Antioxidant effects of açaí seed (*Euterpe oleracea*) in anorexia-cachexia syndrome induced by Walker-256 tumor[1]

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ABSTRACT

PURPOSE: To assess antioxidant effects of açaí seed extract on anorexia-cachexia induced by Walker-256 tumor.

METHODS: A population of 20 lab rats were distributed into four groups (n=5): Control Group (CG), which only received tumor inoculation. Experimental Group-100 (EG-100), with animals submitted to tumor inoculation and treated with seed extract in a 100 mg / ml concentration through gavage. Experimental Group-200 (EG-200), with animals submitted to tumor inoculation and treated with seed extract in a 200 mg / ml concentration. Placebo Group (GP), which received tumor inoculation and ethanol-water solution. We analyzed proteolysis, lipid peroxidation, tumor diameter and weight.

RESULTS: Lipid peroxidation was representative only in the cerebral cortex, where there was more oxidative stress in rats treated with the extract ($p = 0.0276$). For proteolysis, there was less muscle damage in untreated rats ($p = 0.0312$). Only tumor diameter in treated rats was significantly lower ($p = 0.0200$) compared to untreated ones.

CONCLUSIONS: The açaí seed extract showed no beneficial effect on the general framework of the cachectic syndrome in lab rats. However, some anticarcinogenic effects were observed in the tumor diameter and weight.

Key words: Cachexia. Carcinoma 256, Walker. Plants, Medicinal. Euterpe. Rats.

Introduction

Cancer is one of the main causes of death and killed 8.2 million people in 2013[1]. In the past decade, the incidence of cancer increased 30% worldwide and, in Brazil more specifically, there were 576,000 new cases estimated for the year of 2015[2].

One of the aspects associated with cancer that increases patient mortality and determines a worse prognosis is the cachectic syndrome. This syndrome is derived from oxidative stress and is responsible from 30 to 40% of cancer patient's deaths[3]. Cachectic syndrome occurs in chronic pathophysiological processes, such as cancer, AIDS, diabetes mellitus and severe rheumatoid arthritis. In neoplastic cachexia, during the advanced stage of the disease, there is an interaction between the tumor and the host's defense mechanisms and chemical mediators, such as peptide hormones, neurotransmitters and cytokines[4].

The cachectic syndrome's mechanism is directly related to oxidative stress, since cell malignancies are in constant imbalance in oxidation and reduction reactions, damaging the cellular structure and organism's tissue[6]. Oxidative stress results from loss of balance between the production and elimination of ROS (reactive oxygen species), which participates in the elimination of harmful substances or microorganisms from the cell. If the ROS exhibit an imbalance, they can cause damage to DNA, RNA, lipids and proteins, as well as contribute to the onset of various diseases such as cancer[5,6].

Considering the mechanism of action of oxidative stress in cancer, antioxidants may represents a suitable therapeutic option in anorexia-cachexia, but the optimal dose and frequency of supplementation have to be established[7]. The consumption of foods with high levels of antioxidants can help fight diseases caused by oxidative stress, improving a patient's condition. Studies have shown that the use of polyphenolic compounds found in medicinal herbs, tea, fruit and vegetables is associated with low risk of these diseases. Therefore, there is a great interest in plants containing antioxidant as potential therapeutic agents[8].

Among the most promising sources of natural antioxidants, is the *Euterpe oleracea*, also known as the "açaí" berry. The pulp from the açai berry is consumed *in natura* or combined with other ingredients. Açaí is famous not only for its antioxidant and anti-inflammatory properties, but also for its hypocholesterolemic activity, indicated by favorable changes in the enzyme profile and fatty acids in the vascular system[9].

Recently, some researches have indicated that not only its pulp, but also the açaí seed has antioxidant properties against linoleic acid and superoxide anion oxidation. Still, there has not been enough research on the subject yet, which affects toxicological safety in some way[10]. Thus, it is important to conduct studies to understand the effect of the açaí seed in cachectic syndrome caused by cancer.

Methods

The Ethics Committee in Animals Use of the Universidade Estadual do Pará (UEPA), protocol No. 05/15 approved it.

All the animals in the study were taken care of according to the national legislation of Procedures for Scientific Use of Animals (Federal law 11.794 from October 8, 2008) and to the rules of Brazilian College of Animal Experimentation (COBEA). We used 20 male rats of the Wistar lineage (*Ratus norvegicus*) in the experiment, weighting between 250 and 310 grams. The rats came from the Universidade Federal de São João Del Rei's (UFSJ) *vivarium*, kept in a controlled environment in the Laboratory of Morphophysiology Applied to Health, and received ration and water *ad libitum*.

