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Original

Effects of different concentrations of passion fruit peel (*Passiflora edulis*) on the glicemic control in diabetic rat

Estudo dos efeitos de diferentes concentrações de casca de maracujá (Passiflora edulis) no controle da glicemia de ratos diabéticos

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Abstract

Diabetes mellitus, an endocrine disorder, is the major cause of morbidity in developing countries, and it is considered the fourth leading cause of death worldwide. The conventional therapy for diabetes is insulin treatment. The peel of the Passion fruit is rich in fiber and prevents the absorption of carbohydrates, and thus can control and reduce the blood sugar rate. The objective of this study was to analyze the effect of the passion fruit peel flour on the glicemia of diabetic rats, as well as to study the probable action mechanisms. Wistar rats were used in the experiment and were offered the flours of the passion fruit peel in three concentrations: 5, 10, and 15%, and a casein diet as control. The most significant effect on the reduction of the glicemic rate was obtained with the 5% diet. The best values of hepatic glycogen were found in the 5 and 10% diets. The results of this study suggest that the 5% passion fruit flour diet was the one that provided the best reduction of blood glucose levels (59%) and the higher increase of the hepatic glycogen level (71%). The conversion of blood glucose into hepatic glycogen was considered the probable action mechanism involved.
Keywords: *hyperglycemia; diets; passion fruit; diabetes; rats.*

Resumo

Diabetes melittus, uma desordem endócrina, é a maior causa de morbidade em países em desenvolvimento e é considerada a quarta causa de mortes no mundo. A terapia convencional para diabetes é o tratamento com insulina. A casca do maracujá é um material rico em fibras, e impede a absorção de carboidratos, podendo, dessa forma, controlar e reduzir a taxa de açúcar no sangue. O objetivo deste estudo foi analisar o efeito dessa farinha na glicemia de ratos diabéticos, bem como estudar os prováveis mecanismos de ação. Foram empregados ratos Wistar, aos quais foram oferecidas dietas balanceadas contendo farinhas da casca de maracujá em três concentrações, 5, 10 e 15%, e tendo como controle uma dieta com caseína. A dieta que mostrou maior efeito significativo sobre o controle do diabetes foi a de 5%, proporcionando uma redução de cerca de 60% da glicemia dos animais desse grupo e um aumento de 71% do glicogênio hepático. A conversão de glicose sanguínea em glicogênio hepático foi considerada um dos prováveis mecanismos de ação envolvidos.
Palavras-chave: *hiperglicemia; dieta; maracujá; diabetes; ratos.*

1 Introduction

Diabetes Mellitus, an endocrine disorder characterized by hyperglycemia and impaired insulin secretion, results from defects in insulin secretion or insulin action disorder or in both. It is the major cause of morbidity in developing countries (RAVI; RAJASEKARAN; SUBRAMANIAN, 2005). Nowadays, it is considered the fourth leading cause of death worldwide. The public affected by diabetes corresponds to approximately 3% of the world's population.

According to the World Health Organization (WHO), diabetes is expected to increase in the next 10 years thus it is considered a serious public health problem (CARRION; KATO; MOURÃO, 2002).

According to McIntyre (2001), this fact occurs mainly due to changes in lifestyle such as inadequate food intake and sedentary lifestyle resulting in the development of other diseases such as heart disease and obesity among other risk factors.

The genetic predisposition and lifestyle are related to the high rate of occurrence of non-insulin-dependent diabetes mellitus. The contemporary style of life characterized by low physical activity and diets rich in energy, such as carbohydrates and lipids, results in an increase in the levels of glucose in the tissues sensitive to insulin and an exacerbated resistance to this substance (DE PAULA, 2002).

The conventional therapy for diabetes Type I includes exogenous insulin, and diabetes Type II includes hypoglycemic agents. Nevertheless, diet is the best form of prevention. A research conducted in Australia and in the United States indicated that, respectively, 48.5 and 34% of the population

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use non-conventional therapies for the treatment of diseases, including medicinal herbs. In Brazil, it is clear that the treatment of diseases with the infusion of plants and cooking is a common practice despite the lack of statistical data on the subject (PEPATO et al., 2005).

Recent recommendations suggest that the consumption of grains, fruits, and vegetables should be increased to prevent or treat these chronic diseases. Among the possible components of these foods that may have hypoglycemic actions are protein, fibers, and some compounds called antinutrients, such as phytic acid, tannins, lecithins, and inhibitors of enzymes and saponins (COMMITTEE ON DIET AND HEALTH, 1989).

