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Oral Administration of *Euterpe oleracea* Mart., Its Influence over Body Weight and Lymphoid Organs and Alterations in Haematological and Glycaemical Parameters

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MTP, NSRS and JCTC designed the study, wrote the protocol, involved in writing the first draft, participated in experiments and data collection. Authors CCL, FSB, CFS and JSC managed the literature search, analyses of the study and manuscript preparation. Author CBRS, FSB and JSC performed the statistical analysis and also aided in data interpretation and was actively involved in reading the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aims of the study were to evaluate alterations caused by the oral administration of the aqueous extract of *Euterpe oleracea* to non-isogenic adult male mice during 15 and 30 consecutive days through the comparison of body and spleen weights, number of splenic and medullary cells, their hemogram and glycemia indices.
Study Design: Animals were divided into 8 groups (5 animals/group). Treated groups received the AEA concentration (125 mg/ml by gavage) at doses of 5, 50 and 500 mg/kg for 15 and 30 days. The control group received only the vehicle of dilution (1x PBS) in volume of 0.5 ml. After treatment the animals were sacrificed in a CO_2 chamber for testing.
Place and Duration of Study/Methodology: The aqueous extract of concentration of 125 mg/ml of lyophilized açaí pulp was prepared in Laboratory of General and Analytical Chemistry of Federal University of Amapá. Values of body weight, weight of liver and spleen, number of spleen and bone marrow cells, blood count and glucose of the mice were conducted on Drugs Research Laboratory between January and November of 2013.
Results: The statistical analysis was done by ANOVA test two-way with significance level $p<0.05$ in relation to control followed by Tukey post-test. The AEA caused significant changes in body weight, about 22% in animals treated with 500mg/kg. Weight of spleen showed no significant changes, there was statistical difference in blood glucose levels between groups 5 and 500 mg/kg treated for 15 days and punctually in the 500 mg/kg group treated for 15 and 30 days. It was observed that the treatment with the AEA doses (5, 50 and 500 mg/kg) for 30 days increased the number of bone marrow cells. Regarding the number of spleen cells, treatment promoted changes, reducing the amount of cells during the 30 days of treatment, principally at the dose of 50 mg/kg. During the 15 day treatment of 500 mg/kg there was an increase in the number of spleen cells.
Conclusion: The treatment of mice with aqueous extract of açaí pointed that the concentration has significant influence in: 1) glucose concentration in the blood; 2) The increased number of bone marrow cells; 3) and the number of spleen cells. Thus, comparison between groups of animals was compatible with the hypothesis that the increase in body mass is a risk factor for insulin resistance.

Keywords: Açai; Euterpe oleracea mart; spleen; mice.

1. INTRODUCTION

The açaí palm (*Euterpe oleracea*, Mart) is included in the family *Arecaceae* (Palmae), originally from amazonian area, is distributed in the estuary of Amazon, Low Amazon and in the coast of Amapá, Pará, Piauí, Guyana and Venezuela. Açaí palm is highlighted between the palm trees that ornament the flora of the Amazonian and serves as subsistence for the man of the field. These palm tree suffers extensive devastation that happens there are some decades due palm heart exploration [1]. The açaí fruit, produced by açaí palm tree, is characterized by the spherical form, with a diameter from 1.0 to 1.5 cm and violet color almost black.

In the third year, the palm tree fructifies, with maximum harvest in the fifth and in the sixth year, in two harvests yearly: one in the winter of the month January to June and other in the summer of August for the month of December [2].

Several parts of this palm tree are widely used in the popular medicine, as examples, the oil of the fruit, as anti-diarrheal agent [3], in association with the root *Carica papaya*, Citrus sp. (lemon) and *Quassia amara* as antimalarial [4]. Professionals of health and nutrition has dedicated attention for the antioxidant capacity of the açaí and use as functional food, skin cosmetics and even as nutraceuticals [5-7]. The natural products are highlighted as main sources of new therapeutic resources, that is due to different chemical compositions flowed mainly of the secondary metabolism of plants, toxins in animals and microorganisms [8].

