenols (CP).

### Pancreatic lipase activity

Porcine pancreatic lipase type II (Sigma-Aldrich) was dissolved in ultra-pure water at 10 mg/ml; then the supernatant was used after centrifugation at 10,000 rpm for 10 min, at 4°C. The assay buffer was 100 mM Tris-HCl buffer (pH 8.2) containing 0.5% Triton X-100, and *p*-nitrophenyl laurate (420 µM) was used as the substrate. The mixture was heated in water bath at 60°C for 15 min into dissolution of the substrate, mixed well, then cooled to room temperature (Pinsirodom and Parkin, 2001). The reaction medium was maintained in water bath at 37°C for 5 min for the reaction equilibrium. Reaction was started with the addition of porcine pancreatic lipase (2 U/ml), and maintained at 37°C for 30 min. Control was prepared without addition of enzymes. Lipase activity was determined at 410 nm (molar extinction coefficient,  $\epsilon = 1.59 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$ ). One unit of enzyme corresponded to the amount of enzymes that releases 1 µM of *p*-nitrophenol per min. All reactions were carried out in triplicate.

### Porcine pancreatic lipase inhibition

Different total polyphenols concentrations were used for enzyme inhibition. Analyses were performed by adding the diluted GTE and enzyme (2 U/ml), as presented in the previous paragraph. The inhibition of the enzymes was obtained from the determination of slopes of straight lines (Abs × time) for testing of the activity of control enzymes (no sample) and enzymes + inhibitor. The slope of the results from the speed of the product formation per minute of

reaction and the presence of inhibitor cause a decrease in its slope. The inhibition percentage (I) was analyzed as follows:

$$I(\%) = \frac{(A - a) - (B - b)}{(A - a)} \times 100$$

Where: *A* absence of extract, with enzyme and substrate; *a* absence of extract and enzyme; *B* presence of extract, with enzyme and substrate; *b* absence of extract, with enzyme. All experiments were performed in quadruplicate.

### Measurement of kinetic constants

In order to measure the Michaelis-Menten constant,  $K_m$ , the inhibition constant,  $K_i$ , and  $V_{max}$ , a series of substrate concentrations (5 to 650 µmol) were tested in the assay system. Each analysis was performed with and without *C. sinensis* extract. Lineweaver-Burk plots were fitted to determine the mechanism of the effect of the extract on porcine pancreatic lipase activity. The inhibition constant,  $K_i$ , was calculated from the following equation:

$$K_m \cdot \text{app} = K_m (1 + [I]K_i)$$

Where  $K_m \cdot \text{app}$  and  $K_m$  represent the  $K_m$  with or without plant extract,  $[I]$  represents the concentration of plant extract.

### Feed composition

Conventional feed (Nuvilab<sup>®</sup>, Sogorb Ind & Market, São Paulo, Brazil) is a balanced food for laboratory mice and rats, based on recommendations of the National Research Council and National Institute of Health - USA. The basic product composition is: calcium carbonate, corn bran, soy bran, wheat bran, dicalcium phosphate, sodium chloride, vitamins (vitamin A 12,000IU, vitamin E 30.0 mg, vitamin K 3.0 mg, vitamin B 18.2 mg, niacin 60.0 mg, pantothenic acid 20.0 mg, folic acid 1.0 mg, biotin 0.05 mg, choline 600.0 mg), amino acids (D,L-methionine 300.0 mg, lysine 100.0 mg), microelements minerals (50.00 mg iron; zinc 60.00 mg; copper 10.00 mg; iodine 2.00 mg; manganese 60.00 mg; selenium 0.05 mg; cobalt 1.50 mg), antioxidant 100.0 mg.

### High-fat feed preparation

Conventional feed (Nuvilab<sup>®</sup>) was prepared increasing 10% (w/w) of lard (Seara Alimentos S.A., St Catarina, Brazil) in its composition. Conventional feed was triturated and lard was incorporated into the standard feed. To this preparation was added distilled water and then manually extruded to pellets formation. The pellets were dried at 60°C until constant weight. According manufacturer's information, the lard composition is (100 g): calorific value 910 kcal, carbohydrates 0%, proteins 2.0 g, total fat 89 g, saturated fat 30 g, trans unsaturated fat 0.2 g, food fiber 0 g and sodium 26 mg.

