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THE INTAKE OF FIBER MESOCARP PASSIONFRUIT (PASSIFLORA EDULIS) LOWERS LEVELS OF TRIGLYCERIDE AND CHOLESTEROL DECREASING PRINCIPALLY INSULIN AND LEPTIN

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Abstract: *Background:* Diabetes mellitus (DM) is a major risk factor for coronary artery disease, renal failure, retinopathy, and neuropathy. Over the last years, there has been an increasing demand in folk medicine for natural sources that could help in the treatment of chronic diseases, including diabetes. The rind of passion fruit (Passiflora edulis f. Flavicarpa) is traditionally used as a functional food due to its high concentration of soluble and insoluble fiber. *Objective:* The aim of this study was to determine the effect of high-fiber diet albedo of passion fruit on the metabolic and biochemical profile in diabetic rats induced by alloxan (2%). *Design:* The passion fruit mesocarp fiber was dried in an oven with circulating air at 60°C and pulverized. We used 32 adult male rats, divided into 4 groups: Wistar group 1 control (GC), Wistar group 2, 15% fiber (GF15), Wistar group 3, 30% fiber (GF30), Wistar group 4, fiber disolved in water (GFH2O). The ratio of passion fruit was prepared according to the AIN 93M guidelines, varying only the source of dietary fiber. The corresponding diet for each group was offered to the animals for 60 days. *Results:* There was a statically significant decrease in plasma glucose for GFH2O, GF15%, and GF30% groups with 27.0%, 37.4%, and 40.2%, respectively. *Conclusion*: The use of mesocarp fiber of passion fruit at concentrations of 15% and 30% are an important dietary supplement for the treatment of DM due to its potential hypoglycemic effect, and its ability to reduce triglycerides and VLDL-cholesterol levels with a principal reduction of insulin and leptin.

Key words: Diabetes, passion fruit fiber.

Introduction

Diabetes Mellitus (DM) is defined as a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (1). DM is a chronic disease and a serious public health problem due to its high frequency among the population, complications, morbidity, high financial costs, and social aspects involved in the treatment of the disease and the significant deterioration produced in quality of life of the patients (2).

Currently, studies on new hypoglycemic drugs have been performed using especially medicinal plants. Of the several medicinal plants used for the treatment of diabetes only some have been scientifically validated and recommended by the World Health Organization (WHO) (3). Córdova and colleagues (2005) reported the functional properties of passion fruit peel, especially those related to the content and type of fiber (4). Several studies have reported that the genus Passiflora has been used as an aid in the treatment (or control) of diabetes, mainly due to the presence of soluble fibers such as pectin (5, 6). The WHO recommends a diet fiber consumption of 25 - 35 g/day, 35% of which shall consist of 65% soluble and insoluble fiber. According to Pereira (2002), soluble fibers tend to delay gastric emptying and the passage of food through the intestines (7). Therefore, promoting lower glucose absorption, increased insulin

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sensitivity, reduction in plasma cholesterol and blood pressure, and also helping in weight control. These actions are due to the property of soluble fibers to bind water molecules and forming a gel-like substance. This reduces the absorption of fat and sugars and promotes lubrication of the stomach and intestinal wall, thus contributing to the proper functioning of the intestinal transit (3). As for the insoluble fiber, it has been found to accelerate intestinal transit, increasing fecal bulk and slowing the hydrolysis of glucose (3). Thus, a high fiber diet tends to lower the risk of obesity and cardiovascular and gastrointestinal diseases (3). There is no doubt of the great benefits of dietary fiber as a constituent of food.

The action of pectin as a hypocholesterolemic agent in animals has also been demonstrated (2, 8). Moreover, pectin showed a hypoglycemic and plasma insulin secretion-inducing effects in rodents (9, 10). These characteristics and functional properties make the passion fruit peel a potential ingredient for the development of new products such as foodstuffs, mainly due to the considerable amount of pectin in the fruit mesocarp (11, 12).