Studies showed that the açaí extract in culture cells, was capable to inhibit the production of nitric oxide and of Inducible nitric oxide synthase (iNOS), one of main generating enzymes of nitric oxide (NO) produced from the amino acid L-arginin [9-10]. This effect seems to be due to a direct action of the extract in the production of NO, molecule with physiologic activities as vasodilatation and neurotransmission. On the other hand, the NO derived of macrophages has an effect potentially cytotoxic/cytostatic on the tumorous cells [11].

In study conducted by Pacheco-Palencia et al. [12], demonstrated that the chemical composition of the açaí oil has a significant influence on the proliferation of cells, suggesting properties antiproliferative polyphenols in cultures of cancer cells. In another study, accomplished for Del Pozo-Insfran et al. [13], was confirmed the induction of the activity antiproliferative and proapoptotic of polyphenolics constituents of açaí against cells HL-60, that cause leukemia. Besides these studies, were demonstrated that the açaí has a vasodilator effect and that this effect is dependent of the pathway activation NO-cGMP and can also involve the liberation of the endothelial hyperpolarizing factor, what suggests the possibility to use of açaí as medicinal plant to treat cardiovascular diseases [14].

Açaí has unquestionable nutritional properties, being rich in proteins, fibers, fat, vitamin E, minerals, besides having high tenor of anthocyanins cyanidin-3-glycoside. Açaí are used as food and in the popular medicine, for prevention and reduction of the diseases caused to free radicals, such as diseases: cardiovascular, circulatory, inflammatory process and carcinogenic [2,15].

This study evaluates the effect of the consumption of aqueous extract of açaí - AEA (*Euterpe oleracea* Mart), during 15 and 30 consecutive days in adult male mice no isogenic comparing to the corporal mass, the spleen, the number of cells spleen and of the marrow, blood count and glycemia.

2. MATERIALS AND METHODS

2.1 Animals

Were used Mice Swiss male adults no isogenics, with weight between 25 and 30g, originating from Laboratory of Drug Research, Department of Biological Sciences and Health, Federal University of Amapá, Macapá, Brazil. The animals were maintained at rooms with controlled temperature (23±2°C) obeying the clear/darkness cycle of 12 hours (light period 07:00-19:00). Animals were maintained in fast by 12 hours before the experiments, with free access to the water. This study was approved by the Committee of Ethics in Research of the Federal University of Amapá (UNIFAP), being this presented to the Committee of Ethics for evaluation and emission of the opinion embodied, approved for the protocol n°002A/2012, of August 6, 2012.

2.2 Preparation Aqueous Extract of Açai *Euterpe oleracea* Mart. (AEA)

The pulp of the fruit was collected from the Cooperative of farmers of the Municipal districts Macapá and Mazagão, belonging to the state of Amapá-Brazil. The pulp was submitted to lyophilization process in the Laboratory of Research in Drugs of the Federal University of Amapá. The extracted powder of the referred process was diluted to concentration of 125 mg/ml in PBS 1x for the treatment of animals. Different doses (5, 50 and 500mg/kg) and periods (15 and 30 days) of the administrations were chosen to evaluate the effects of the concentration and time in the analyzed parameters.

2.3 Treatment of Animals

Animals were divided in 8 groups (5 animals/group). Three groups received the aqueous extract (125 mg/ml by gavage) in doses of 5, 50 and 500 mg/kg (125 mg/ml by gavage) in doses of 5, 50 and 500 mg/kg during 15 and 30 days. The control group just received the dilution vehicle (PBS 1x) in the volume of 0.5ml. In the end of the treatment the animals were sacrificed in a camera of CO2 for further testing.

2.4 Evaluated Parameters

2.4.1 Body, Liver and Spleen weights

The animals were weighted in the end of treatment and their body weights were compared with their respective controls. After the sacrifice of the animals the spleen was removed and weighted.

