

The effect of a high calorie diet containing a peanut candy on weight loss

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Abstract

The consumption of peanuts and other oilseeds (nuts) as walnuts, almonds, chestnuts, hazelnuts and pistachios promote weight loss. Mice, male (20 g - 35 g) were treated on a standard diet (SD, n = 08) and a test diet (high calorie) (HD, n = 08). The HD standardized in this experiment was composed of a mixture of commercial feed Labina® flour and processed foods, peanut paçoca (peanut candy), in the proportion of 30% commercial feed Labina® and 70% peanut paçoca. Analyses were made on the chemical composition of the diets, evaluating feed efficiency (FE) and mice's satiety. Antitryptic activity was done. Also been investigated the pancreas through organ histology and biochemical analysis of blood serum. The group HD showed weight loss when compared to the group SD. The FE was greater in the group SD when compared to the group HD. The mice on the group HD had a lower average food intake at the end of fifteen weeks. This research shows that consumption of peanut paçoca, food derived from peanut, could help to promote satiety and reduced weight gain, without causing damage to the pancreas, or cause changes in biochemical parameters analyzed, even with a diet high in trypsin inhibitor.

Keywords

Obesity

Peanut paçoca

Satiety

Trypsin inhibitor

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Introduction

Obesity stands out among the diseases caused by nutritional inadequacies and is growing in many regions of the world, reaching epidemic levels (Must *et al.*, 1999; Bmi and Bmi, 2004; Azagury and Lautz, 2011). The increasing prevalence of obesity is considered a major cause of cardiovascular disease and diabetes type 2 (Bmi and Bmi, 2004; Hossain *et al.*, 2007).

Because it is an important public health problem, which affects not only adults but children and adolescents (Caballero, 2007; Selassie and Sinha, 2011), it is necessary to reduce the intake of foods high in simple sugars, trans fat and with high levels of saturated fats, and in return, to stimulate the consumption of foods with significant amounts of minerals, vitamins, fiber and essential polyunsaturated

fatty acids, nutrients typically found in oil seeds and nuts (Venkatachalam and Sathe, 2006).

Several studies have shown that the consumption of peanuts and other oilseeds (nuts) as walnuts, almonds, chestnuts, hazelnuts and pistachios reduces the risk of cardiovascular disease, mainly by reducing LDL-c (low density lipoprotein) and triglycerides, while maintaining stable levels of blood glucose (Jiang *et al.*, 2006; Kocyigit *et al.*, 2006; Ros, 2009, 2010).

Some studies have shown that these seeds promote weight loss. The responsible mechanism for not gaining weight when ingesting these oilseeds is still being investigated, the effects being attributed mainly to satiety (Hollis and Mattes, 2007). In a study of acute preload, peanuts exerted a strong suppression of hunger and caloric compensation (Hollis and Mattes, 2007). Still, according to some

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authors (Kirkmeyer and Mattes, 2000), snacks containing peanuts and peanut butter show up as an effective way to control hunger without leading to weight gain. However, it is unclear whether or not all foods derived from peanuts would show this effect, since there are few studies related.

The reason for its satiety power, has been proposed to be its lipid composition, fiber (Traoret *et al.*, 2008; Devitt *et al.*, 2011), and there are reports in the literature that suggest that the presence and influence of trypsin inhibitors can lead to weight reduction (Komarnytsky *et al.*, 2011; Nakajima *et al.*, 2011; Chen *et al.*, 2012; Ribeiro *et al.*, 2015). In this context it is noteworthy that the trypsin inhibitors increase the concentration of the cholecystokinin (CCK) hormone, which has satiety power. Further studies have confirmed the presence of trypsin enzyme inhibitor in peanuts (Ahmed and Applewhite, 1988; Maleki *et al.*, 2003), and peanut products, such as in the Japanese peanut, peanut butter, and peanut nougat (Araújo *et al.*, 2014).