### Animal and experimental conditions

Female rats (*Rattus norvegicus*), 4-months-old, Wistar strain (240 to 320 g), free of specific pathogens, were obtained from the Animal Experimentation Center (Hermínio Ometto Foundation – Uniararas, Araras, Brazil). The plain and experimental conditions complied with the Ethic and Research Committee of Uniararas (protocol n° 007/2012, May 09th, 2012). Animals were maintained in a controlled temperature room, 12:12 h artificial light-dark cycle and *ad libitum* access to the feed and water. After a random selection,

**Table 1.** Screening of plant extracts with *in vitro* inhibition activity of the porcine pancreatic lipase.

Plant extract	Popular name	Plant part used	Volume used	Activity Inhibition (%)
<i>Echinodorus macrophyllus</i>	Lather hat	Dried leaves and stalks	250 µl crude extract	+
<i>Camellia sinensis</i>	Green tea	Dried leaves and stalks	125 µl dilution 1:3	76.65±2.04
<i>Camellia sinensis</i>	White tea	Dried leaves and stalks	125 µl dilution 1:3	31.18±0.39
<i>Vitis vinifera</i>	Grape	Seed	750 µl dilution 1:3	+
<i>Fragaria ananassa</i>	Strawberry	Dried fruit	250 µl crude extract	14.05±1.93
<i>Solanum melongena</i>	Eggplant	Fresh fruit	750 µl dilution 1:3	+
<i>Baccharis trimera</i>	Gorse	Dried leaves	125 µl dilution 1:5	46.47±1.02
<i>Cymbopogon citratus</i>	Lemon grass	Fresh leaves	125 µl dilution 1:5	29.84±0.70

All extracts were prepared in methanol solvent. Activity inhibition is the relative activity of inhibitor trials with *versus* without inhibitor. Enzymatic assays were carried out at 37°C, 10 min, using p-nitrophenyl laurate as substrate. Dilutions were necessary due to the color of the extracts that interfere in the enzymatic analysis. Symbol: + represent the plant extracts that increased the spectrometric absorbance. Assays carried out in triplicate.

**Table 2.** Effects of catechin polyphenols concentrations on porcine pancreatic lipase activity.

Catechin polyphenols concentrations (µg/ml)	Relative activities (%)
14	69.5±5.2
28	70.2±2.3
42	73.0±2.1
56	76.0±6.4
70	78.0±1.2
84	80.0±4.4
98	75.0±3.2
112	52.0±3.6
126	54.0±2.9

Activity inhibition is the relative activity of inhibitor trials with *versus* without inhibitor. Enzymatic assays were carried out at 37°C, 10 min, using p-nitrophenyl laurate as substrate. Assays were carried in quadruplicate.

the rats were introduced to the standard diet (n = 10) for 15 days. The animals were weighed and randomly distributed into five groups of six subjects each. A randomized trial was carried out to assess the results. Group I received only a conventional diet (CD). Group II received only a high-fat diet (HFD). Group III received a high-fat diet and orlistat™ (1.67 mg/kg) (HFD+OR). Group IV received a high-fat diet and 22.5 mg of dry GT/ml (0.51 mg/ml CP) (HFD+CP 22.5). Group V received a high-fat diet and 112.5 mg of dry GT/ml (2.55 mg/ml CP) (HFD+CP 112.5). Intragastric administration of the extract and orlistat™ was performed with the aid of a gavage needle once a day for a period of 15 days. Every other day throughout the experiment, the feed and water intake was controlled, and individual weighing of the animals was performed at the end of treatment. Beyond the period of treatment, the animals were anesthetized with 0.3 ml of a mixture of ketamine 50 mg/ml and 2% xylazine (3:1). Blood was collected by cardiac puncture and visceral fat was removed and weighed.