2.4.2 Determination of the number cells of the spleen and of marrow

The spleen was removed, weighted and later macerated. The suspensions of obtained cells were diluted in PBS1x in the proportion of 1:50. Then, 90 µl of this suspension was mixed with crystal violet 10 µl (0.05% and acetic acid to 30%). This mixture was used to quantify the spleen cells on camera of Neubauer, using optical microscope [16]. To quantify blood marrow cells, the femur of the animal was extracted and cut in their distal ends. Cells were extracted from the spinal canal by washing with 1 ml of PBS1x. Blood marrow cells were quantified with the same methodology used for quantifying the spleen cells.

2.4.3 Hematological evaluation and glucose dosage in the blood

The blood was obtained by retro-orbital bleeding through method of the capillary tube and transferred for blood count tubes containing EDTA and analyzed in CELL-DYN 3700 ®. The glucose levels were certain with the aid of digital glucometer (Biocheck). The obtained values represent the glucose concentration in mg/dl.

2.5 Statistical Analysis

The statistical analysis was done by ANOVA test two-way with significance of the 5% in relation to control followed by Tukey post-test to do comparisons of the means, using BioStat 5.0 software.

3. RESULTS

Data in the Tables 1 and 2 show the results of the studied parameters. Table 1 displays the variation of the corporal weight in mice. Table 2 displays the results for the hematological data (erythrocytes, hemoglobin, liver weight, spleen weight, number of spleen cells and of the medulla and glucose concentration).

Table 1. Comparison of mean initial and final body weight modifications in all groups (control, 5, 50 and 500mg/kg) during the period of 15 and 30 days

Period	Body weight	Group for dosage			
		Control	5mg/kg	50mg/kg	500mg/kg
15 days	Initial (g)	$37.40\pm0.54^{*A}$	$46.00\pm1.00^{*B}$	$44.22\pm1.10^{*B,C}$	$44.02\pm1.15^{*C,D}$
	Final (g)	$44.40\pm1.51^{*A}$	$49.40\pm0.54^{*B}$	$51.60\pm1.51^{*C}$	$54.10\pm1.41^{*D}$
30 days	Initial (g)	$45.68\pm1.43^{\#A}$	42.45 ± 1.91^{B}	37.08 ± 0.88^{C}	$40.31\pm1.93^{\#B,D}$
	Final (g)	$51.80\pm3.01^{\#A}$	45.48 ± 2.02^{B}	40.11 ± 3.21^{C}	$44.21\pm2.53^{\#B,D}$

Different characters, in the same line, indicate that there are significant differences and in the columns significant differences for initial and final values are indicated by "" for 15 days and "#" for 30 days. According to the ANOVA ($p<0.05$)*

Table 2. Results for hematological data (erythrocytes, hemoglobin, spleen weight, number of cells of the spleen and of the medulla and glucose concentration) in all groups (control, 5, 50 and 500mg/kg) during the period of 15 and 30 days