Insofar as new studies are done on the health claim of peanuts, various foods with this legume are increasingly being consumed in Brazil, such as: peanut oil, peanut paste and peanut paçoca, consumed as dessert. In this sense studies that clarify whether the benefits attributed to peanut extend to their products are very important, so that society becomes to know them and broadly enjoy all its qualities. Thus, this study aims to assess the weight of swiss mice subjected to a diet with peanut paçoca, the peanut candy typical Brazilian.

Materials and Methods

Materials

The enzyme trypsin and bovine serum albumin, the substrate N-benzoyl-DL-arginine-p-nitroanilide (BAPNA) and the reagents acrylamide and N,N'-methylenebisacrylamide were purchased from Sigma® Chemical (St. Louis, MO) and Dimethylsulfoxide (DMSO) from RECTAPUR GPR® (VWR International, BDH) BDH Prolabo®. The kits for serum dosage were diagnostic kit transferases of Labtest® and CELM® Kit (São Paulo, Brazil). The other reagents were purchased commercially and always with high analytical grade. The peanut paçoca was from local supermarket (RN, Brazil). The feed used was a commercial feed Labina® which was acquired at Purina® (Paulínia, SP, Brazil).

Experimental model and animals

The experimental protocol of this study was submitted to the Committee on Animal Research and

Ethics (CEUA), Federal University of Rio Grande do Norte - UFRN. For the development of the protocol No. 021/2011 we adopted the ethical principles and procedures according to the Standard Guide for Care and Use of Laboratory Animals (1996). This study was conducted in the Laboratory and Animal Facility of the Department of Biochemistry, Federal University of Rio Grande do Norte-UFRN.

Young adult mice, male Swiss strain (20 g - 35 g) were obtained and maintained in polypropylene cages (n = 8 per cage) in the vivarium of the Center for Biosciences UFRN with a temperature of $23 \pm 2^\circ\text{C}$, dark-light cycle of 12 hours and humidity between 45 and 55%, controlled. After 6 days of adaptation, mice were randomly divided into two groups: treated with standard diet (SD), referred to as standard group (SG, n = 08) and test diet high calorie (HD), named test group (TG, n = 08).

Diets

The experimental diets of the mice followed the specifications of the guide Nutrient requirements of the laboratory rat. The standard diet used the commercial feed Labina®, Table 01. The standardized high calorie diet of this experiment was composed of a mixture of the commercial feed Labina® flour and the processed food, peanut paçoca, in a proportion of 30% commercial feed Labina® and 70% peanut paçoca, as shown in Table 01. The nutritional composition of peanut paçoca consists of: 113 kcal, 12 g carbohydrate, 3.3 g protein, 5.8 g total fat, 0.7 g saturated fat, 0 trans fat, 0.9 g fiber feed, 41 mg of sodium in 22 g (one unit). To analyze the centesimal chemical composition, nutritional information provided on labels of commercial feed Labina® and peanut paçoca was added to the program Diet Pro®.

Table 1. Centesimal composition and contribution percentage of macronutrients in 100 g of standard diet (SD) and high calorie diet (HD), in accordance with the program Diet Pro®

Variables	Diets			
	^a SD	%	^b HD	%
Energy (Kcal)	350		454	
Protein (g)	22	22	15	15
Lipids (g)	16	16	18	18
Carbohydrate (g)	52	52	58	58
Ash (g)	11	11	4	4
Humidity (g)	9	9	5	5
Total (g)	100		100	

^a SD (commercial feed Labina®); ^b HD [(30% commercial feed Labina® and 70% peanut paçoca (peanut candy)].

Feed efficiency of animals

In order to analyze the ability to convert consumed

food energy into animal body weight, the calculation of FE was performed with 16 mice (8 SG and 8 TG). This calculation was obtained by dividing the average weekly weight gain of the animals in each group by the total energy (kcal) intake, multiplied by 100 (Campbell, 1963). The energy taken in was calculated by multiplying the amount of ingested food by the value of each diet.

$$\text{FE} = \frac{\text{weekly weight gain}}{\text{total consumed energy}} \times 100$$

Preparation of crude extract and protein fractionation of peanut paçoca

The peanut paçoca was macerated in a cold mill (6°C) until the formation of fine grained flour, of about 40 mesh. Total proteins of this flour were extracted in Tris-HCl buffer 50 mM, pH 7.5, at a ratio of 1:10 (w/v). The mixture was subjected to constant stirring, for 3 hours at room temperature. Afterwards, the material was centrifuged at 12,000 g for 30 min at 4°C. The supernatant was named Crude Extract (CE) and its precipitate was called (PCE).