#### Biochemical analysis

The blood samples were centrifuged at 3500 rpm for 20 min to obtain the serum. Then, the analyses of total cholesterol,

triglycerides, HDL-cholesterol and LDL-cholesterol were performed with kits for measurement of lipids and AST (aspartate aminotransferase) and ALT (alanine aminotransferase) with kits for hepatic enzyme according to the manufacturer's instructions.

#### Analysis of fecal fat

The fat present in the feces of animals was extracted following the modified methodology proposed by Bligh and Dyer (1959). All samples were performed in triplicate.

#### Statistical analysis

Statistical analyses were performed using Prism 3.0 software and the results were subjected to analysis of variance (ANOVA) and means compared by t-test, adopting a significance level of 5% (p < 0.05).

## RESULTS

### Plant extracts with inhibitory activity against porcine pancreatic lipase *in vitro*

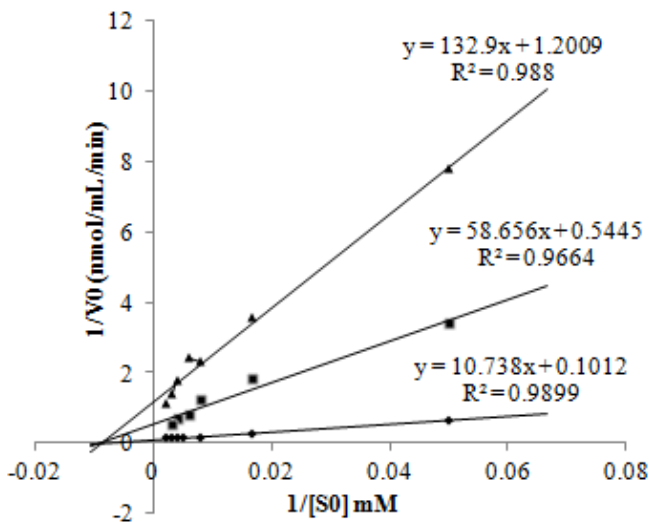
Initially, eight methanolic plant extracts were analyzed for *in vitro* lipase inhibition. According to the results obtained in these experiments, *C. sinensis* extract was identified with the greater inhibitory activity of porcine pancreatic lipase (76.7 ± 2.04%), followed by *B. trimera* (46.4 ± 1.02%), *C. sinensis* (white tea) (31.18 ± 0.39%), *C. citratus* (29.84 ± 0.7%) and *F. ananassa* (14.05 ± 1.93%) (Table 1). *E. macrophyllus* and *V. vinifera* showed an activation effect of the enzyme. *S. melongena* did not inhibit the pancreatic lipase activity. In view of these results, the GTE was selected for characterizing the *in vitro* inhibitory effect on pancreatic lipase activity and to evaluate the effect on the female rats fed with a high-fat diet.

Crude GTE used for the experiments showed an equivalent of 1.70 ± 0.10 mg/ml CP. The inhibitory action of the GTE was evaluated using concentrations of 14 to 126 µg/ml CP, as shown in Table 2. The inhibitory effect

**Table 3.** Serum parameters in female rats fed treated with *C. sinensis* extract in female rats fed with high-fat diet.

Group	Total triglycerides (mg/dl)	Total Cholesterol (mg/dl)	LDL-Cholesterol (mg/dl)	HDL-Cholesterol (mg/dl)
Group I	170.6±23.3 <sup>b</sup>	98.8±15.8 <sup>a</sup>	64.4±8.9 <sup>b</sup>	28.0±1.1 <sup>a</sup>
Group II	227.8±21.3 <sup>c</sup>	129.4±13.9 <sup>a</sup>	98.9±7.3 <sup>c</sup>	36.9±1.8 <sup>a</sup>
Group III	149.3±14.8 <sup>b</sup>	123.7±14.9 <sup>a</sup>	68.9±7.8 <sup>b</sup>	34.4±1.9 <sup>a</sup>
Group IV	127.7±12.6 <sup>a</sup>	94.5±12.4 <sup>a</sup>	32.7±1.7 <sup>a</sup>	27.6±0.9 <sup>a</sup>
Group V	123.4±18.5 <sup>a</sup>	91.7±14.7 <sup>a</sup>	27.0±1.2 <sup>a</sup>	32.5±1.3 <sup>a</sup>