Period	Parameters	Control	Group for dosage		
			5mg/kg	50mg/kg	500mg/kg
15 days	Erythrocytes (μ^3)	9.78 ± 2.68^{A}	5.24 ± 0.70^{A}	6.76 ± 2.43^{A}	3.95 ± 0.64^{A}
	Hemoglobin (g/dL)	13.86 ± 0.78^{A}	13.62 ± 0.74^{A}	13.74 ± 0.4^{A}	13.30 ± 0.69^{A}
	Liver weight (g)	2.46 ± 0.15^{A}	1.92 ± 0.11^{B}	2.86 ± 0.25^{C}	$2.83\pm0.08^{C,D}$
	Spleen weight (g)	0.23 ± 0.05^{A}	0.19 ± 0.05^{A}	0.21 ± 0.05^{A}	0.21 ± 0.02^{A}
	Medullary cells number (10^7/ml)	3.64 ± 0.15^{A}	3.30 ± 0.11^{B}	$3.76\pm0.22^{A,C}$	2.75 ± 0.29^{D}
	Spleen cells number (10^7/ml)	3.86 ± 0.20^{A}	4.13 ± 0.05^{A}	3.86 ± 0.73^{A}	4.30 ± 0.17^{A}
	Glucose (mg/dl)	108.40 ± 24.85^{A}	138.42 ± 35.11^{B}	169.60 ± 24.20^{C}	202.00 ± 24.23^{C}
30 days	Erythrocytes (μ^3)	4.70 ± 0.43^{A}	4.75 ± 0.52^{A}	4.72 ± 0.34^{A}	4.73 ± 0.23^{A}
	Hemoglobin (g/dL)	13.83 ± 0.72^{A}	14.03 ± 1.12^{A}	14.23 ± 1.04^{A}	13.84 ± 0.95^{A}
	Liver weight (g)	1.23 ± 0.27^{A}	1.84 ± 0.12^{B}	1.65 ± 0.20^{B}	1.69 ± 0.21^{B}
	Spleen weight (g)	0.18 ± 0.04^{A}	0.15 ± 0.03^{A}	0.17 ± 0.05^{A}	0.14 ± 0.02^{A}
	Medullary cells number (10^7/ml)	4.26 ± 1.48^{A}	6.50 ± 1.01^{B}	6.12 ± 1.05^{B}	6.44 ± 0.43^{B}
	Spleen cells number (10^7/ml)	5.20 ± 0.36^{A}	4.13 ± 0.45^{A}	3.80 ± 0.60^{A}	4.53 ± 0.49^{A}
	Glucose (mg/dl)	110.00 ± 21.53^{A}	113.02 ± 10.36^{B}	121.36 ± 16.85^{C}	130.72 ± 9.20^{C}

Different characters, in the same line, indicate that there are significant differences between the means. According to the ANOVA ($p<0.05$)

The differences between initial values of the body weight for 15 days do not have meaning in the context discussed, since the measures were performed before any administration, these

values were used to compare initial and final situations. The body weight also showed significant differences between initial and final values for the control and for all doses in 15 days. In 30 days these values were only significant for the control group and to dosage of 500mg/Kg.

Significant differences between the final values of body weight for the control group and the different doses show an increase in body weight with increasing dosage for periods of 15 and 30 days, but reduced body mass gain compared to control in both periods. And in the shortest period lower body mass gain was observed.

In Table 2, hemoglobin, spleen weight, erythrocytes and spleen cells for the 15 to 30 days, showed no significant differences, compared with the control and with different dosages. The parameters liver weight and medullary cells number (30 days) presented differences between the control group and dosages, but not between dosages, showing that administration of açai extract exerts significant influence compared to the control. Differences between control group and the dosages, and also between each dosage for the values of liver weight, medullary cells number (15 days), and glucose (15 and 30 days), show that concentrations of these dosing also influence the parameters values.

4. DISCUSSION

Regarding the significant differences between the values of body weight for different dosages at 15 and 30 days, the increase in body weight gain due to the increase of dosages can be attributed to the presence of proteins and fats in the composition of açaí. Positive effect can be attributed to the açaí because of the large amount (32% of the chemical composition) of fats. These fats are represented largely of saturated fatty acids, they cause a reduction of total cholesterol and low density lipoprotein (LDL) in the circulation by promoting increased expression or activity of LDL receptors in liver [17,18,19].

The low weight gain compared to control observed at 15 and 30 days (mainly for 30-day period, with less weight gain) can be attributed to high concentrations of dietary fiber, which provide a physical barrier in the absorption nutrients and increase the fecal excretion [20].