The crude extract was sequentially fractionated with ammonium sulfate at 0-30% (F1), 30-60% (F2), and 60-90% (F3) saturation. In the following, each fraction was maintained for about 10 h at 8°C temperature and centrifuged at 12,000 g for 30 min at 4°C, in order to obtain the precipitated proteins. The precipitates were resuspended in Tris-HCl buffer 50 mM, pH 7.5, then dialyzed against the same buffer and kept at -20°C until the fulfillment of the experiments.

Determination of soluble proteins

The proteins were quantified by the Bradford method (Bradford, 1976) using bovine serum albumin (BSA) as standard. The reading of protein quantification was performed on a spectrophotometer at 595 nm.

Antitrypsin activity from extract and protein fractions

The antitrypsin activity of the PCE, CE, F1, F2 and F3 were determined in accordance to Kakade *et al.* (1969), the aliquot of 20 µL bovine trypsin solution (0.3 mg/ml 2.5 mM HCl) was pre-incubated with 560 µL of Tris-HCl buffer 50 mM, pH 7.5, 120 µL of HCl 2.5 mM and 100 µL CE, F1, F2 and F3 for 15 minutes at 37°C. Afterwards, the reaction was started by adding 500 µL of substrate solution (BAPNA 1.25 mM). The reaction was processed for 10 minutes under the same incubation conditions and then stopped by adding 120 µL of acetic acid 30%. All assays were performed in triplicate and

blank tests were performed. The absorbance was measured at 410 nm. The results were expressed in µg of soluble protein/µL and used to calculate specific activities expressed in IU unit (inhibition)/mg of soluble proteins. The IU was defined as the amount of inhibitor capable of decreasing the value of 0.01 nm absorbance in the antitryptic test.

Histological and morphometric analysis of pancreas

In 16 mice (8 SG and 8 TG), anesthetized with xylazine 2% and ketamine 5%, the thoracic and abdominal cavity was opened and the pancreas was removed. The removed organ was rinsed in saline, dried on suitable and absorbent paper. The data of animals weight and organs were used for the calculation of absolute and relative body weight, contributing to its macroscopic characterization according to Ohmuraya *et al.* (2005).

After opening the abdominal cavity, the macroscopically evaluated pancreas and the removed pancreas fragments were fixed by immersion in 10% buffered formalin. During the processing of the tissue, it was subjected to dehydration. Then diaphanization was performed in xylene and impregnation by paraffin. The addition was performed on paraffin blocks. Histological sections were prepared by microtomy, at 4 µm, and stained with routine technique of hematoxylin eosin (HE). Histological analysis of the fragments was performed by adopting the criteria: randomly choosing three cuts, which were fixed on slides, previously prepared with organosilane (Ohmuraya *et al.*, 2005).

Biochemical analysis of blood serum

At the end of the treatments, 16 mice (8 SG, 8 TG) were put on fasting for 12 to 15 hours and anesthetized with xylazine 2% and ketamine 5%. Blood was collected by cardiac puncture and stored in Falcon tubes. Serum was separated by centrifugation at 3,000 x g for 10 minutes and used for the determination of glucose (GL), triglyceride (TG), total cholesterol (COL), high density lipoprotein (HDL-c) and LDL-c. The method employed in the assays was the enzymatic colorimetric, using the CELM® kit (São Paulo, Brazil). The LDL-c were calculated according to the formula described by Friedwald *et al.*, (Johnson *et al.*, 1997), $\text{LDL-c} = \text{COL} - (\text{VLDL} + \text{HDL-c})$, considering that $\text{VLDL} = \text{triglyceride}/5$, when TG is less or equal to 400 mg/dL. In concentrations with TG higher than 400 mg/dL, the VLDL concentrations were estimated by the mean value of the present group. The measures were read in a spectrophotometer.