Experimental conditions: Group I received only conventional feed (control negative), Group II received only high-fat diet, Group III received high-fat diet and orlistat (1.67 mg/kg), Group IV received high-fat diet and 0.51 mg/ml of *C. sinensis* extract, Group V received high-fat diet and 2.55 mg/ml of *C. sinensis* extract. Experiments were conducted during 16 days. The values are means ± SD.,  $n = 10$ .  $p < 0.05$  compared to Group II.



**Figure 1.** Lineweaver-Burk plots of the *p*-nitrophenol released from *p*-nitrophenyl laurate in the presence *C. sinensis* extract. (◆) 0 µg/ml, (■) 14 µg/ml, and (▲) 28 µg/ml. Enzymatic assays were carried out at 37°C, 10 min. Assays were carried in quadruplicate.

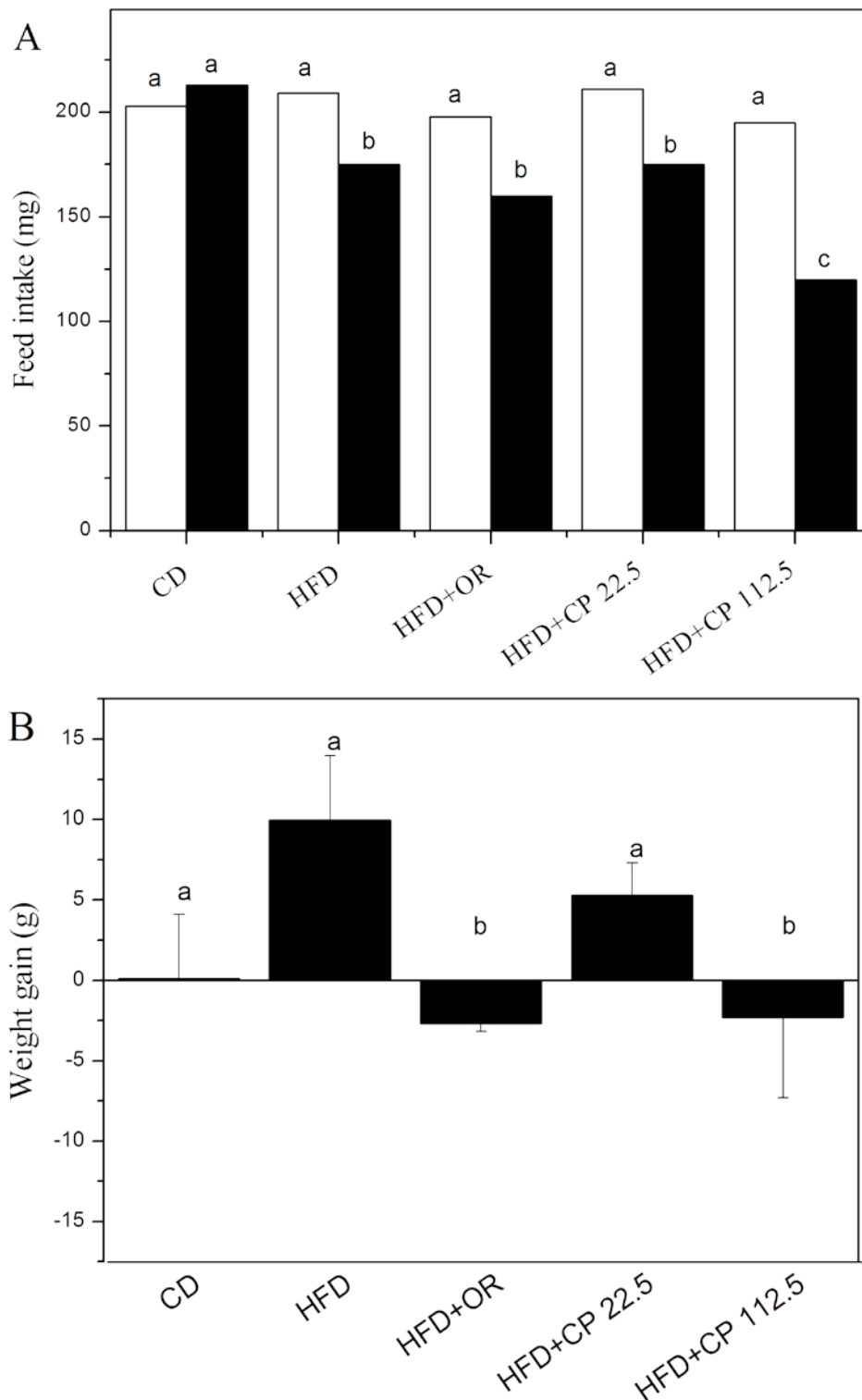
of a wide range of concentration of GTE (14 to 98 µg/ml CP) did not vary significantly ( $p > 0.05$ ), remaining at approximately  $72.82 \pm 3.60\%$ , while values above this concentration range (112 and 126 µg/ml CP) reduced the activity of the pancreatic lipase in only  $52.67 \pm 0.71\%$ . Inhibition mechanism of pancreatic lipase was examined at different concentrations of the *p*-nitrophenyl laurate substrate using a extract concentration equivalent to 14 µg/ml CP (Figure 2). Kinetic data were analyzed using the double-reciprocal plot of Lineweaver-Burk and Michaelis-Menten constants  $K_m$  and  $V_{max}$  were determined (Figure 1).  $K_m$  and  $V_{max}$  values showed that methanolic GTE is a non-competitive inhibitor, as the  $V_{max}$  was reduced from 8.57 to 1.70 nmol/ml/min without changing significantly the  $K_m$ .  $K_i$  value of *C. sinensis* on the enzyme was 3.392 µg/ml CP.

