

Effect of *Solanum sessiliflorum* Dunal (Maná-cubiu) Extract in Diet-induced Metabolic Syndrome in Rats

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Abstract

Obesity is a multifactorial disease, defined by the excessive accumulation of body fat, causing negative consequences for health. Metabolic syndrome (MS) is characterized by the association between abdominal obesity, insulin resistance, hypertension, and hyperlipidemia. Experimental models are essential to elucidate the pathophysiology, development, and treatment of the disease. The objective of the study was to evaluate the effects of maná-cubiu extract (*Solanum sessiliflorum* Dunal) in experimental models of diet-induced MS. Male Wistar rats received a high-fat, high-calorie diet for 24 weeks, with exception of the healthy control group, which received commercial AIN-93 chow, and both groups were supplied with filtered water. After this period, the animals began to receive daily gavages containing 150 mg of extract/kg or 300 mg of extract/kg for another 8 weeks. The control group received only metformin (150mg/kg). Body weight, bioelectrical impedance (BIA), body circumferences and the Lee index were used to assess body composition. The study was approved by the Ethics Committee under protocol n°501. The experimental group treated with 150mg/kg of the extract showed weight reduction in the first month of treatment, followed by an average reduction of 54 grams per animal. The group treated with 300mg/kg of the extract suffered weight reduction only after the second month of treatment (on average 9g/animal). This same group and the group treated with metformin were the only ones to reduce the percentage of body fat, comparing the two evaluation moments. The results suggest that maná-cubiu has a potential therapeutic use in obesity, and that the evaluation methods in animal models need to be better standardized.

Keywords: *Solanum sessiliflorum dunal*, mana-cubiu, metabolic syndrome, obesity, diabetes type 2

1. Introduction

Obesity has become a global public health problem and has drastically increased in recent decades. It is a multifactorial disease, defined as the excessive accumulation of body fat, causing negative consequences for health (Spinelli & Monteleone, 2021). In general, the assessment of body composition is essential for assessing the nutritional status and monitoring the progression of obesity during medical and dietary interventions (Holmes & Racette, 2021). When other chronic non-communicable diseases are associated with obesity, the metabolic syndrome (MS) can be established, defined by the World Health Organization (WHO) as a pathological condition characterized by the association between abdominal obesity, insulin resistance, hypertension, and hyperlipidemia. These conditions are interrelated and share pathophysiological mechanisms. Its development is related to eating habits, such as high caloric intake, low fiber consumption, and sedentary lifestyle (Saklayen, 2018).

Experimental models are fundamental for elucidating the pathophysiology, development and treatment of

different types of diseases, due to the similar way to their behavior in human organisms (Costa et al., 2014). The high-fat diets induction model has the objective to develop obesity, hyperinsulinemia and alteration of glucose homeostasis via insufficient compensation by the pancreatic islets. The fat content of these diets can vary from 20 to 60%, being used for both MS and obesity models (Wong et al., 2016; King, 2012). When choosing an animal model for a diet-induced obesity study, it is important to consider that rats and mice respond differently to this type of diet; in addition, strain, sex and age may affect the response to the obesogenic diet, with young and male animals being more sensitive to obesity-related comorbidities. The period of supply of high-fat food is another determining factor for the development of the disease and can vary from eight days to 27 weeks. The markers for evaluating the alterations promoted by the induction are, normally, the body weight, fat gain, parameters related to inflammation, concentration of hormones, blood glucose, lipid profile, and markers of liver health. However, as there are no specific cutoff points for these parameters for animals, it is imperative that studies include a non-obese (healthy) control group, so that results can be compared (De Moura & Dias et al. 2021).

Pharmacological agents are the most used form of treatment for MS and DM2, and their comorbidities, such as hypertension, dyslipidemia and hyperglycemia. Despite its effectiveness, this method has a number of possible side effects, such as coughing, dizziness, headaches, palpitation, angioedema, and others. For this reason, there is a growing scientific interest in studying bioactive and dietary compounds that act in the prevention and treatment of this disease, decreasing its symptoms and avoiding future complications of the condition (Saheli, et al., 2019; Alam, et al. 2014).