Blood samples were also subjected to enzymatic

assays for glutamic-pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT), using the diagnostic kit transferases of Labtest®.

Statistics

The weight, food intake and feed efficiency were analyzed using the multifactor ANOVA for finding possible differences between groups (TG) and (SG) over the 14-week experiment. When significant differences were detected, the post-hoc Tukey test was applied. The biochemical parameters (glucose, total cholesterol, HDL-c, LDL-c, Triglycerides, GOT and GPT) were analyzed and compared between the different groups (TG) and (SG) using the t-Student test. Data were analyzed for normality and homoscedasticity using the Kolmogorov-Smirnov test and Levene test, respectively. Data that did not fit the assumptions of the test were transformed (log 10, ln, and square root) and evaluated using the corresponding nonparametric tests. All data were analyzed using the software Statistica 7 (Stat Soft, Tulsa, OK, USA).

Results and Discussion

Despite the dense energy value of the “nuts”, research has shown that some of the energy in these seeds is not available or there even may be, in their composition, some component that allows higher metabolic activity, causing weight loss than expected (Traoret *et al.*, 2008; Devitt *et al.*, 2011). Among those seeds stands out worldwide the peanuts. Some studies (USDA, 2012) show that 100 grams of roasted peanuts without adding oil provide approximately 615 kcal, 20 g carbohydrate, 51 g fat, 26 g protein, 2 g ash and 8 g fiber.

Analyzing peanut *paçoca*, a food traditionally used as a dessert in Brazil, and becoming one of the most popular sweets derived from peanuts, we could observe weight loss in the group of swiss mice subjected to HD (30% commercial feed Labina® and 70% peanut *paçoca*), the TG. This reduction possibly happened due to satiety, demonstrated by the decreased intake of the HD by the TG during the experiment, when compared to SG with the SD ($t = 27.2$, $p = 0.05$) (Figure 1A). It is important to say that the amount of feed in grams offered to the mice was equal for both groups.

The same that happened to the group that consumed peanut *paçoca* (TG), a food derived from peanuts, occurred in other studies, where mice that consumed peanut butter and peanut oil in their diet had weight loss when compared to the SG (Kirkmeyer and Mattes, 2000; Johnston and Buller,

2005; Coelho *et al.*, 2006; Hollis and Mattes, 2007) also pointing signs of satiety in weight control. Satiety reflected in a lower food intake (amount of food), defined as a factor that determines a lower feed rate; it is estimated that between 55-75% of the energy consumption from peanuts is offset by a lower food intake, regarding the satiety process (Hollis and Mattes, 2007).

This satiety result is clearly demonstrated in Figure 1B, when it was expected that the TG, with the HD, to have a greater weight gain due to the fact that HD presents 29% more calories, when compared with the SD ($f = 46$, $p < 0.05$). In the analysis of the macronutrients, higher percentages of lipids and carbohydrates, 18% and 58% respectively, were found for the HD in 100 g, offering 4.5 Kcal/g (Table 01).

In the study it can be seen that the differences in weight gain between the SG and the TG were due to a lower feed efficiency observed in the group submitted to HD when compared to the SD ($f = 16.68$, $p < 0.05$) (Figure 1C). However, this lower FE is a result of satiety caused by HD shown in Figure 1A, since the mice that underwent HD consumed a smaller volume of feed and consequently had a lower energy value. Some authors suggest the existence of a distinct effect on satiety, according to the type of product derived from peanuts (Coelho *et al.*, 2006). In this study we could confirm this with a dessert, the peanut *paçoca*.

Some authors (Hollis and Mattes, 2007) cited several factors that may explain the mechanism by which peanut exerts this effect on body weight. First, because it has a high content of dietary fibers and proteins, it promotes suppression of hunger and energy compensation, so the energy consumed is not significantly increased. Second, by not being totally absorbed, especially in the fresh form, it drags other nutrients in feces. And third, because of its high level of unsaturated fatty acids, it increases energy expenditure of individuals due to the higher metabolic rate (Jiang *et al.*, 2002; Johnston and Buller, 2005).

