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Effects of water-soluble extract of açaí (*Euterpe oleracea* Mart.) on Wistar rats with induced obesity, diabetes, and cholesterol

Efeitos do extrato hidrossolúvel de açaí (Euterpe oleracea Mart.) em ratos Wistar com obesidade induzida, diabetes e colesterol

Efectos del extracto soluble en agua de açaí (Euterpe oleracea Mart.) en ratas Wistar con obesidad inducida, diabetes y colesterol

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Resumo
Dentre as inúmeras espécies encontradas no bioma amazônico, o açaí é valorizado por seu sabor característico e alto teor de nutrientes; em particular, possui um alto teor de antocianinas. O presente estudo teve como objetivo avaliar o efeito do extrato hidrossolúvel de açaí em ratos induzidos a obesidade, diabetes e colesterol, sendo respectivamente administrado o glutamato monossódico na concentração de 4 mg / g de peso do animal, alloxan mg/kg in 0.9% salina por via intraperitoneal. Para os ensaios biológicos, estes foram compostos com um total de 50 animais, divididos em 10 grupos, compostos por 5 roedores cada. O grupo controle foi constituído por ratos saudáveis e alimentados por ração comercial. Foi realizada a determinação da composição proximal, fibra alimentar e antocianina no extrato de açaí. Este extrato foi então suplementado na ração para os diferentes tipos de animais modelo que permaneceram em confinamento por 28 dias. A caracterização do extrato de açaí revelou elevados teores de fibra alimentar insolúvel (3,31 g / 100 g) e antocianina (45 mg / 100 g). Também houve efeito positivo na suplementação alimentar durante os 28 dias de confinamento, com redução do peso e do índice glicêmico observado nos animais ($p < 0{,}05$).
Palavras-chave: Índice glicêmico; Compostos bioativos; Flavonóides; Nutrição alimentar; Índice lipídico.

Abstract
Among the countless species found in the Amazon biome, açaí is valued for its characteristic flavor and high nutrient content; in particular, it has a high content of anthocyanins. The present study aimed to evaluate the effect of the water-soluble extract of açaí in rats induced to obesity, diabetes and cholesterol, being administered monosodium glutamate at a concentration of 4 mg/g of animal weight alloxan mg/kg in 0.9% salina by intraperitoneally. For the biological tests, these were composed with a total of 50 animals, divided into 10 groups, composed of 5 rodents each. The control group consisted of healthy rats fed on commercial food. The determination of the proximal composition, dietary fiber and anthocyanin in the açaí extract was carried out. This extract was then supplemented in the feed for the different types of model animals that remained in feedlot for 28 days. The characterization of the açaí extract revealed high levels of insoluble dietary fiber (3.31 g / 100 g) and anthocyanin (45 mg / 100 g). There was also a positive effect on feed supplementation during the 28 days of confinement, with a reduction in weight and glycemic index observed in animals ($p < 0.05$).
Keywords: Glycemic index; Bioactive compounds; Flavonoids; Food nutrition; Lipid index.

Resumen
Entre las innumerables especies que se encuentran en el bioma amazónico, el açaí es valorado por su sabor característico y alto contenido de nutrientes; en particular, tiene un alto contenido en antocianinas. El presente estudio tuvo como objetivo evaluar el efecto del extracto hidrosoluble de açaí en ratas inducidas a la obesidad, diabetes y colesterol, a las que se les administró glutamato monosódico a una concentración de 4 mg / g de peso animal y alloxan mg/kg in 0.9% salina por vía intraperitoneal. Para las pruebas biológicas, estas se compusieron con un total de 50 animales, divididos en 10 grupos, compuestos por 5 roedores cada uno. El grupo de control consistió en ratas sanas alimentadas con alimentos comerciales. Se realizó la determinación de la composición proximal, fibra dietética y antocianina en el extracto de açaí. A continuación, este extracto se complementó en el pienso de los diferentes tipos de animales modelo que permanecieron en el corral de engorde durante 28 días. La caracterización del extracto de açaí reveló altos niveles de fibra dietética insoluble (3,31 g / 100 g) y antocianina (45 mg / 100 g). También hubo un efecto positivo en la suplementación alimenticia durante los 28 días de confinamiento, observándose una reducción del peso y del índice glucémico en los animales (p <0.05).
Palabras clave: Índice glucémico; Compuestos bioactivos; Flavonoides; Nutrición alimentaria; Índice de lípidost.