The maná-cubiu (*Solanum sessiliflorum* Dunal) is a fruit original from the Amazon region, found in Brazil, Peru and Colombia. The fruit belongs to the Solanaceae family, as same as potato (*Solanum tuberosum* L.) and tomato (*Solanum lycopersicum*) (Sequi, 2016). Some compounds have already been identified in these species, mainly present in its exocarp, such as p-coumaric acid, p-hydroxydihydrocoumaric acid, naringenin, methyl salicylate, fatty acids, such as methyl and ethyl esters (Cardona et al., 2011). Studies on the antioxidant capacity of maná-cubiu have detected alkaloids, organic acids, phenols and flavonoids, in addition to anthocyanins, gums, tannins and mucilage (Nascimento, et al., 2022; Faria, et al., 2021; Mascato et al., 2015). Due to its composition, the potential therapeutic of use the fruit to alleviate certain conditions, such as diabetes and hypercholesterolemia has already been investigated (Maia et al., 2015; Yuyama et al., 2005). Thus, the objective of this study is to evaluate the effect of maná-cubiu extract in experimental models of MS induced by a high-calorie and high-fat diet, associated with fructose consumption.

2. Method

2.1 Obtaining Maná-cubiu Extracts

The ethanolic extract was prepared from the lyophilized and crushed fruits, in a hot closed system in a Soxhlet apparatus, using 80% ethanol solvent (Carvalho, 2001). After adding the sample and ethyl alcohol to the Soxhlet, the entire system was heated (80 °C) and left at continuous reflux, then that the solvent could properly extract the bioactive compounds from the fruit. After obtaining, the extract was concentrated in a rotary evaporator and taken to dryness in a water bath at 50 °C, until the complete evaporation of the solvent. For complete water extraction, the dry extract was lyophilized (Carvalho, 2001). The extracts were kept under refrigeration (5 °C) until the moment of dilution and administration via gavage to the animals, at doses of 150mg/kg and 300mg/kg.

2.2 Induction of Metabolic Syndrome in Animals

All experiments involving animals followed ethical norms and were approved by the Committee for Ethics in Research on the Use of Animals in July 2019 (CEUA/Positivo University, protocol n°501).

Male Wistar rats (n=40), weighing approximately 250g, were provided by the vivarium of Universidade Positivo, Curitiba, Parana, Brazil, and were randomly divided into five experimental groups: healthy control group (SA, n=8), metabolic syndrome with and without treatment group (SM, n=8), metabolic syndrome treated with metformin group (ME, n=8), metabolic syndrome treated with maná-cubiu extract 150 mg/kg group (MN 150 mg/kg, n=8), and metabolic syndrome treated with maná-cubiu extract 300 mg/kg group (MN 300 mg/kg, n=8).

The animals were kept two by two in cages with water and food available ad libitum, in an air-conditioned environment (22 °C) and with a light/dark cycle of 12h/12h.

The induction of the metabolic syndrome lasted 24 weeks, in which all experimental groups received a high-calorie and high-fat diet, in addition to a fructose solution (20% - 4 kcal/mL – Lowúcar®, Brazil) in water, with the exception of the healthy control group (SA), which received commercial AIN-93 chow (Presence®, Brazil) and filtered water, containing 3.9 kcal/g, being 20% protein, 10% fat and 70% carbohydrate. The diet

used to induce obesity consisted of 5.9 kcal/g, with 18.2%, 66.8% and 15% of calories coming from proteins, fats and carbohydrates, respectively (PragSolu ções®, Brazil).

2.3 Intervention

After the 24 weeks of obesity induction, the animals began to receive daily gavages with 150 mg of extract/kg/or 300 mg of extract/kg for eight weeks. During the intervention, the animals continued to receive a high-calorie, high-fat diet and fructose added to the water (ad libitum) up to (20%). Control groups received purified water (ad libitum).

2.4 Body Composition Analysis

For the evaluation of body composition, the parameters of body weight, bioelectrical impedance (BIA), body circumferences and estimation of Lee's index were evaluated.