Due to the high prevalence of diabetes, especially in developed countries, this disease is considered the seventh leading cause of death in the world. It is essential to search for new sources of efficient alternatives to treat this disease. In this context, the present study evaluated the effect of passion fruit mesocarp fibers on plasma levels of glucose, insulin, leptin, triglycerides, total cholesterol and its fractions in rats with alloxan induced diabetes type I.

Materials and methods

Raw materials and vegetable diet preparation

The fiber of passion fruit dehydrated albedo was developed by the Biotechnology Program PPGBiotec of the Federal University of Amazonas (UFAM), and incubated by the company "Divine Fruit" in the industrial district of Manaus. The fruits were purchased from producers in the municipality of Manaus-Am and selected by the stage of maturity and firm shells. Albedo (the white part of the peel) was removed from the flavelo (yellow part of the rind) and the pulp. The albedo was subjected to the process of inactivation using the bleaching method, steam heated to a temperature of 90ºC for 5 minutes and dehydrated in an oven with circulating air at 60ºC for 4 hours. The dried albedo was pulverized in Hammer Mill and sieved to obtain desired particle size. The formulation of rodent Labina was produced according to the AIN 93M which is in agreement with the data from Reeves et al. (1993), varying only the source of dietary fiber (13).

Biological assays and animal experimentation

We used 32 adult male rats (Rattus norvegicus) of the Wistar strain, with 1 month of age. The animals were obtained from the animal house of the Universidade Federal do Amazonas (UFAM) and kept in individual plastic cages, under the light-dark cycle of 12 hours with free access to food (Labina Rat Chow®) or feed on the passion fruit and water free (ad libtum) except for 12 hours before the experiments, which were performed with fasted animals under appropriate conditions of light and temperature. This project is under the approval of the Ethics Committee on Animal Experiments (EAEC-UFAM) under protocol No. 069/2010, strictly following the international standards for laboratory animal care.

The animals were divided into four groups (n = 8): Group 1 - Control (CG): diabetic rats fed commercial feed; Group 2 - 15% Fiber (GF15): diabetic rats fed with a diet prepared with 15% of passion fruit mesocarp fiber; Group 3 - Fiber 30% (GF30): diabetic rats fed with a diet prepared with 30% of passion fruit mesocarp fiber; and Group 4 - Fiber dissolved in water (GFH2O): diabetic rats fed with commercial feed dissolving passion fruit mesocarp fiber in water.

Induction of Diabetes Mellitus

DM was induced by administration of an aqueous solution of alloxan monohydrate (Sigma-Aldrich, St. Louis, USA) diluted to 2% in a solution of 0.05 M sodium citrate, pH 4.5 after 24 h of fasting (13), injected intraperitoneally at a dose of 42 mg/kg. Rats were considered diabetic if they had blood glucose levels greater than or equal to 120 mg/dL (14, 15).

Food consumption

Animals were housed in individual cages and fed the experimental diets ad libitum. Food intake was recorded daily and calculated based on the remains checked the following day.

Body Mass Index

Animals were weighed at the beginning and at the end of the experiment to monitor the clinical course.

Biochemical profile

Blood glucose levels were determined at the beginning and at the end of the experiment, using the ACCU-CHEK® device, using the blood taken from the tail tip of the animals. The other biochemical parameters including: triglycerides, total plasma cholesterol, plasma HDL-

Cholesterol, LDL and VLDL plasma-cholesterol, total protein, plasma leptin, and insulin levels were determined using appropriate kits. The determination of plasma levels of LDL and VLDL were obtained from the Friedewald equation [16]. = TG VLDL / LDL cholesterol = total cholesterol - (VLDL + HDL)

Statistical Analysis

Data analysis was performed using Systat 12.0, employing the Steam and Leaf test to check for "outliers". These values were removed from the analysis. All data were tested for normality using the Shapiro-Wilk test. Since the data was not normally distributed, we applied the nonparametric Kruskal-Wallis test assuming a reliability of 95% ($p < 0.05$). ANOVA was used to compare the difference between groups.

Results

In the experiment conducted in a period of sixty days, there was no statistically significant difference between the treated groups, in relation to food intake and body mass gain, when compared with the control group ($p > 0.05$) (Table 1). These results are in agreement with Janebro et al. (2008) , where healthy and diabetic rats treated with the passion fruit peel fiber also showed no statistically significant differences in relation to food intake and body mass gain (Table 1) (2).