### Effect of the *C. sinensis* extract on the weight gain

*In vivo* studies, the groups that received a high-fat diet consumed less feed compared with Group CD, and Group HFD+CP 112.5 consumed significantly less feed at the final experiment (Figure 2A). The reduction of high-fat diet intake did not cause weight loss in animals in Group HFD; by contrast, the animals significantly ( $p < 0.05$ ) increased weight gain relative to Group CD (Figure 2B). These results suggest that Group HFD had a higher energy intake while consuming the least amount of feed. Group HFD+CP 22.5 diet did not significantly prevent the weight gain compared with Group HFD+OR, but showed a clear prevention trend. Group HFD+CP 112.5 diet prevented the weight gain of the rats and promoted a significant loss of  $2.80 \pm 0.82$  g to the final experiment. Group HFD+OR showed weight loss of  $4.4 \pm 1.7$  g compared with Group HFD. These results show that the response of animals to the treatment with orlistat to promote weight loss is similar to that observed in humans due to its ability to inhibit the pancreatic lipase and reduce lipid absorption. Differences in weight gain of the treatments were also observed in the excretion of lipids in feces (Figure 3). Group HFD+OR and Group HFD+CP 22.5 increased the lipid excretion in feces in 54.6 and 31.4%, respectively, in final experiments. However, animals of Group HFD+CP 112.5 lost weight without increasing the fecal excretion of lipids.