1. Introduction

The development of new research into foods, by using the fruits of the Amazon biome, has deservedly attracted much attention: these foods contain a significant amount of vitamins and minerals, and have a high biological value, with significant metabolic effects that may promote population health. Among these nutrients, we have previously highlighted those with functional properties, such as antioxidant compounds, which contain a wide range of substances such as phenolic compounds, vitamins, carotenoids, and minerals (Almeida et al., 2011). According to Udnai et. al., the (2011) açaí berry stands out for its high content of polyphenols, especially anthocyanins, ranging from 50 to 180 mg/100 g of pulp. Some authors report the quantification of total phenolics in some agroindustrial co-products, such as acerola bagasse, with 247.62 mg gallic acid/100 g (Sousa et al., 2011) and grape marc with 7475.0 mg gallic acid/100 g (Rockenbach et al. al., 2011). Studies have shown that the consumption of polyphenol-rich foods, especially those of the flavonoid class, has been associated with a low risk of developing various diseases owing to the antioxidant properties present in the food (Devalaraja et al., 2011).

The growing consumer interest in healthier products, such as fruits and vegetables, reflects the evidence from several scientific studies that have demonstrated the consumption of these foods results in beneficial effects to health, including the prevention of chronic diseases related to oxidative stress (Baxter et al., 2005). In the present study, it was found that the effects of the anti-inflammatory drugs on the metabolic syndrome were not significant. This was favored by the high content of compounds with bioactive properties such as polyphenols, the anthocyanins group, which are phenolic compounds, and are responsible for the purple, red, and blue pigmentation in the plants, and contribute significantly to the physiological processes in the organism (Santos Buela et al., 2014). Diabetes mellitus is a syndrome of multiple etiology, due to lack of insulin and/or the inability of insulin to adequately exercise its effects, and results in insulin resistance. It is characterized by the presence of chronic hyperglycemia, often accompanied by dyslipidemia, hypertension, and endothelial dysfunction. (McLellan, 2007). Basu et. al. (2010) reported that as a result of hyperglycemia, there may be a high concentration of cholesterol and free fatty acids in the plasma that lead to the development of micro and macrovascular lesions; these are responsible for the appearance of chronic complications that are frequently associated with diabetes, such as retinopathy, nephropathy, neuropathy, and macroangiopathies, with myocardial infarction and stroke as manifestations.

Therefore, given the potential action of anthocyanins found in fruits, the present study aimed to test the effect of water-soluble extract of acai (*Euterpe oleracea* Mart) on wistar rats induced to obesity, diabetes, and cholesterol.

2. Methodology

2.1 Obtaining the raw material extract

The fruits were obtained from the local market of Manaus and transported to the National Institute of Amazon Research (INPA) Food Analysis Laboratory where they underwent a selection process. Selected fruits were then sanitized in running water and sanitized with hypochlorite at 200 ppm for 30 mins, and rinsed again to remove excess hypochlorite. The pulp was then softened in water at 50ºC and added to the juice extractor with an additional 40% of water. After pulping, the juice was stored in sterile trays and frozen at -80ºC, lyophilized, and stored at -18 ° C.

The açaí extracts consisted of the samples that underwent the lyophilization process and were reconstituted with water.

2.1.1 Chemical analyses of the extract

The characterization of the extract was performed in accordance with the standard analytical procedures of the Adolfo Lutz Institute. General procedures and determinations AOAC (2012): humidity; ashes; lipids; crude protein; carbohydrate (by difference); total fibers (enzymatic-gravimetric method); soluble and insoluble fibers (enzymatic-gravimetric method); minerals (atomic absorption spectrometry).

The quantification of total anthocyanins was performed in accordance with the method described by De Rosso and Mercadante (2007). The crushed samples were mixed with 95% ethanol/1.5 N HCl and homogenized. The mixture was stored in the dark at 4ºC for 16 h, vacuum filtered, and washed thoroughly with acidified ethanol. The absorbance at 535 nm was measured by using a UV-visible spectrophotometer at 535 nm. The extraction was performed in triplicate.

2.1.2 Biological assays

The experimental groups were composed of a total of 50 animals, divided into 2 groups composed by 5 animals each, for each analyzed variation (control, diabetes with and without açaí extract, cholesterol with and without açaí extract), and 50 animals were used in each plot (Animal Use Ethics Committee - CEPA of the National Institute of Amazonian Research - INPA, No. 050/2017). The control group consisted of healthy rats fed on commercial food.