We used açaí seed extract from the açaí seeds of the medicinal plant garden of EMBRAPA. The Chemical Engineering School (UEPA) prepared the extract (açaí with ethanol-water solution).

The 20 animals were distributed randomly into four groups, each group containing five rats:

- Control Group (CG), which received only tumor inoculation;
- Placebo Group (PG), which received tumor inoculation and ethanol-water solution;
- Experimental Group-100 (EG-100), which received tumor inoculation and açaí seed extract in a 100mg/ml concentration
- Experimental Group-200 (EG-200), which received tumor inoculation and açaí seed extract in a 200mg/ml concentration.

For the inoculation of the Walker-256 Tumor, we made a trichotomy of the rats' right hemidorsum. After this process, we made antisepsis with alcoholic PVPI and injected, with a 3 ml syringe, 1 ml of tumor cells in the animal's hemidorsum at a 1 x 10^7 cells/ml concentration.

After the inoculation, we delivered açaí seed extract in the animals for 14 days, with an appropriate syringe (gavage cannula). Group EG-100 was given 100mg/ml, while group EG-200 was given 200mg/ml.

In the gavage period, we kept rats isolated in metabolic cages (Figure 1) in order to evaluate food ingestion. We also weighed the animals daily to quantify weight loss and food intake. This evaluation aimed to register the cachectic syndrome development in these rats.

FIGURE 1 - Metabolic cage.

We performed euthanasia on the 14th day after tumor inoculation when the cachectic process was completely settled in the animal. The chosen method was decapitation with a guillotine. Then we performed tumor mass exeresis, which was weighed with a precision balance and had its diameter quantified with a pachymeter.

The oxidative stress was measured indirectly by the quantification of TBARS (Thiobarbituric Acid Reactive Substances) in the cerebral cortex, liver and soleus muscle of the rats, using the Winterbourn method (1985). The quantity of TBARS produced was measured in a spectrophotometer with a 532 nm wave length.

We homogenized the soleus muscle and a portion of the liver to evaluate the proteolysis and later we quantified proteins with the Bradford method with spectrophotometer at 695 nm wave length.

Finally, the obtained data was analyzed by one-way analysis of variance – ANOVA test using Bioestat software. P values < 0.05 were taken to indicate statistical significance. Kruskall-Wallis (KW) test was also performed using a significance level of 5% to reject the null hypothesis.

Results

The quantification of oxidative stress using the TBARS technique in animal's cerebral cortex had higher results in the Experimental Group (EG-100), and significantly lower results in the Control Group (CG), suggesting that the açaí extract caused deleterious effects in the tissue (Table 1). The quantification of oxidative stress in muscle and liver tissues showed no statistically significant differences between groups.

TABLE 1 - Thiobarbituric acid reactive substances (TBARS) levels in cerebral cortex.

	CG	EG-100*	EG-200*	**PG***
R1	0.37	1.00	0.63	0.64
R2	0.38	0.47	0.37	0.48
R3	0.27	0.86	0.73	0.47
R4	0.54	0.59	0.54	0.74
R5	0.34	0.59	0.54	0.53
Media	**0.38**	**0.70***	**0.56***	**0.57***
Standard Deviation	**0.10**	**0.22**	**0.13**	**0.12**

Source: Research protocol. *p<0.05 (ANOVA).

Protein concentration in rats' muscle was higher in the Control Group (CG) and Placebo (GP) and lower in the animals treated with açaí (EG-100 and EG-200), showing an accentuated proteolysis in these last two groups (Table 2). Liver protein concentration did not show a statistically significant difference between groups.

TABLE 2 - Protein concentration in muscular tissue.

	CG	EG-100*	EG-200*	**PG**
R1	2.18	1.94	2.12	2.13
R2	2.16	1.98	1.86	2.25
R3	2.44	1.95	2.18	2.07
R4	2.13	1.53	1.36	1.66
R5	2.18	1.82	1.57	1.98
Media	**2.22**	**1.84***	**1.82***	**2.02**
Standard Deviation	**0.13**	**0.19**	**0.35**	**0.22**

Source: Research protocol. *p<0.05 (Kruskal Wallis).

For the tumor, there were smaller diameters in the Experimental Groups (EG-100 and EG-200) compared to the

Control Group (CG), which received no treatment (Table 3). A similar trend was observed in tumor mass values, which were lower in EG-100 and EG-200 and higher in the GC, although the difference between them was not statistically significant (Figure 2).

TABLE 3 - Diameter of tumor mass, in centimeters.