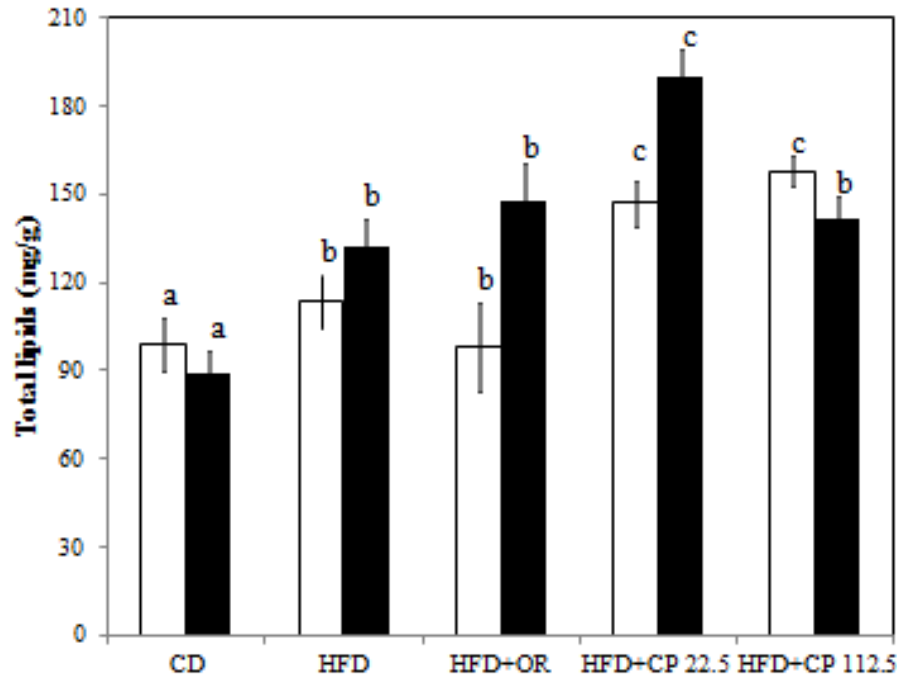
### Effect of *C. sinensis* extract on the changing levels of lipids and lipoproteins

The treatments in Group HFD+OR, Group HFD+CP 22.5 and Group HFD+CP 112.5 reduced triglyceride levels significantly ( $p < 0.05$ ) during the experimental period (Table 3). The results observed in Group HFD+OR and Group HFD+CP 22.5 could be associated with the increase of lipid excretion, while in Group HFD+CP 112.5 the triglycerides reduction could be related to the feed intake reduction, increased thermogenesis, fat oxidation and energy expenditure. The total cholesterol level was



**Figure 2.** The effect of *C. sinensis* extract on feed intake (A) and body weight gain (B) in female rats fed the experimental diets. Group CD received only conventional feed (negative control), Group HFD received only high-fat diet (positive control), Group HFD+OR received high-fat diet and orlistat (1.67 mg/Kg), Group HFD+CP 22.5 received high-fat diet and green tea extract (22.5 mg/mL of GT), Group HFD+CP 112.5 received high-fat diet and green tea extract (112.5 mg/mL of GT). Experiments were conducted during 16 days. Legend: (□) Initial experiment, (■) Final experiment. The values are means  $\pm$  SD.,  $n = 10$ .  $p < 0.05$  compared to Group II.





**Figure 3.** Total lipids of the feces in female rats fed treated with *C. sinensis* extract fed with high-fat diet. Group CD received only conventional feed (negative control), Group HFD received only high-fat diet (positive control), Group HFD+OR received high-fat diet and orlistat (1.67 mg/Kg), Group HFD+CP 22.5 received high-fat diet and green tea extract (22.5 mg/ml of GT), Group HFD+CP 112.5 received high-fat diet and green tea extract (112.5 mg/ml of GT). Experiments were conducted during 16 days. The values are means  $\pm$  SD.,  $n = 10$ .  $p < 0.05$  compared to Group II. Legend: (□) initial lipids in the feces, (■) final lipids in the feces.

not changed significantly, but tended to decrease in animals in Group HFD+CP 22.5 and Group HFD+CP 112.5. LDL-cholesterol decreased significantly ( $p < 0.05$ ) in Group HFD+CP 22.5 and Group HFD+CP 112.5 compared with Group CD and Group HFD, while Group HFD+OR showed no change in this parameter. However, the results showed that the HDL-cholesterol and visceral fat did not differ significantly. The activities of serum hepatic aminotransferases (aspartate transaminase and alanine transaminase) of all treatments were not altered, indicating that likely the GTE did not cause a hepatotoxic effect in these animals (data not shown).