Fifteen animals were selected for the induction of diabetes, which was performed by the intraperitoneally administration of alloxan at a concentration of 42 mg/kg in 0.9% saline after a 12 h fast (overnight) with ad libitum water. Eight days after induction, the animals were fasted for 12 h overnight and the glycemic levels were measured. A small hole was drilled with a lancet in the rodent’s syringe and a drop was placed on the glycoprotein tape which was then taken to the meter (Accu-Check Active, Roche®, Manheim, Germany). Animals with a glycemic level of >125 mg/dL were considered diabetic.

The acai extract was administered through gavage; 2.5 mL of the reconstituted concentrated extract in 50 mL water (equivalent to a concentration of 5%) were administered from the cage drinkers by using a stainless steel needle, 1.2 mm diameter cannula and 2.3 mm packed in a 3.0 mL plastic syringe. In the control group, the animals received only water.

The glycemic of the animals was measured on Days 0, 14, and 28. Drops of blood were sampled and measured in a portable blood glucose meter for control purposes. During the experiment, the feed consumption of the animals was monitored every 2 days and the data collected were calculated from the formula: Food consumption = [Ration (g) – Scraps (g)]; weekly weight was also measured.

The rats were housed in metabolic cages for an average period of only 4 h in order to acquire urine samples to verify parameters; there was no need for a longer period than this. The samples were conditioned in cryogenic tubes and kept refrigerated until analysis.

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To induce high cholesterol, monosodium glutamate at the concentration of 4 mg/g animal weight was administered intraperitoneal.

At the end of the experiment, the animals underwent were euthanized by the use of anesthetic dissociative solution administered by intraperitoneal injection (ketamine 0.2 mL/25 g body weight and xilazine 0.2ml/25 mg of animal weight, in a proportion of 3:7 respectively).

2.2 Experimental and statistical design

The biological experiment had a simple 2×3×3 design (two 0% and 5% concentrations; three collection times 0, 14, and 28 days; three variables analyzed: obesity, glucose, and cholesterol) and the Tukey 5% test was analyzed by using the statistical package SISVAR (Ferreira, 2010) in 100 animals. The mean data obtained in the characterization of extracts were presented followed by standard deviation.

3. Results and Discussion

The values obtained in this experiment further evidence the importance of the process of adding values to native species through the development of new pharmaceutical and/or chemical food products, which can be means of improving the health of the population as a whole, as well as a source of income and improvement of regional socioeconomic indices.

The values obtained for the characterization of the açaí extract administered to Wistar rats as control in the parameters related to the metabolism of glycemic and cholesterol are presented below (Table 1): the data indicate a high percentage of lipids (22.17%), insoluble fiber (3.31%), anthocyanin (45 mg/100 g), in addition to the low value of total carbohydrates (18.87%). The values presented above for the anthocyanin data are higher than the data found by Coutinho et. (2017), of 12.05–24.98 mg/100 g, Fernandes et al., (2016), of 36.38 mg/100 g, and lower than the data found by Rufino et al. al., (2010), of 111 mg/100 g. The differences found may be related to the processing method used to obtain the extract, as climatic conditions, soil characteristics, and stages of maturation. However, an higher value of insoluble dietary fiber was observed compared with other authors; this may be related to the processing used, variety of fruit, and climatic effects, among others. Although not the focus of the study, the dietary fiber content increases the nutritional value of the product, as the fibers have the ability to decrease the time of the fecal cake in the intestinal tract, which reduces the absorption of nutrients and aids in the perch of weight. According to Becker et. al., (2014), the consumption of fiber-rich foods has been associated with weight loss, as well as in the prevention of chronic noncommunicable diseases.

Table 1: Proximal composition, food fiber, and anthocyanin contents of açaí extracts.

Variable	Content with Standard deviation
Moisture (%)	55.00±0.01
Protein (%)	2.35 ± 0.00
Lipids (%)	22.17 ± 0.30
Ash (%)	1.61 ± 0.00
Total carbohydrates (%)	18.87 ± 0.30
Soluble fiber (g/100g)	0.68 ± 0.01
Insoluble fiber (g/100g)	3.31 ± 0.02
Total fiber (g/100g)	3.99± 0.01
Anthocianyn (mg/100g)	45.00 ±0.30

Source: Authors. (2021).