Some studies reported that the consumption of dietary fiber may reduce the risk of disease in populations, especially the prevention of cardiovascular and gastrointestinal diseases, colon cancer, hyperlipidemia, diabetes, and obesity among others (CHAU; HUANG, 2004).

Fibers have physiological effects related to laxation, increase the fecal cake, and reduce cholesterol and blood glucose levels due to their solubility in water (CERQUEIRA et al., 2008).

Several studies showed a protective effect of fibers on the development of insulin resistance and type 2 diabetes (SALMERON, 1997a, b; MEYER et al., 2000; PEREIRA et al., 2002).

In another study, Nandini, Sambaiah and Salimath (2000) found the greatest effect in reducing levels of glucose in the blood of diabetic rats with diet enriched with soluble fiber (guar gum). Those same fibers showed a significant effect of reducing blood glucose and insulin resistance, however, their impact on the risk of developing type 2 diabetes was not clearly defined (CHAMP et al., 2003).

Results showed that diets enriched with the flour of eggplant peel decreased blood glucose levels in diabetic animals. These results indicated that the soluble pectin in the diet contributed to the reduction in the glucose rate of these animals (DERIVI et al., 2002).

Another study, with a diet rich in fiber, also showed beneficial effects on the systolic blood pressure and levels of lipids in the blood suggesting a higher intake of fiber to prevent disease complications (JUE et al., 2004).

However, Laerke et al. (2007), on a trial with diabetic rats using potato pulp and soluble fiber, found no significant results in reducing blood glucose levels of cholesterol or plasma.

According to Córdova et al. (2005), some studies have shown the functional properties of the passion fruit peel, especially those related to the content and type of fiber. Due to these characteristics and functional properties, passion fruit has no longer been considered an industrial waste since it can be used in the development of new products.

The peel of passion fruit is rich in fiber, minerals, and especially pectin. Pectin is a fraction of soluble fiber in the gastrointestinal tract that forms a gel preventing the absorption of some nutrients. In the case of diabetes, it prevents the absorption of carbohydrates and can thus control and reduce the blood sugar rate (SACHS, 2002).

The purpose of this study was to determine the chemical composition and the concentration of antinutrients compounds, and also to evaluate the effect of different concentrations of the passion fruit peel on the blood glucose levels in diabetic rats.

2 Materials and methods

The chemical analysis and biological experiment were conducted at the Laboratory of Bromatology and Experimental Nutrition of the Agri-food Industry, Food and Nutrition Department, Luiz de Queiroz College of Agriculture (ESALQ/USP).

The passion fruits investigated in this experiment were the IAC-275 yellow variety from the Agronomic Institute of Campinas (IAC).

2.1 Material processing

The fruits were cleaned out and only the peel was used for the production of the flour. The peel of the fruit was rinsed with distilled water, placed in trays, and dried in a circulating forced air incubator at 55 and 60 °C for 48 hours. The dry peels were ground to a fine powder using a blender (Tecnal T345, Piracicaba - SP, Brazil) and stored in clear polyethylene bags at 10 °C prior to use.

2.2 Chemical analysis

The chemical analysis of the dry matter, total protein, ether extract, and ash were conducted with three replicates for each sample according to AOAC (ASSOCIATION..., 1975). The fiber content was determined through a method described in the literature (ASP et al., 1983), which used enzymes such as α-amylase to hydrolyze starch and pepsin followed by pancreatin to break down proteins.

The minerals were determined according to the methodology described by Sarruge and Haag (1974).

2.3 Antinutritional factors

The concentration of tannins was determined through the protocol described by Price, Hagerman and Butler (1980); the standard curve was drawn using catechin and the results were expressed in mEq of catechin.

The total polyphenols were determined by the method proposed by Swain and Hillis (1959) using the reagent Folin-Ciocalteau.

2.4 Preparation of diets

The diets (Table 1) were prepared in accordance to Reeves, Nielsen and Fahey Junior (1993) and contained 15% of protein each, and were divided into normal and diabetic control (casein) and three tests (casein + flour of passion fruit peel) in three different concentrations: 5, 10, and 15%.

2.5 Biological experiment

A total of 48 male Wistar rats (Rattus norvegicus), weighing between 200 to 220 g, were selected for the biological assay.