## DISCUSSION

Among extracts studied, *C. sinensis* presented a strong *in vitro* inhibitory effect on lipase pancreatic. Other plant extracts, such as *Baccharis trimera*, *C. sinensis* (white tea), *Cymbopogon citratus* and *Fragaria ananassa* also had significant, but weaker inhibitory effect on the pancreatic lipase activity, whereas *Solanum melongena* were ineffective. This result confirms the inhibitory effect of GTE on the pancreatic lipase activity suggested by

Gondoin et al. (2010). The main inhibitory effect of the pancreatic lipase was attributed to catechin galatte, epigallocatechin gallate or epicatechin compounds present in the major fraction in the GT (Juhel et al., 2000; Martins et al., 2010). However, *Echinodorus macrophyllus* and *Vitis vinifera* showed activator effects on pancreatic lipase activity. These results were also observed for other plant extracts, suggesting that the lipase activation occurs due to the stabilization of the non-polar surface of the active site by the contact of the extract with a polar environment (Kato and Tosa, 1983; Nagen et al., 1995; Souza, 2009). Our results showed that using *C. sinensis* extract up to 98  $\mu\text{g/ml}$  inhibited pancreatic lipase, but above this concentration the inhibition decreased to 52.67%. These results are contradictory to those presented by Curiel (2011), which characterized the inhibitory effect of GT to be dose-dependent. Moreover, the author did not observe any reduction in inhibition of pancreatic lipase at higher concentrations of GT. Martins et al. (2010) suggested that the inhibition by polyphenols depended on how the substrate was presented to the lipases, and that the phospholipid species, especially the choline moieties, profoundly affected the lipase inhibitory activity of *Ilex*

*paraguariensis*. Thus, it was noted that the extract used in this study was prepared by methanol extraction, while Curiel (2011) used a *C. sinensis* infusion.

Pancreatic lipase inhibition by *C. sinensis* was first-time demonstrated to be non-competitive inhibition, and the  $K_i$  value of *C. sinensis* on the enzyme was 3.392  $\mu\text{g/ml}$  of CP. The inhibitory mechanism of polyphenols on pancreatic activity remains unclear. Won et al. (2007) showed a non-competitive inhibition of pancreatic lipase using licochalcone A, and a  $K_i$  value of 11.2  $\mu\text{g/mL}$ . Martins et al. (2010) verified that the inhibition of pancreatic lipase by *I. paraguariensis* was of a competitive type, and had a  $K_i$  value of 12.9 mmol/ml (3.0 mg/ml maté tea). Gholamhoseinian et al. (2010) observed a non-competitive inhibition of pancreatic lipase for *Rosa damascene*, *Quercus infectoria* and *Eucalyptus galbie* and mixed inhibition for *Levisticum officinale*, using methanolic extracts. Chanmee et al. (2013) observed that a *Solanum stramonifolium* compound, named carpesterol, presented a competitive inhibition of pancreatic lipase.

In the *in vivo* experiments a reduction in feed intake was observed for Groups HFD+OR, HFD+CP 22.5 and HFD+CP 112.5 compared with Group HFD. These results can be associated with substances known to increase hepatic fatty acid oxidation, such as beta-adrenergic agonist, and decrease voluntary food intake in rats (Kahler et al., 1999). Kao et al. (2000) showed that rats treated with (-)-epigallocatechin-3-gallate (EGCG) by intraperitoneal injection had a reduction in food intake of 50 to 60% versus control rats. Belza et al. (2007) conducted a short-term trial in normal weight men. Subjects consumed 8% less energy at an *ad libitum* meal 4 h following the consumption of 500 mg GTE versus placebo. Given the evidence that catechin may increase hepatic fat oxidation, it is plausible that appetite may be altered by GT (Rains et al., 2011). Therefore, the reduction in feed intake by Group HFD+OR can be related to the consumption of a high-fat diet. Consumption of medium-chain fatty acids and 1,3-diacylglyceride oil, ingredients that increase hepatic fatty acid oxidation, has been shown to reduce food intake in human subjects (St-Onge and Jones, 2002).