The body weight of the animals was measured on a GEHAKA® – BG 8000 digital scale, using a plastic container for storing the animals while the weight was read (FIGURE 7). The animals were weighed individually, in the morning and with a tared scale, to deduct the weight of the apparatus. To perform the bioimpedance, the protocol described by Hall et al. (1989) was followed, with some modifications, the animals were sedated using inhaled isoflurane (2.5%) and immobilized in prone position on a rigid non-conducting structure for better positioning of the electrodes. Mercur® conductive gel was used to position the electrodes on the four body extremities of the animals (lower and upper limbs) connected to the device (RJL Systems BIA Analyzer, Quantum X model) to read the Reactance and Resistance measurements, used to calculate the percentage of fat-free mass, total body solids, and total body fat.

From these data, it was possible to obtain the percentage of body fat from equation 1:

$$\% \text{ Body Fat} = (\text{Total Body Fat} \times 100) / \text{Weight}$$

3. Results

The data obtained in the analysis of body measurements showed that there was a difference in the abdominal circumference of the rats in the healthy control group (SA) in relation to the groups treated with metformin (ME) and with the fruit extract high dose (MN300) (Table 1).

Table 1. Waist Circumference

Waist Circumference	ME Control	SM Control	SA Control	MN 150	MN 300
Post-induction	23,75 ± 2,62a	24,56 ± 2,88a	21,8 ± 1,64a	22,56 ± 3,02a	24,4 ± 2,37a
Post-treatment	26,5 ± 3,46a	23,13 ± 3,4a	20,29 ± 2,36b	23,06 ± 2,73a	25,06 ± 2,74a

Results expressed as mean ± standard deviation. Different letters on the same line represent statistical difference according to the two-way ANOVA statistical test, followed by Bonferroni ($p < 0.05$). Metabolic syndrome group treated with metformin (EM Control); Group with untreated metabolic syndrome (SM Control); Group without metabolic syndrome and without treatment (SA Control); Group with metabolic syndrome treated with man ácubiu extract at a dose of 150mg/kg (MN 150); Group with metabolic syndrome treated with man ácubiu extract at a dose of 300mg/kg (MN 300).

The measurement of the thoracic circumference of the animals showed a reduction in the measurements between the two evaluation moments in all groups, apart from the SA control group, which maintained a stable gain throughout the entire evaluated period, considering the standard deviation (16.43 cm and 17.36 cm) (Table 2).

Table 2. Chest Circumference

Chest Circumference	ME Control	SM Control	SA Control	MN 150	MN 300
Post-induction	18,8 ± 1,42a	24,5 ± 1,04a	16,43 ± 0,93a	17,29 ± 1,29a	18,06 ± 1,35a
Post-treatment	17,5 ± 1,83a	17,25 ± 1,04a	17,36 ± 1,55a	16,88 ± 0,74a	17,4 ± 1,47a

Results expressed as mean ± standard deviation. Different letters on the same line represent statistical difference according to the two-way ANOVA statistical test, followed by Bonferroni ($p < 0.05$). Metabolic syndrome group treated with metformin (EM Control); Group with untreated metabolic syndrome (SM Control); Group without metabolic syndrome and without treatment (SA Control); Group with metabolic syndrome treated with man ácubiu extract at a dose of 150mg/kg (MN 150); Group with metabolic syndrome treated with man ácubiu extract at a dose of 300mg/kg (MN 300).

The results referring to the bioimpedance test (BIA) revealed that the groups of diabetic animals treated with the fruit extract at a concentration of 300mg/kg (MN 300) and the group treated with metformin (ME control) were the only ones that obtained a reduction in the percentage of body fat, comparing the two evaluation moments. Even the group treated with the lowest extract dose (MN 150) had a small change in this parameter, while the groups that did not receive any type of treatment (SM and SA controls) significantly increased their percentages of adipose mass (Table 3).

Table 3. Bioimpedance

	ME Control	SM Control	SA Control	MN 150	MN 300
% Fat Post-induction	60,97±8,02 ^b	40,44±11,6 ^a	27,35±9,13 ^{ab}	45,45±5,04 ^{ab}	49,59±8,54 ^b
% Fat Post-treatment	42,40±18,07 ^b	59,54±8,57 ^a	48,39±8,03 ^{ab}	46,15±11,03 ^{ab}	47,54±10,91 ^{ab}

Results expressed as mean ± standard deviation. Different letters on the same line represent statistical difference according to the two-way ANOVA statistical test, followed by Bonferroni ($p < 0.05$). Metabolic syndrome group treated with metformin (EM Control); Group with untreated metabolic syndrome (SM Control); Group without metabolic syndrome and without treatment (SA Control); Group with metabolic syndrome treated with man ácubiu extract at a dose of 150mg/kg (MN 150); Group with metabolic syndrome treated with man ácubiu extract at a dose of 300mg/kg (MN 300).