	CG	EG-100*	EG-200*	**PG***
R1	4.5	4.5	3.8	4.3
R2	4.1	4.5	3.7	4.0
R3	5.0	4.7	3.6	4.4
R4	4.0	3.9	3.4	3.5
R5	4.9	4.5	3.0	4.0
Media	**4.50**	**4.42***	**3.50***	**4.04***
Standard Deviation	4.53	3.03	3.16	3.51

Source: Research protocol. *p<0.05 (ANOVA).

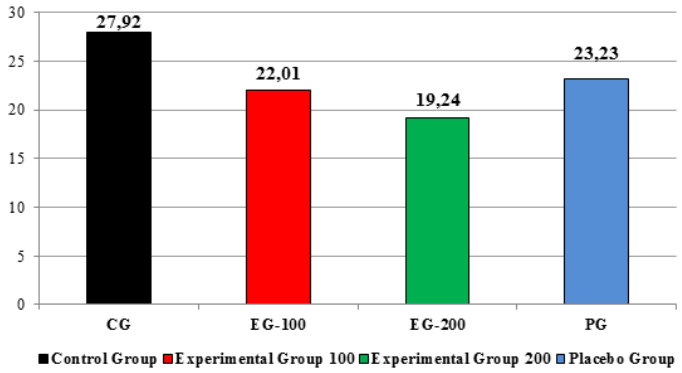

FIGURE 2 – Arithmetic mean of tumor weight, in grams (p>0.05, ANOVA).

Discussion

As a functional food, the açaí berry has antioxidant and anti-inflammatory effects, as well as other benefits represented by several nutritional components, thus acts as a health promoter when properly used[11].

A study conducted in 2014 assessing many types of fruits with great antioxidant potential, including açaí, determined that a fruit concentrate was able to promote beneficial effects in experimental animals. Among the results, it was observed that the levels of Thiobarbituric Acid Reactive Substances (TBARS) were lower in lab rats whose diets had high levels of açai fruit concentrate. Positive results were found in both the blood serum and liver of those animals[12].

However, the results found in the research above differ from what was found in this study, as the animals in Group EG-100 showed high levels of TBARS compared to the Control Group. As a result, we can show that the açaí seed extract caused deleterious effects of increased oxidative stress in those animals' bodies.

Regarding protein concentration, it is known that açaí can significantly reduce serum levels of carbonyl protein coming from cellular oxidative stress. In a 2009 publication, it was observed in experimental animals that the addition of açaí pulp to the diet could reduce the levels of carbonyl proteins in serum by 47%[13].

In this paper, we aimed to analyze the total proteins concentration of the soleus muscle. However, unlike the research cited in the paragraph above, the protein concentration in rats' muscles was higher in the Control and Placebo Groups and lower in the groups with animals treated with açaí, which shows increased proteolysis and cell damage in the groups treated with extract.

The açaí berry is known for its many antioxidants, including anthocyanins, which have a variety of functions including anticarcinogenic, anti-inflammatory and antioxidant activity, as well as the prevention of cardiovascular diseases[14].

A study conducted with C-6 cells from rats' glioma and MDA-468 human breast cancer cells was made in order to prove the anticarcinogenic and antioxidant activities of anthocyanins from açaí extract. An extract with high levels of anthocyanins from açaí was able to induce apoptosis of C-6 cells from rats' glioma. However, apoptosis was not observed in breast cancer cells, although there is strong scientific evidence of this phenomenon[15].

Similar results were found in the research of Fragoso *et al.*[16], which determined that açaí powder reduces the carcinogenesis of rats' colon. In this research, there was a reduction in the multiplicity of tumors and the number of invasive tumors when the animals were fed açaí powder. In addition, it promoted proliferation inhibition of tumor Ki-67.

This scientific evidence relates to what was found in this study, given that the diameters and masses of Walker-256 tumors were lower in animals treated with the açaí seed extract. Thus, we hypothesized that substances present in açaí seed can provide anticarcinogenic effects.

In the study, there was no statistically significant difference in daily weight and food intake among the animal groups analyzed. This data corroborates the study of Fragoso *et al.*[13], but disagrees with what was found by Souza *et al.*[14], which determined that consumption of açaí pulp reduced food intake of rats in hypercholesterolemic diet and reduced weight gain in animals on the same diet and even those from the Control Group.

Although we have found results differing to what literature shows, our study stands out for being original and seeking new mechanisms of action against cancer deleterious effects, using a material that would theoretically be disposable and unusable. Further new research should be conducted to elucidate the subject and promote greater knowledge about the benefits of the açaí seed.

Conclusions

The açaí seed extract did not cause major changes in the parameters studied in lipid peroxidation and muscle proteolysis, when submitted by gavage and in an ethanol-water solution. However, based on the diameter and tumor weight data, we observed anticarcinogenic effects.

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