To test the effectiveness of the extract on obesity, diabetes, and cholesterol, the consumption of food was also observed throughout the experiment; a lower percentage of consumption was observed for the animals with diabetes that were fed the açaí extract. It was observed that the mean consumption of the control group was 744 g, approximately 74 g per animal. For the diabetic group administered the açaí extract by gavage, the average consumption was 616 g, approximately 62 grams per rodent. Consumption in the diabetic group that was administered only water by gavage was 831 g, approximately and 83 g per rat. These values may be related to the fiber content in the extract, which increases satiety and leads to a lower consumption; this was could be corroborated with the weight loss of the same animals (Table 2). In contrast, the results by Samuel Wu et al. (2013) in rats after 10 weeks on a hypercaloric diet in the presence or absence with aqueous extract of lychee flower, rich in anthocyanin, did not identify the difference between the control group and the treatment groups, indicating that anthocyanin did not contribute to a decrease in body weight. However, Titta et al. (2009) observed a reduction in weight and body fat accumulation, and resistance to obesity in animals treated with a hypercaloric diet associated with the red-orange liquid extract.

According to Cani et al., (2004) the short chain fatty acids generated by the fermentation of the fibers can affect the satiety through effects on the production and secretion of hormones. Rats fed a diet supplemented with fermentable dietary fiber showed a reduction in the plasma concentration of ghrelin, a hormone associated with hunger and appetite, compared to the group of animals that did not receive this supplementation. Thus, a decrease in food consumption by rodents may be due to the functional characteristics of the açaí berries and its fiber content.

Table 2: Effects of supplementation of the animals receiving and not receiving açaí extract

Group	**Control**	**Diabetes with açai extract**	**Diabetes without açai extract**	**Cholesterol with açai extract**	**Cholesterol without açai extract**
Weight (g)	180.2b	144,0c	197.4a	92.5e	125.5d

* Averages followed by the same letter do not differ by $p < 0.05$. Source: Authors. (2021).

With regard to the data obtained for glycemic levels, the animals fed the açaí extract presented the lowest indices throughout the experiment (Table 3). This may be associated, not only with the anthocyanin present in the extract, but also with the soluble fiber content, as these compounds also have a significant effect on the glycemic indices, in addition to the significant weight loss in accordance with the abovementioned data (Table 1). According to Li et. al. (2015), components present in foods, such as dietary fiber and phytochemicals such as polyphenols, appear to interfere positively in postprandial glycemic and insulinemic response, reducing the rate of glucose uptake in the intestine through the modulation of enzymes such as α-glycosidase, which affects the bioavailability of carbohydrates and reduces their intestinal absorption, exerting a hypoglycemic effect. Epidemiological studies have shown that diets rich in dietary fiber are associated with a reduced risk of diabetes and cardiovascular diseases (Fung et al., 2002).

Table 3: Effects of supplementation on glycemic index of animals receiving and not receiving açaí extract

	Glycemic Index mg/dl
Control	193.88cb
Diabete with açaí	146.79[a]
Diabete without acai	172.67b

* Averages followed by the same letter do not differ by $p < 0.05$. Source: Authors. (2021).

In relation to the lipid levels (Table 4) (cholesterol and triglycerides), a significant improvement was only observed in relation to the triglycerides, which were observed to decrease. However, in relation to cholesterol, no positive effect was observed from the açaí extract supplementation. This may be related to the content of lipids found in the açai extract (Table 1). According to Li et al., (2015), the accumulation of fat in rats may be related to the confinement process, which may generate oxidative stress in obese rats. Severino et al. (2002) reported that the increase in lipid content that may or may not be associated with increased liver fat has been associated with altered metabolic conditions in which insulin resistance is the predominant characteristic.

Table 4: Effects of supplementation on cholesterol index of animals receiving and not receiving açaí extract.

	Control	**Cholesterol with extract açaí**	**Cholesterol without açaí extract**
Triglicerides	39.9b	32.5^{a}	42.1bc
Cholesterol	31.8^{a}	42c	38.6b

* Averages followed by the same letter do not differ by $p < 0.05$. Source: Authors. (2021).

4. Conclusion

The consumption of lyophilized açaí extract during a 28-day period in diabetes, obesity, and cholesterol in Wistar rats (*Rattus norvegicus* albinus) was shown to be efficient for weight loss, as evidenced by satiety, with lower feed intake and improved in glycemic indices. However, it was not as effective for the reduction of cholesterol. The research itself highlights the importance of adding value to species in our biodiversity through processes that are adequate and bring optimal benefits to those who use them. It is also important to point out here that results like these demonstrate the importance of our biodiversity as a means of obtaining essential nutrients and as a way of improving the socioeconomic conditions of the populations that survive in the agro-extractive system.

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