In the beginning of the experiment, the animals were anesthetized with halothane, after a 24-hour fast for the induction of diabetes, with an injection of Alloxan monohydrate (4 $mg.kg^{-1}$ body weight, dissolved in saline solution) in the dorsal vein of the penis. The dose was enough to cause diabetes of moderate intensity, according to Lazarow and Palayes (1946). The development of diabetes was controlled the daily by determining the level of glucose in urine using a glucose test-tape (Glukotest) which allowed a diagnosis in animals that were kept alive. Eight animals were selected for initial sacrifice, after fasting for 12 hours, for blood collection through heart puncture. The blood sample was called time 0 (T0), and the liver was also collected for further analysis.

The remaining 40 animals were divided into 5 groups of 8 animals each and maintained in individual cages at room temperature (between 22 and 23 °C) with daily cycle consisting of 12 hours of light and 12 hours of darkness.

During the experiment, which lasted 30 days, they were fed the 5 diets and water was provided "ad libitum". Feed consumption and the animals' weight were recorded weekly. After thirty days of the experiment, new samples of blood were collected.

2.6 Biological analysis

The animals were sacrificed for blood and liver collection, after fasting for 12 hours, using halothane as anesthetic. For the blood glucose analysis, the blood was collected and immediately transferred into a test strip and checked using a blood glucose monitoring system (Accu-Chek). For the analysis of glycogen in liver, the method proposed by Hassid and Abraham (1957) was used.

2.7 Statistical analysis

The experimental design was performed randomly, according to the methodology described by Pimentel Gomes (2000), with 5 groups of 8 animals each and 5 treatments. The F test was applied for the variance analysis and the Tukey test was used to identify the statistic differences between the means.

3 Results and discussion

3.1 Chemical analysis

Analyzing the data in Table 2, it seems that the peel of the passion fruit has low levels of protein and ether extract, but these values were higher than those found by Oliveira et al. (2002), and probably this difference may be linked to the variety of the fruit studied.

The amount of soluble and insoluble fibers in the peel of the fruit deserves attention, which has also been mentioned by Bueno, Castilho e Costa (2005) and Córdova et al. (2005), who found similar levels.

The passion fruit peel showed a significant content of Fe, K, Zn, and Mn (Table 3).These micro-nutrients are important for the metabolism, so the peel of the passion fruit can be considered an alternative source for the intake of these minerals. In addition, it represents a potential for new food products such as cereals and juices that can be fortified with this flour (CÓRDOVA et al., 2005).

Table 1. Composition of control and test diets supplemented with flour of passion fruit peel (PFPF).

Components	Control	5%	10%	15%
Caseín[1]	15.00	15.00	15.00	15.00
Peel 5%[7]	–	5.00	–	–
Peel 10%[7]	–	–	10.00	–
Peel 15%[7]	–	–	–	15.00
Soybean Oil[3]	3.50	3.50	3.50	3.50
Mineral Mixture[2]	1.00	1.00	1.00	1.00
Vitamin Mixture[2]	0.25	0.25	0.25	0.25
∝ Cystine[4]	0.30	0.30	0.30	0.30
Terti Butil Hidroquinone[5]	0.0014	0.0014	0.0014	0.0014
Corn Starch[6]	Complete to 100%			

[1]Synth; [2]Reeves, Nielsen and Fahey Junior(1993); [3]Lisa®; [4]Synth; [5]Sigma; [6]Maizena®, [7]IAC – 275.

Table 2. Results of chemical analysis of the passion fruit peel used in the diets (g.100 g^{-1} of dry matter).

Analysis	Result
Humidity	6.96
Ether extract	0.74
Ash	8.30
Protein	9.8
Insoluble fiber	54.27
Soluble fiber	3.49

Table 3. Levels of minerals found in the passion fruit peel used in the diet.

Minerals	$g.Kg^{-1}$
Calcium	3.30
Phosphorus	1.51
Potassium	33.66
Magnesium	0.80
Sulfur	1.06
Sodium	2.81
	$mg.Kg^{-1}$
Copper	1.33
Iron	41.10
Zinc	11.60
Manganese	17.45

3.2 Antinutritional factors

The antinutritional compounds (Table 4) found in the peel of the passion fruit can be of great importance because, depending on the quantities, they can influence the decrease of blood glucose.

According to the Committee on Diet and Health (1989), antinutrients such as phenolic compounds can prevent or treat chronic diseases, and the amount found in the peel of the passion fruit can have particular importance in the effect on the blood glucose of the diabetic rats.