Administration of 22.5 mg/ml of GT (Group HFD+CP 22.5) showed a clear trend of weight gain prevention compared with Group HFD+OR and increased fecal lipid excretion, while administration of 112.5 mg/ml of GT (Group HFD+CP 112.5) prevented the weight gain of the rats and promoted a significant loss of  $2.80 \pm 0.82$  g in the final experiment. The weight gain reduction of Group HFD+CP 112.5 can be related to the feed intake, but can also be associated with other action mechanisms of GT already reported in the literature. GT catechins may stimulate thermogenesis and fat oxidation through inhibition of catechol O-methyl-transferase, resulting in increased energy expenditure that promotes weight reduction (Diepvens et al., 2005; Phung et al., 2010). In

addition to catechins, the mixture of GTE and caffeine, which has been reported *in vitro*, has thermogenic effects and can stimulate fat oxidation, in part via sympathetic activation of the central nervous system (Diepvens et al., 2005). The fact that a catechins-caffeine mixture stimulates energy expenditure cannot be completely attributed to its caffeine content because the thermogenic effect of a catechins-caffeine mixture is greater than that of an equivalent amount of caffeine (Hursel et al., 2009). In healthy men supplemented with GTE containing 270 mg EGCG and 150 mg caffeine, energy expenditure increased significantly by 4% compared with caffeine alone, and fat oxidation was 41% for GT compared with 33% for caffeine alone (McKay and Blumberg, 2007).

The *C. sinensis* extract prevented the high-fat diet-induced increases in body weight and decreased the serum triglyceride and LDL-cholesterol concentrations, but did not significantly alter the total cholesterol and HDL-cholesterol. Beside this results, the treatments did not demonstrate alteration in the hepatic enzymes (aspartate transaminase and alanine transaminase), what can indicate that the animals that received GTE did not present hepatic disorder effect. In a similar study, Chanadiri et al. (2005) investigated the effectiveness of GT catechins in the disorder of lipid metabolism, antioxidant status and excess body weight by administration of a high-fat diet in rats for 7 weeks. The results showed that the group that received the GT catechins corrected the biochemical parameters of lipid metabolism (total cholesterol, triglyceride and LDL), visceral fat and activity of antioxidant enzymes. Jang and Choung (2013) showed that rats fed with a high-fat diet initially exhibited significantly higher triglycerides, total cholesterol, LDL-cholesterol and free fatty acids, and lower HDL-cholesterol and HDL-cholesterol/total cholesterol ratio. But, with the administration of *L. japonica* extract or tea catechin, these parameters decreased to near normal levels in serum and liver, indicating that oral administration of the extract or tea catechin suppresses the accumulation of body fat in a dose-dependent manner, resulting in improved lipid profiles in serum and liver without any renal or hepatic toxicity. Ikeda et al. (2003), and Murase et al. (2002) show that GT catechins may significantly decrease body weight, visceral fat and hepatic triacylglycerol concentration, in addition to significantly increase the activity of  $\beta$ -oxidation of fatty acid in the liver and decrease the activity of the enzyme fatty acid synthetase, explaining the decrease in liver triacylglycerol and visceral fat deposition. Finally, our results suggest that GTE has an anti-obesity preventive function by inhibiting the hydrolysis of dietary fat in the small intestine, subsequently reducing intestinal absorption of dietary fat; however, although mechanistic studies have suggested that tea decreases lipid and carbohydrate absorption, increases lipid metabolism, inhibits de novo lipogenesis, and increases carbohydrate utilization, the relative



importance of these mechanisms to human disease remains unclear (Grove and Lambert, 2010).

## Conclusion

This study demonstrated that the *in vitro* inhibition effect of *C. sinensis* extract was not dose-dependent and it was also demonstrated by the first-time that the methanolic green tea extract presented a mechanism of non-competitive inhibition on porcine pancreatic lipase. *In vivo* study, green tea extract decrease the feed intake by the animals but the weight reduction of female rats fed a high-fat diet was promoted by the polyphenols of the extract following the increase of the lipids in feces. Beside of this, green tea extract presented benefic effects on lipid metabolism that was not observed with the treatment with orlistat; and so, green tea extract can be administrated to control the biochemical parameters as total cholesterol, triglyceride and LDL, and visceral fat.

## Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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