In Table 2, the evaluation of total cholesterol, HDL-cholesterol, and LDL-cholesterol levels did not showed statistically significant differences between the experimental groups when compared to the control group. A hypoglycemic response occurred in the GFH2O, GF15%, and GF30% groups with a 27%, 37.4%, and 40.2% reduction, respectively. There was no change in total protein concentration for groups GFH2O (15.2% increase), GF15% (30.4% decrease) and GF30% (25.3% decrease). There was a reduction in triglyceride levels for GFH2O, GF15%, and GF30% groups of 26.8%, 61.7%, and 74.4%, respectively. HDL-cholesterol levels showed a 11% and 7.6% decrease in the GFH2O and GF15% groups, while the GF30% showed an increase of 19.1%. VLDL-cholesterol levels were reduced in GFH2O, GF15%, and GF30% groups by 26.9%, 61.9%, and 74.4%, respectively. Insulin levels were also decreased in GFH2O, GF15%, and GF30% groups by 36.8%, 89.5%, and 73.7%, respectively. The same effect was seen for leptin levels for GFH2O, GF15%, and GF30% groups with 38.2%, 94.1%, and 91.2% reduction, respectively ($p < 0.05$).

It is important to establish that there was significant change ($p < 0.05$) between the levels of glucose, total protein, triglycerides, and VLDL-cholesterol between the groups GF15% and GF30%%, with lower percentages in relation with the GC and GFH2O groups. In addition to these findings, it can be seen that the levels of triglycerides and VLDL-cholesterol were also lower in the GF30% group when compared to the GF15% group (Table 2).

Discussion

The fiber of passion fruit peel has been the subject of great speculation. In 2007, Ramos and colleagues

Table 1

Food intake and body mass gain on Winstar rats with different diets

	GC	GFH2O	GF15%	GF30%
Food intake g/day	26.70±0.40[a]	26.12±0.50[a]	28.07±0.27[a]	27.55±0.30[a]
Body mass gain g/60days	152.28±12.41[a]	142.20±5.68[a]	158.14±17.54[a]	139.40±12.89[a]

*a: Difference in relation to control group, median ± stantard error ($p > 0.05$)

Table 2

Comparison of biochemical parameters (mean ± SE) between the groups

Metabolic data	GC (n=8)	GFH2O (n=8)	GF15% (n=8)	GF30% (n=8)
Glucose (mmol/L)	8.95 ± 0.25[a]	6.49 ± 0.26[b]	5.60 ± 0.37[c]	5.36 ± 0.32[c]
Triglycerides (mmol/L)	0.90 ± 0.14[a]	0.66 ± 0.06[b]	0.35 ± 0.05[c]	0.23 ± 0.02[d]
Total cholesterol (mmol/L)	2.17 ± 0.09	2.27 ± 0.10	2.26 ± 0.23	2.37 ± 0.11
HDL-cholesterol (mmol/L)	1.46 ± 0.18[a]	1.30 ± 0.17[a]	1.35 ± 0.22[a]	1.74 ± 0.18[b]
VLDL-cholesterol (mmol/L)	0.41 ± 0.06[a]	0.30 ± 0.03[b]	0.16 ± 0.02[c]	0.11 ± 0.01[d]
LDL-cholesterol (mmol/L)	0.47 ± 0.08[a]	0.66 ± 0.11[b,c]	0.74 ± 0.18[c]	0.52 ± 0.11[a,b]
Total Protein	79.00 ± 5.00[a]	91.00 ± 4.00[b]	55.00 ± 1.00[c]	59 ± 4.00[c]
Insulin (μU/ml)	1.9 ± 0.50[a]	1.2 ± 0.30[b]	0.2 ± 0.10[c]	0.5 ± 0.30[c]
Leptin (mg/ml)	3.4 ± 0.70[a]	2.1 ± 0.20[b]	0.2 ± 0.00[c]	0.3 ± 0.10[c]

*Different letters indicate statistical differences between the parameters evaluated by using ANYWAY ANOVA and Tukey post-test ($p < 0.05$).

demonstrated a significant reduction in fasting blood glucose levels and triglycerides upon supplementation with yellow passion fruit peel during the first four weeks of the study (17). As shown in Table 2, the plasma insulin levels of groups ingesting feed-based fibers (GF15% and GF30%) were significantly lower than GC and GFH2O groups. Plasma levels of leptin alone showed a significant reduction when compared to the control group.