3.3 Biological analysis

A significant statistical difference was observed for dietary consumption indicating lower consumption for the normal control group. However, in the diabetic control group and test groups, there was high consumption rate (Figure 1), probably due to glucose deprivation thus providing higher consumption of the diet (NANDINI; SAMBAIAH; SALIMATH, 2000).

The animals showed statistical differences in weight gain (Figure 2). The diabetic group showed no significant weight gain corroborating with Nandini, Sambaiah and Salimath (2000), who observed small weight gain in animals under hyperphagic conditions. However, the group that consumed 15% of PFPF did not show a high weight gain probably due to the high concentration of fibers in the diet.

Analyzing Figure 3, it can be observed that the test diet with 5% of the flour of the passion fruit peel reduced the blood glucose by 59% in diabetic rats reaching the normal glycemic amount (112.6 mg.dL^{-1}).

It is likely that the mechanisms of action that helped to reduce the blood glucose levels were the transformation of blood glucose into liver glycogen, which maintained normal levels of glucose in the bloodstream, as well as the presence of soluble fiber, tannins, and phenolic compounds.

The presence of soluble fiber (pectin) found in fruits, mainly in the peel, can beneficially influence the diabetic patient, and according to Del-Vechio et al. (2005) these fibers reduce blood glucose. The benefits attributed to the fibers are the properties to increase the intestinal transit time, lower gastric emptying,

Table 4. Antinutritional (mg.g^{-1} of fresh matter) found in the peel of the passion fruit.

Antinutricional	Result
Tannin	0.17
Phenolic compounds	4.20

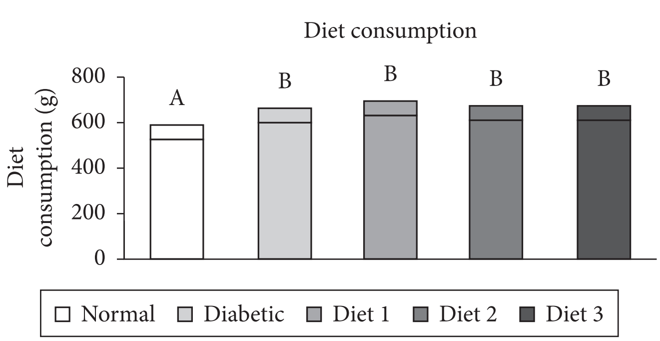

Figure 1. Total dietary consumption (g) by diabetic male Wistar rats treated with passion fruit peel flour during 30 days of experiment. (*) Values of 8 animals, where: Normal = 15% casein diet; Diabetic = 15% casein diet; Diet 1 = 5% of PFPF; Diet 2 = 10% of PFPF; Diet 3 = 15% of PFPF. Averages with different letters in the graph differ statically at the level of 5%.

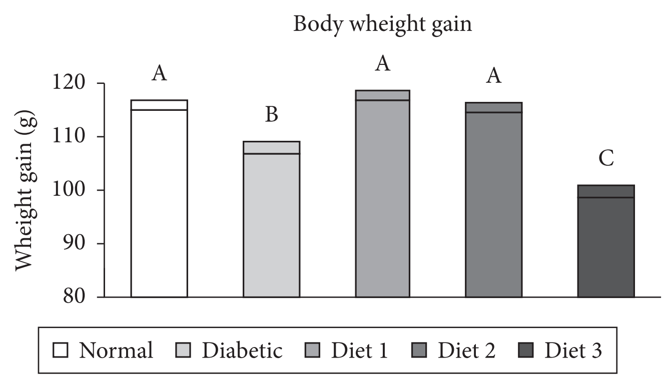

Figure 2. Total body weight gains (g) of diabetic male Wistar rats treated with passion fruit peel flour during 30 days of experiment. (*) Values of 8 animals, where: Normal = 15% casein diet; Diabetic = 15% casein diet; Diet 1 = 5% of PFPF; Diet 2 = 10% of PFPF; Diet 3 = 15% of PFPF. Averages with different letters in the graph differ statically at the level of 5%.