According to the data obtained by analyzing the Lee index, there was a slight increase in the results between the two evaluation moments for all groups of the experiment (Table 4).

Table 4. Lee Index

	ME Control	SM Control	SA Control	MN 150	MN 300
Lee Index Post-induction	222,13±22,20 ^a	224,22±21,53 ^a	199,09±16,34 ^a	208,08±23,29 ^a	218,31±17,85 ^a
Lee Index Post-treatment	230,04±19,18 ^a	231,22±23,69 ^a	212,65±17,23 ^a	212,30±20,57 ^a	227,21±13,75 ^a

Results expressed as mean ± standard deviation. Different letters on the same line represent statistical difference according to the two-way ANOVA statistical test, followed by Bonferroni ($p < 0.05$). Metabolic syndrome group treated with metformin (EM Control); Group with untreated metabolic syndrome (SM Control); Group without metabolic syndrome and without treatment (SA Control); Group with metabolic syndrome treated with man ácubiu extract at a dose of 150mg/kg (MN 150); Group with metabolic syndrome treated with man ácubiu extract at a dose of 300mg/kg (MN 300).

Considering that a peak body weight of the animals can be observed between the 16th and 20th week from the beginning of the administration of the induction diet, the present study opted for a period of 24 weeks, before starting the intervention phase, with the administration of extracts and medication for the control group, corresponding to eight weeks, thus totaling 32 weeks of experimentation. In this study, the evaluation of the animals' body weight was evaluated during the eight months of duration, and the results were increasing in weight gain for all groups, however with oscillations. Considering only the treatment period, that is, the last two months of the experiment, the only group that presented weight loss after 60 days of ingestion of man ácubiu extract, was the MN300 group. The other experimental groups, as well as the control groups, showed weight gain between the 30th and 60th days of treatment (Table 5).

Table 5. Body Wait Monitoring

	ME Control	SM Control	SA Control	MN 150	MN 300
1 st month	321,2±41,3	335,9±20,9	313,4±34,9	315,7±41,2	318,1±37,4
2 nd month	355,4±48,5	388,2±24	353,4±32,9	357,9±57,3	363±28,6
3 rd month	408,2±61,6	449,6±40,2	378,8±49	386,9±56,2	402,7±44,3
4 th month	477,5±69,2	524,3±44,5	420,9±61,6	438,8±71,6	473,9±54,1
5 th month	528,4±83,9	590,3±57,1	445,9±64,4	476,7±89,6	519,8±71,8
6 th month	553,9±97,6	624,3±61,9	548,1±68,3	508,4±99,5	561±81,3
7 th month*	458,4±88,5	689,7±81,9	517,2±74,4	435,9±55,9	592,3±70,7
8 th month*	470,2±91,3	691,5±84,3	526,8±76,1	490,6±55,9	583±70,8

Results expressed as mean ± standard deviation. Different letters on the same line represent statistical difference according to the two-way ANOVA statistical test, followed by Bonferroni ($p < 0.05$). *Period of administration of extracts. Metabolic syndrome group treated with metformin (EM Control); Group with untreated metabolic

syndrome (SM Control); Group without metabolic syndrome and without treatment (SA Control); Group with metabolic syndrome treated with man-á-cubiu extract at a dose of 150mg/kg (MN 150); Group with metabolic syndrome treated with man-á-cubiu extract at a dose of 300mg/kg (MN 300).

It is also possible to observe that the animals in the control group treated with metformin (ME control) obtained increasing weight gain in the months that preceded the administration of the drug, while the control group with the disease and without treatment (SM control) maintained this weight gain in all weighing procedures until the last month of the experiment. In relation to the groups that received the fruit extract, the MN150 group showed weight reduction in the first month of treatment, followed by an average reduction of 54 grams per animal. While the MN 300 group experienced a weight reduction only after the second month of treatment, with a reduction of, on average, nine grams per animal.