The data presented in Table 2 shows that intake of mesocarp fiber of passion fruit (Passiflora edulis) in proportions of 15% and 30% led to a decrease in plasma glucose when compared to the group of Type 1 diabetic rats treated with commercial feed after sixty days of experimental diets. These results are in agreement with previous studies that show that feeding passion fruit peel flour to normal and diabetic rats can effectively control diabetes, as it is a byproduct rich in pectin (2). It was also reported by other study that feeding hamsters with fiber flour of Passiflora edulis; whose constitution resembles the passion fruit peel, resulted in decreased levels of triglycerides, cholesterol and liver lipids (9). Other studies have assessed the importance of including food products that promote improvement in glucose tolerance and reduction in total cholesterol and plasma triglyceride levels in diets of diabetic patients (18, 19).

A preclinical study using passion fruit husk fiber in the diet of normal and diabetic rats showed a reduction in blood glucose after four weeks of feeding. This effect was due to the action of soluble fiber on glucose absorption in the gastrointestinal tract and increased insulin secretion (10, 20). The use of the extract from Passiflora mollissima showed a hypoglycemic effect in diabetic rats treated for eight days, causing a reduction of almost 50% in blood glucose concentration. Another study stated that a long term diet supplemented with fiber (without addition of carbohydrate), improved glucose homeostasis (3).

Ramos et al. (2007) observed by means of a pilot clinical study that treatment with passion fruit peel flour (P. edulis fo. Flavicarpa) resulted in decreased cholesterol levels in women between 30 and 60 years of age who had hypercholesterolemia (cholesterol ≥ 200 mg / dL) (17). Ramos (2004) showed that the dry extract of passion fruit bark exerts a positive effect on glycemic control in the treatment of diabetes type II, due to the presence of totally degradable fibers in the body which help to decrease the levels of blood glucose and cholesterol (21). This suggests that the use of dry extract of passion fruit peel can be used as adjuvant therapies. This effect is attributed to the property of insoluble fiber and other soluble constituents of albedo yellow passion fruit (Passiflora edulis) to reduce total cholesterol and fractions, plasma level of triglycerides, and glucose (3). Since fibers are substances of plant origin which are not digested and absorbed by the body they can resist the action of human digestive enzymes; therefore, reaching the colon intact and being partially or totally hydrolyzed and fermented by the colonic bacterial flora in the large intestine (22). According to Zeraik et al. (2010), due to the high content of pectin in the passion fruit peel it can also induce plasma insulin and leptin secretion, in addition to its hypoglycemic and hypocholesterolemic effects (23). This finding confirms the results of this study in which we observed a reduction of these parameters in GF15% and GF30% groups, when compared with the control and GFH2O groups probably generated by hypoglycemic factors in these rats.

Thus, the hypoglycemic action of the passion fruit peel (Passiflora edulis f. Flavicarpa) can also be attributed to the presence of pectin, a fraction of soluble fiber which is able to absorb water and form a viscous gel that can delay gastric emptying and bowel traffic. The fiber content found in wet passion fruit peel corresponds to 1.58 (g/100g) of soluble fiber (4, 24).

Conclusion

Our results showed that the mesocarp fiber of passion fruit (Passiflora edulis), at concentrations of 15% and 30%, could be an important dietary supplement for the treatment of diabetes due to its hypoglycemic potential. Mesocarp fiber of passion fruit also promotes the reduction of triglycerides, VLDL -cholesterol, insulin, and leptin levels. However, more specific studies of the biochemical mechanisms involved in the observed effects are needed. In addition, a more detailed analysis of the benefits of fiber toxicity and the passion fruit peel should be performed, to assess any risk in the human population.

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