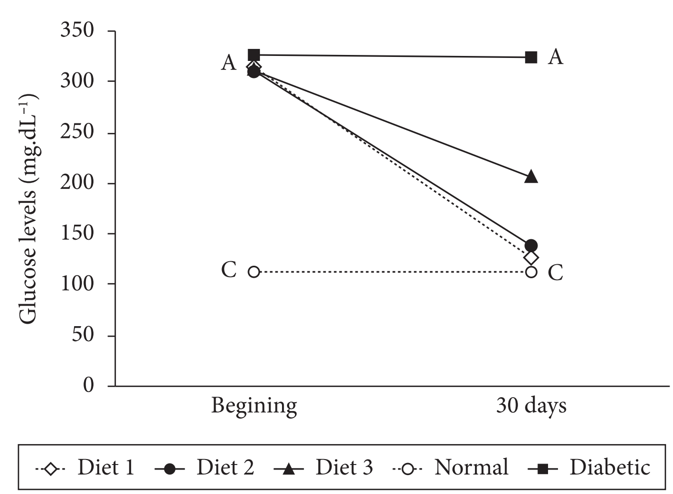

Figure 3. Levels of plasma glucose (mg.dL^{-1}) in diabetic male Wistar rats treated with passion fruit peel flour during 30 days of experiment. (*) Values of 8 animals, where: Normal = 15% casein diet; Diabetic = 15% casein diet; Diet 1 = 5% of PFPF; Diet 2 = 10% of PFPF; Diet 3 = 15% of PFPF. Averages with different letters in the graph differ statically at the level of 5%.

delay absorption of glucose by slowing access to the absorptive epithelium, and hence to reduce postprandial glycaemia. In addition, the fibers with the highest level of viscosity such as soluble fibers are known for providing slower absorption of sugar in the intestine by interacting with intestinal enzymes (NANDINI; SAMBAIAH; SALIMATH, 2000).

Figure 4 shows the values of hepatic glycogen after 30 days of experiment.

The results of hepatic glycogen showed a significant increase in the diabetic rats fed the flour of the passion fruit peel. The values of liver glycogen for the 5% of PFPF group were almost equal to the values of the normal rats. One possible explanation for these results is that in the diabetic control group, the insulin insufficiency of the animals affected the glucose capture in peripheral tissues sensitive to the hormone thus accumulating the glucose in the extracellular liquid. Consequently, the synthesis of glycogen decreased, and probably its degradation was stimulated as a source of energy for the cell reflecting the significant reduction in glycogen content.

The presence of the passion fruit peel flour in the diet reduced the digestion and absorption of carbohydrates and increased the sensitivity of muscle and adipose tissue to insulin remaining allowing greater gluconeogenesis (production of liver glycogen), thereby contributing to the reduction of hyperglycemia of these animals (SACHS, 2002).

It is interesting to note that these positive results, both for glucose and hepatic glycogen, were not directly proportional to the increase in the concentration of the passion fruit peel flour in the diet. This study showed that under concentrations higher than 10%, the consumption of the peel did not have the desired physiological effect.

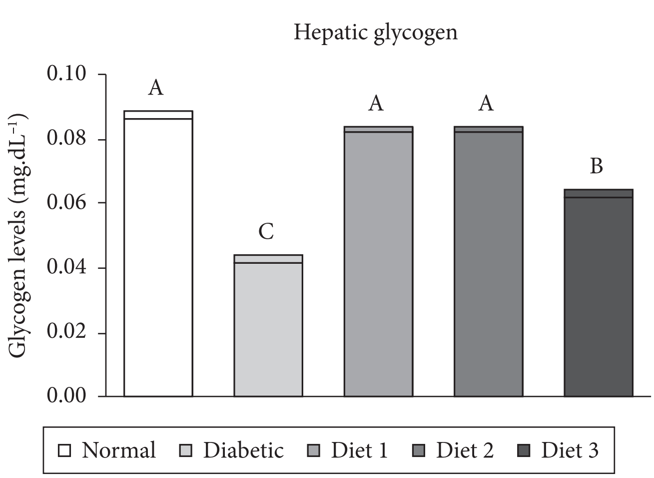

Figure 4. Levels of hepatic glycogen ($mg.dL^{-1}$) in diabetic male Wistar rats treated with passion fruit peel flour during 30 days of experiment. (*) Values of 8 animals, where: Normal = 15% casein diet; Diabetic = 15% casein diet; Diet 1 = 5% of PFPF; Diet 2 = 10% of PFPF; Diet 3 = 15% of PFPF. Averages with different letters in the graph differ statically at the level of 5%.

4 Conclusion

The results of this study showed that the passion fruit peel affects the metabolism of carbohydrates, and can positively influence the metabolic control of diabetes by preventing or delaying complications associated with this disease.

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