The lower weight gain promoted by eating peanuts can also be due to the presence of protease inhibitors found in these seeds (Ahmed and Applewhite, 1988; Pusztai *et al.*, 1997), beyond the specific nutritional composition of peanuts. Some authors show that the signal for the release of CCK, a hormone involved in the process of satiety, depends on the concentration decrease of pancreatic enzymes in the lumen (occurring through the action of these protease inhibitors), the signal is probably mediated by a peptide originated from the monitor jejunum or pancreas. In the absence of inhibitors, the peptide

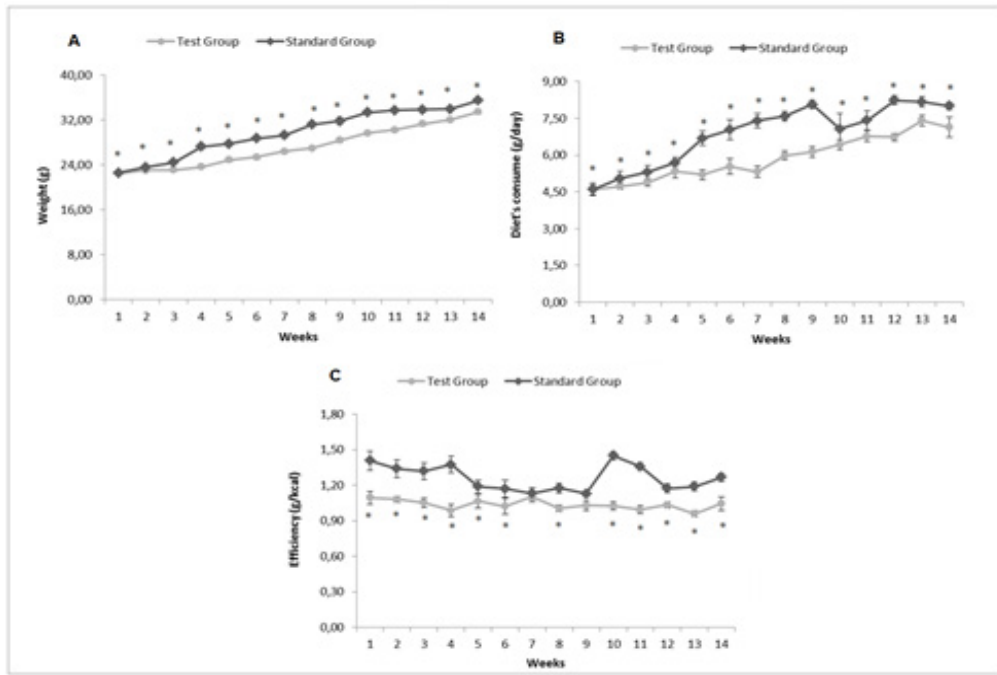


Figure 1. A) Average food intake in grams per day (g/day) of Swiss mice submitted to a Standard Diet (SD) and a high calorie diet (HD) for 14 weeks. B) Evolution of body weight in grams (g) of Swiss mice submitted to a Standard Diet (SD) and a high calorie diet (HD) for 14 weeks. C) Average feed efficiency in grams per kilocalories (g / kcal) of Swiss mice submitted to a Standard Diet (SD) and a high calorie diet (SH) for 14 weeks. Data are expressed as mean \pm standard deviation of the group of animals (Standard Group - SG, n = 08, Test Group - TG, n = 08). *P < 0.05 vs. standard group. The SD used was the commercial feed Labina® (Purina®, Paulínia, SP, Brazil). The standardized HD in this experiment was made of 30% commercial feed Labina® and 70% peanut paçoca (peanut candy). *p < 0,05 vs SG. ANOVA two-way com test post-hoc de Tukey

monitor is broken and inactivated by proteases present in the lumen. When the inhibitors are present, the level of proteases is low and insufficient to inactivate the peptide monitor. The process of stimulating the endocrine cells of the gastrointestinal tract is started (Liener, 1986; Garthoff *et al.*, 2002; Komarnytsky *et al.*, 2011; Nakajima *et al.*, 2011; Ribeiro *et al.*, 2015), thereby increasing the levels of CCK, which suggested its effect on the control of hunger and satiety.