Total corporal mass causes important effect on the metabolism of the body, since a decrease or increase of the mass of organs can affect the vital functions in the organism and may indicate systemic toxicity, by changing the relative mass organs, hematological and biochemical blood changes [21,22]. The absence of systemic toxicity can be justified by the no significant differences observed for the hemoglobin, spleen weight, erythrocytes and spleen cells parameters. However, the lack of changes in the spleen weight of the animals, suggests that visceral deposits of triglycerides not exert influence on this organ in relation to other areas of body, increasing the offer of acids fatty free in the blood, that stimulate the gluconeogenesis and inhibit the hepatic purification of the insulin, contributing to elevate the glycemia Table 2 [23].

Glucose levels were significantly different both between periods as between dosage. The animals of this group were classified as diabetics, when compared to the control group 15 and 30 days, once the capillary glycemia possesses a parameter glycemic for mice that it varies among 70 to 110 mg/dL [24]. However, açaí extract exerted hypoglycemic effect in obese patients, according to pilot study by Udani et al. [25]. These pilot study also evaluated the effect of the aqueous extract of açaí in the cellular dynamics of the lymphoid organs, bone marrow and spleen. These organs participate actively of the immune answer: marrow

as the central organ for the production of white and red globules and antigens of the spleen [26].

Treated animals during 30 days showed increased in the number of marrow cells compared to control for all dosages, evidence of an effect immunostimulant on production and/or maturation of cells. Similar results were obtained for the extracts of the species *Silybum marianum* [27], *Pulicaria crispa* [28] e *Tinospora cordifolia* Miers [21]. The açaí is rich in anthocyanins, cyanidin 3-glucoside and cyanidin 3-rutinoside are the main [17]. Anthocyanins are flavonoids, compounds containing phenolic hydroxyls, described as powerful antioxidants [29]. Flavonoids may promote hypolipidemic, antiatherosclerotic, antiinflammatory effects, besides having a high power immunomodulatory [30].

Although do not differ significantly, the values for spleen cells number, have changed, reducing the amount of cells during the 30 days of treatment, mainly in the dose of 50 mg/kg. The reduction of number cells the spleen was also evidenced for Currier (2001) [31], in the treatment conducted in mice with extract of *Crimson echinacea*. This result suggests that the treatment of 30 days, in spite of being an intermediate dose, induces the increase of the process of cells apoptosis in this organ. The apoptosis (solemnity-destruction) is the cellular death that happens in a programmed way, without affecting the neighboring cells. This phenomenon also occurs naturally removing unnecessary cells [26], being increased by factors, as mentioned above. This phenomenon is part of several physiologic or pathological processes of the organisms, from the fetal development, as the control of depletion of lymphocytes T, regression of tumors, periodic renewal of lineages normal cells, the loss of fabric neuronal or heart and the loss of leukocytes induced by the infection of human immunodeficiency virus (HIV) [32].

Investigations antiproliferative and proapoptotic effects from açaí were conducted by Del Pozo-Insfran, Talcott and Percival (2006) [33]. In this study the authors demonstrated that polyphenols present in acai induce mortality of human leukemic cells (HL-60) by proapoptotic mechanisms, such as activation of caspase-3. For the 15 days the dosage of 500 mg/kg increased the number of cells spleen. Considering the observed increase, the highest dose might have been capable to induce a larger activation and cellular proliferation. Significant difference between dosage and spleen cells number, suggests proportional relationship between the two variables, this was also certain for Sheyab et al. [34] and Tang et al. [35].

5. CONCLUSION

The mice treatment with the *Euterpe oleracea* Mart extract (açaí) allows to infer that the concentration has significant influence on the values of following parameters: 1) glucose concentration in the blood, high values that classify the animals as diabetics; 2) the increase of number cells marrow, with evidence of immune stimulatory effects modulated by açai; 3) and the spleen cells number: the lower extract concentration caused a reduction on cells number (apoptosis) and greater extract concentration resulted in an increase of number cells (cellular proliferation). Therefore, the comparison among the groups of animals was compatible with the hypothesis that the increase of corporal mass represents risk factor for resistance to the insulin.

CONSENT

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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