4. Discussion

Bioimpedance was one of the parameters evaluated by Angđoco et al. (2012) in rats treated with diets rich in lipids and sucrose in order to correlate the direct analysis of the carcass with the observed biochemical and anthropometric parameters. In the study, 24 male Wistar rats were fed with a standard diet, rich in lipids or rich in sucrose for a period of four weeks. The control group received standard AIN-93 diet; the group with a high-fat diet received a diet containing 50% lipids, with 70% coming from saturated fats; and the high-sucrose diet group was fed a higher proportion of simple carbohydrates, containing 3.50% corn starch and 59.85% sucrose. The higher intake of lipids led to an increase in the percentage of liver fat and cholesterol and reduced the amount of total body water in the group with a high-fat diet, but without changes in anthropometric measurements or changes in bioimpedance parameters. In the group fed with a diet rich in sucrose, no changes were observed in anthropometry and bioimpedance. However, there was a positive association between carcass fat and body mass index, Lee index and abdominal circumference. Oppositely of what was observed in this study.

Aiming to compare the body mass and the Lee index in rats submitted to a commercial diet in relation to a diet supplemented with sucrose, a sample of 40 animals was divided into two groups, during three months for intervention. Twenty animals in the control group received only commercial food and water and the other twenty in the experiment group received the same supplemented with sucrose 300 g/l in water, both groups submitted to weekly body weight monitoring. As a result, there was a statistically significant difference between the 14th and the 78th day, indicating that sucrose interfered with the weight gain of the rats. The average weight was higher in the experimental group in all periods, having the initial weight as reference, however, there was no significant difference when comparing the Lee index (control group 322.3 ± 11.4 ; experimental group $323.9 \pm 12,3$) (Malafaia et al., 2013).

The influence of the sweet taste of the diet was also evaluated in obese rats fed with the commercial diet in three different experimental groups: the first, only received the commercial diet; the second received the same diet, with a 30% caloric reduction; and the last group, with a commercial diet associated with the practice of moderate physical activity on a treadmill. The nine-week experiment demonstrated greater body weight gain and Lee's index in animals that received only the isolated commercial diet, in both sexes. The calorie-restricted commercial diet significantly decreased the weight gain and Lee's index especially in females, while this relationship was observed for Lee's index in males (Alvarez-Monell, et al. 2022).

Regarding body composition and body water content, bioimpedance analysis is widely used in humans, as it is a fast, non-invasive and reproducible method. However, few studies have used this technique in laboratory animals. Results presented previously in other studies demonstrated that resistance data do not correlate with chemically determined carcass fat, suggesting that BIA is not sensitive enough to measure body composition in rats or detect differences in groups receiving different diets. Furthermore, reactance was negatively correlated with carcass fat and found wide intragroup variation, showing the heterogeneity of these animals and their different responses to the same diet (Angđoco, et al. 2012). Due to the absence of a specific and consensus marker, for mice and rats, to define the presence or absence of obesity, some studies have established their own parameters, such as a difference of 15% or 20g in body weight between test and control groups, determination of the adiposity index, creation of cut points, and the calculation of the body mass index. The majority of the studies consider differences in total body weight gain as the main parameter to assess the outcome of obesity development (De Moura e Dias et al., 2021).

The body fat is accumulated due to different metabolic pathways, such as genes, as well as diet. Wistar rats have differential expression of genes in the subcutaneous adipose tissue compared to Sprague-Dawley rats, resulting in greater accumulation of body fat. Consuming a high-fat diet generates greater uptake of fatty acids and greater

lipogenesis, increasing the size and number of cells in adipose tissue, impairing the secretion of hormones such as leptin, adiponectin and ghrelin, which are linked to obesity. Thus, the DIO experimental model can cause the metabolic and morphological changes that characterize human obesity (De Moura e Dias, et al., 2021).

The reduction in body weight observed, in the first month after starting treatment (7th month), in the experimental group treated with metformin in this study, may be related to action of the drug itself. Metformin was developed in the 1950s as an oral hypoglycemic drug used in the treatment of type 2 diabetes, that is, non-insulin dependent. In addition to the direct effect on glycemic control, this drug is capable of inhibiting food intake in obese patients, both in those with diabetes, type 1 and 2, and in non-diabetics. This action could be explained by the side effects of its use, such as nausea, vomiting, diarrhea, abdominal pain and taste changes, or even as a response to the increase in glucagon-like peptide-1 (GLP-1). As a third hypothesis, metformin seems to act on the central nervous system (CNS) by inhibiting the expression of neuropeptide Y (NPY) and agouti-related protein (AgRP) in the hypothalamus, substances known to regulate appetite (Wen-Shan, et al., 2012).