According to some authors (Degen *et al.*, 2001; Tai *et al.*, 2010), satiety and the reduction of the size of the meals is triggered by the cholecystinin hormone by binding to receptors in the gut, generating a signal that is transmitted by the vagus nerve to the brainstem, where it is relayed to the brainstem. Cholecystinin is also produced directly by the brain, where it acts as a neurohormone in reducing food intake.

The peanuts is known to be rich in trypsin inhibitors (Maleki *et al.*, 2003; Dodo *et al.*, 2004) and peanut products (Araújo *et al.*, 2014), therefore the speculation that the cause of satiety can be attributed to the presence of these inhibitors seems plausible.

We investigated the presence of protease inhibitors in protein fractions obtained from extracts

of peanut paçoca. Were detected antitryptic activities in the crude extract (2.58 IU/mg), precipitate of crude extract (0.652 IU/mg), and protein fractions in F1 (5.51 IU/mg), F2 (18.5 IU/mg) and F3 (3.97 IU/mg) (Figure 2).

According to some authors (Oliveira *et al.*, 2009) the production of peanut paçoca is obtained by first roasting the seeds and then by grinding them to obtain thin dough, finally, the homogenization of the thin dough occurs with a mixture of specific ingredients. Therefore, it was expected inhibitors not to be present, assuming that inhibitors proteases are mostly thermolabile, however it is known that thermal heating has proved itself quite efficient for the whole grains, but with decreased effects or even ineffective when it comes to flour or purified inhibitors (Carvalho and Sgarbieri, 1997; Machado *et al.*, 2013). Observed in the present study the significant antitrypsin activity and consequently the existence of the trypsin inhibitor in the protein fraction of peanut paçoca.

Some studies attribute to trypsin inhibitors responsibility for the low nutritional value of raw vegetables (Silva and Silva, 2000; Carvalho *et al.*, 2002). Consumption of foods containing inhibitors

Table 2. Serum biochemical parameters of Swiss mice submitted to a Standard Diet *(SD) and a high calorie diet *(HD) for 14 weeks

	Standard Group (SG)		Test group (TG)		t	P
	Average	SEM	Average	SEM		
Fasting Blood Glucose (mg / dl)	123.70	9.20	134.00	11.25	0.833	0.426
Total Cholesterol (mg / dL)	50.67	2.56	48.17	3.08	0.623	0.546
HDL-c (high-density lipoprotein) cholesterol (mg / dl)	56.17	5.31	63.33	4.26	0.518	0.639
LDL-c (low-density lipoprotein) cholesterol (mg / dl)	37.80	6.98	36.20	1.20	0.225	0.005
Triglycerides (mg / dL)	55.83	4.57	56.83	1.05	0.792	0.006
GOT (glutamic oxaloacetic transaminase) (U / L)	76.50	4.23	82.00	3.51	1.000	0.340
GPT (glutamic pyruvic transaminase) (U / L)	30.17	1.35	32.83	2.67	0.889	0.394

*For the SD we used the commercial feed Labina® diet (Purina®, Paulínia, Brazil). The standardized HD in this experiment was of 30% commercial feed Labina® and 70% peanut paçoca (peanut candy).