Treatment with metformin (300mg/kg for 15 days) improved changes in male Wistar rats that received subcutaneous injections of monosodium glutamate (4.0g/kg body weight) to induce obesity, reducing the response induced by norepinephrine, which was shown to increase in the mesenteric arteriolar bed of obese rats. Among the results resulting from the use of the drug are the lowest Lee index, less fat accumulation, control of dyslipidemia, insulin resistance and hyperinsulinemia. The lean mass weight of rats treated with metformin was not different from control rats, but cholesterol, triglycerides and LDL cholesterol levels were restored to control levels after treatment with the drug (Lobato, et al., 2012).

In general, fructose and sucrose, widely used as food or beverage sweeteners, provide less satiety than other types of sugar, thus encouraging excessive intake. However, obesogenic diets did not always result in an increase in the body weight of animals in experimental groups compared to rats receiving control diets. Differences in energy intake between dietary groups can make it difficult to interpret the metabolic consequences of a given diet or a tested therapeutic compound. Likewise, differences in diet composition have a direct impact on metabolic and pathophysiological outcomes in rodent models of diet-induced obesity (Kotz & Hörstmann et al., 2022).

Comparatively, the most used diets for induction of the DIO experimental model were evaluated for their obesogenic and inflammatory consequences. The commercial diet and the high-fat diet with 45% lard were tested in male Wistar rodent models, along with the control group, for 15 weeks. Although both high-fat diets resulted in increased adiposity and hepatic steatosis, the body weight of the animals in the group that received the cafeteria diet increased significantly and remained high compared to the other two groups, as did the animals in the commercial diet, as same as the values obtained in the TOTG evaluation (Sampey, et al., 2011).

Studies demonstrate that the high intake of fats and sucrose, by itself, can also result in a lower food intake in experimental groups, even if the energy consumption remains equivalent to the control groups. This change in food intake may result from the mechanism of regulation of food intake in rats in response to higher and higher energy density in the diet. Fat intake results in an increase in hepatic fat and serum cholesterol, in addition to a reduction in total body water, demonstrating that high-fat diets alter the lipid profile of animals. However, these biochemical changes are not always reflected in anthropometric variables, which may depend on the duration of the study intervention or dietary variation with different proportions of fat for comparison purposes (Ang & Coco, et al. 2012). In DIO animal models, a decrease in motivational behavior towards sweet taste and a reduction in its consumption have been reported for male rats. As well as the increased preference for low concentrations of sucrose and diet-induced weight loss (Alvarez-Monell, et al. 2022).

Skalick et al. (2001) investigated the influence of housing conditions on the body composition of elderly rats. Male Sprague-Dawley rats were divided into two groups of 32 animals each, a group with four animals in each box and the other with only one animal housed. Both groups were maintained with ad libitum feeding from five to 23 months of age, with monthly monitoring of body weight and quarterly monitoring of body composition by bioimpedance analysis. As a result, body weight increased in both groups over time, however, from six months of age onwards, the group with more animals per housing gained more significant weight. Both fat-free mass and total body fat increased in both groups, especially after eight months of age. The amount of fat, expressed as a percentage of body weight, increased with age, but stabilized after 14 months of age. The difference between groups was significant for ages 11 to 20 months, concluding that animals lose lean tissue and gain fat as they age.

The age and gender of the animals are factors that can interfere with the development of obesity, in DIO models

the disease develops better in younger animals, hence the importance of starting the diet in the first years of life. Weight gain also appears to be faster in younger animals. In rodents considered elderly, there is less inflammation, causing less glycemic and hepatic alterations (De Moura e Dias, et al., 2021). As the species reaches adulthood close to two months of life and the induction time used in this study to induce MS was six months, after the treatment period the animals were already at an advanced age, totaling 11 months, the which may explain the slower behavior in body weight gain, including the animals in the SA group, for which there was no intervention.

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