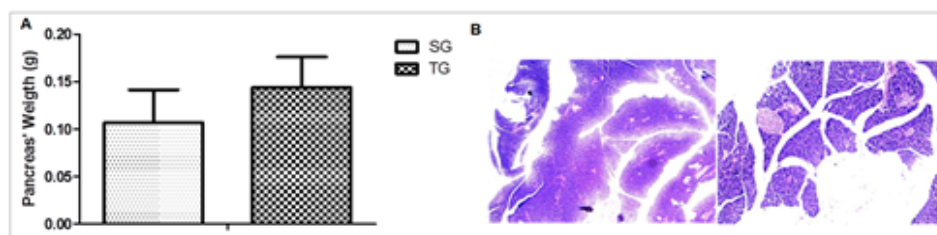


Figure 2. A) Histology of the pancreas of Swiss mice submitted to a Standard Diet (SD) and a high calorie diet (HD) for 14 weeks. (a) Test Group - TG, n = 08 and (b) Standard Group - SG, n = 08. The tissue was stained with hematoxylin and eosin. The group was examined and the representative results are shown (original magnification, X312). B) Morphometry of the pancreas of Swiss mice submitted to a Standard Diet (SD) and a high calorie diet (HD) for 14 weeks. Test Group - TG, n = 08 and Standard Group - SG, n = 08. After the removal of the pancreas, the removed organ was rinsed in saline, dried on suitable absorbent paper and weighed. For the SD we used the commercial feed Labina® (Purina®, Paulínia, SP, Brazil). The standardized HD in this experiment was made of 30% commercial feed Labina® and 70% peanut paçoca (peanut candy)

can influence the digestibility of the protein of these foods in 25% (Carvalho *et al.*, 2002). Besides impairing the digestion of proteins, the presence of this type of inhibitor in the gastrointestinal tract can lead to an increased enzyme secretion by the pancreas, with consequent hypertrophy and hyperplasia of this organ, causing metabolic changes of the pancreas; the presence of trypsin inhibitor in legumes is also associated with a reduction in the growth rate (Al-Wesali *et al.*, 1995; Vasconcelos *et al.*, 2001).

Due to the presence of the trypsin inhibitor in peanut paçoca, a histopathological analysis was performed on the pancreas of mice, and thus it was demonstrated that the pancreas of SG animals, submitted to a SD, showed no pathological alteration, since 37.5% the TG, that underwent the HD, showed

increased organ (Figure 2A) and hyperplasia of acinar glands (Figure 2B), probably due to transitory higher enzyme secretion conditioned by the presence of the inhibitor (Kirkmeyer and Mattes, 2000). Nevertheless, no changes were found in the islets of Langerhans. Besides pancreatic histopathology analysis, a biochemical serum analysis of mice was performed (Table 02), and GPT and GOT were dosed, since they are sensitive indicators of liver damage, however no significant difference was shown between the results presented by the SG and TG groups.

Other biochemical parameters were also measured to reflect possible changes due to the consumption of HD, however no significant difference was shown between the results presented.

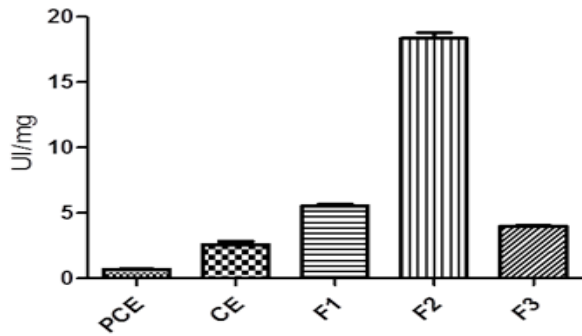


Figure 3. Inhibition of trypsin activity by the precipitated crude extract (PCE), crude extract (CE) and protein fractions saturated with ammonium sulfate in the range of 0-30% (F1), 30-60% (F2) and 60-90% (F3), obtained from the flour of the peanut paçoca (peanut candy). The results were expressed in IU (Inhibition unit)/mg of soluble proteins, using 100 μ L of PCE, EC, F1, F2 and F3, and BApNA as substrate

The analysis of the results showed that the mice that received food with peanut paçoca had a lower weight gain (g) when compared to the group that underwent the SD. Thus, this research shows that consumption of foods derived from peanuts often with high content of dietary fibers and proteins or by not being totally absorbed, due to the presence of protease inhibitors and, still because of its high level of unsaturated fatty acids, it increases energy expenditure, could help to promote satiety and reduced weight gain. In short, we wish that this study may generate hypotheses and lead to more studies on the effect of foods derived from peanuts in weight control.

Considering it is essential for future studies to elucidate and define the quantities needed for such an effect to be determinant in human food.

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